UNITED STATES DISTRICT COURT
DISTRICT OF SOUTH CAROLINA
COLUMBIA DIVISION

AMY ELIZABETH WILLIAMS as the PERSONAL REPRESENTATIVE of the ESTATE FOR [redacted] and AMY ELIZABETH WILLIAMS individually, Plaintiffs, vs. QUEST DIAGNOSTICS, INC., ATHENA DIAGNOSTICS, INC., ADI HOLDINGS, INC. Defendants.	CA. NO. 3:16-CV-00972-MBS **FIRST AMENDED COMPLAINT**

Pursuant to Fed. R. Civ. P. 15(a)(2), and with leave from this Honorable Court, the Plaintiffs submit the following First Amended Complaint:

Plaintiffs, Amy Elizabeth Williams as the Personal Representative of the Estate for [redacted] (hereinafter "[redacted]") and Amy Elizabeth Williams individually (hereinafter "Williams ID") (and when necessary, collectively hereinafter as "Plaintiffs"), by and through their counsel, complaining of the above named defendants herein, respectfully allege and show unto this Honorable Court as follows:

JURISDICTIONAL FACTS

1. Plaintiff, Williams PR, is the duly appointed Personal Representative of the Estate of her deceased child, [redacted] ("[redacted]" or "Decedent"), as will appear more fully in the records of the Probate Court for the County of Richland, South Carolina in case No. 2015-

ES-40-00520. Williams PR brings this action on behalf of the Estate of [redacted] and for those damages recoverable by the statutory beneficiaries of [redacted].

2. Plaintiff, Williams ID, is presently a citizen and resident of Horry County, South Carolina; however, Williams ID lived with [redacted], in Richland County at the time of [redacted]'s death.

3. The Defendant Quest Diagnostics Incorporated (hereinafter "Quest") is, upon information and belief, a foreign corporation, organized in the state of Delaware, with its principal place of business in Madison, New Jersey. Quest is a large publicly traded company with a recently valued market capitalization of approximately Nine Billion U.S. Dollars, which offers a variety of laboratory testing products and laboratory services to clients residing in every U.S. state as well as certain clients living in a number of foreign countries. Quest, at all times relevant hereto, was doing business in South Carolina through its agents and subsidiaries, and through this persistent course of conduct, Quest derives substantial revenue from services rendered in South Carolina. Moreover, both the negligent conduct and purposeful actions and inactions of Quest, related to the Plaintiffs herein and as better described below, foreseeably connect Quest with the forum state of South Carolina such that Quest should reasonably anticipate being subjected to the jurisdiction of this Court, thus this court has in personam and subject matter jurisdiction over the defendants. Likewise, venue is proper in Richland County.

4. The Defendant Athena Diagnostics Incorporated (hereinafter "Athena") is, upon information and belief, a foreign corporation, organized in the state of Delaware, and authorized to conduct business in South Carolina as a registered foreign corporation with the South Carolina Secretary of State. Athena, at all times relevant hereto, was doing business in South Carolina through its agents, and through this persistent course of conduct, Athena derives substantial

revenue from services rendered in South Carolina. Moreover, both the negligent conduct and purposeful actions and inactions of Athena, related to the Plaintiffs herein and as better described below, foreseeably connect Athena with the forum state of South Carolina such that Athena should reasonably anticipate being subjected to the jurisdiction of this Court, thus this court has in personam and subject matter jurisdiction over the defendants. Likewise, venue is proper in Richland County.

5. The Defendant ADI Holdings Incorporated (hereinafter "ADI") is, upon information and belief, a foreign corporation, organized in the state of Delaware, and at all times relevant hereto, ADI was doing business in South Carolina through its agents and subsidiaries, and through this persistent course of conduct, ADI derives substantial revenue from services rendered in South Carolina. Moreover, both the negligent conduct and purposeful actions and inactions of ADI, related to the Plaintiffs herein and as better described below, foreseeably connect ADI with the forum state of South Carolina such that ADI should reasonably anticipate being subjected to the jurisdiction of this Court, thus this court has in personam and subject matter jurisdiction over the defendants. Likewise, venue is proper in Richland County.

6. Quest, Athena, and ADI (when referred to collectively herein after, the "Defendants") are part of an interrelated amalgamation of corporate interests. Quest, a large publicly traded entity, owns all outstanding shares of ADI following its purchase of ADI in 2011. ADI, in turn, owns all outstanding shares of Athena.

RELEVANT AGENTS AND ACTORS

7. Narasimhan Nagan, Ph.D. (hereinafter "Nagan") is, upon information and belief, a resident and citizen of Massachusetts. Nagan was employed by Athena as Director of Genetics, and at all times relevant hereto was an agent and servant of Athena, and therefore Athena is

liable for the acts of Nagan under the doctrine of respondeat superior. Moreover, both the negligent and purposeful actions, and inactions, of Nagan foreseeably caused the harms to the Plaintiffs as described below.

8. Hui Zhu, Ph.D. (hereinafter "Zhu") is, upon information and belief, a resident and citizen of Massachusetts. Zhu was employed by Athena as Director of Genetics, and at all times relevant hereto was an agent and servant of Athena, and therefore Athena is liable for the acts of Zhu under the doctrine of respondeat superior. Moreover, both the negligent and purposeful actions, and inactions, of Zhu foreseeably caused the harms to the Plaintiffs as described below.

9. Sat Dev Batish, Ph.D. (hereinafter "Batish") is, upon information and belief, a resident and citizen of Massachusetts. Batish was employed by Athena as Chief Director of Genetics, and at all times relevant hereto was an agent and servant of Athena, and therefore Athena is liable for the acts of Batish under the doctrine of respondeat superior. Moreover, both the negligent and purposeful actions, and inactions, of Batish foreseeably caused the harms to the Plaintiffs as described below.

10. Joseph J. Higgins, M.D. (hereinafter "Higgins") is, upon information and belief, a resident and citizen of Massachusetts. Higgins was employed by Athena and served as the Clinical Laboratory Improvement Amendments ("CLIA") Laboratory Director and license holder (#22D0069726) for Athena, and at all times relevant hereto was an agent and servant of Athena, and therefore Athena is liable for the acts of Higgins under the doctrine of respondeat superior. Moreover, both the negligent and purposeful actions, and inactions, of Higgins foreseeably caused the harms to the Plaintiffs as described below.

FACTUAL BACKGROUND FOR RELIEF

11. The factual assertions contained in the averments, which follow in paragraphs Eleven (11) through Thirty Seven (37), are herein referenced by the affidavit of Robert Cook-Deegan, M.D., who is a research professor in the Sanford School of Public Policy at Duke University, with secondary appointments in Internal Medicine (School of Medicine), and Biology (Trinity College of Arts & Sciences). He is also the founding director for Genome Ethics, Law & Policy in Duke's Institute for Genome Sciences & Policy. This affidavit and all of its attachments are incorporated within this complaint and affixed hereto as Exhibit A, as if set forth herein verbatim.

12. [redacted] was born on August 23, 2005, and developed normally and relatively healthfully throughout his first four months. By December 23, 2005, at his four month check-up, [redacted]'s health records indicate that he began suffering from febrile focal motor seizures. Accordingly, Williams ID sought treatment for [redacted]'s condition from a variety of service providers. Despite his treatments, [redacted]'s condition developed into frequently occurring afebrile seizures of varying types, including tonic-clonic, atonic and absence seizures. These often reached status epilepticus. The medical treatments given to [redacted] included the prescription of *sodium channel blocking medications*, including: Carbamazepine (Tegretol) and Lamotrigine (Lamictal**)**.

13. These *sodium channel blocking medications* are used to treat frequently occurring seizures of varying types and were prescribed by treating neurologist, Timothy Scott Livingston, M.D. (hereinafter "Dr. Livingston"), while Dr. Livingston was employed with the University of South Carolina Medical School. However, the administration of these medications proved ineffective at treating [redacted]'s condition.

14. In an attempt to more accurately diagnose the exact nature of [redacted]'s condition, a series of tests were performed, including: Five (5) muscle biopsy tests, Seven (7) different genetic tests, Ten (10) metabolic tests, and Two (2) cell culture tests as diagnostic workup for [redacted]'s history of refractory seizures and developmental delay.

15. Many of these tests were undertaken at the direction of treating clinical geneticists, John McKinley Shoffner, M.D. (hereinafter "Dr. Shoffner"), and, Frances Dougherty Kendall, M.D. (hereinafter "Dr. Kendall"), while both were employed at Horizon Molecular Medicine in Atlanta, Georgia. From this diagnostic workup, performed by Dr. Shoffner and Dr. Kendall, [redacted] was diagnosed with "probable mitochondrial encephalomyopathy."

16. Working under this original mitochondrial diagnosis, Dr. Shoffner sought confirmation by systematically ruling out other possible causes for [redacted]'s condition of frequently occurring afebrile seizures. Among the other possible causes of [redacted]'s condition included a severe form of epileptic encephalopathy also known as severe myoclonic epilepsy of infancy– Dravet Syndrome, also known as *Severe Myoclonic Epilepsy of Infancy* ("SMEI").

17. As part of the process of dismissing Dravet Syndrome and confirming Dr. Shoffner's and Dr. Kendall's mitochondrial diagnosis, deoxyribonucleic acid ("DNA") was extracted from a blood sample. The extracted DNA was provided to Athena's laboratory for what is known as an SCN1A DNA Sequencing Clinical Diagnostic Test for the very limited purpose of "detecting an existing disease, illness, impairment, symptom or disorder" on the particular gene where a connection to Dravet would likely be found.

18. Sodium channel, voltage gated, type I alpha subunit (designated "SCN1A") is a human gene that provides instructions for making sodium channels. These channels, which are most often found in brain and muscle cells, transport positively charged sodium atoms (sodium ions)

into cells. This process plays a key role in a cell's ability to generate and transmit electrical signals from one nerve cell (neuron) to another. Without an effective mechanism for controlling the flow of sodium ions (charged particles) from one neuron to the next in the brain, seizures can occur. Seizures arise when neurons that excite electrical impulses are not balanced by neurons that inhibit electrical impulses. Defects in the sodium channel foster seizures because the sodium channel is located in a neuron that inhibits electrical impulses. When the sodium channel is defective, the neuron does not inhibit electrical impulses effectively, so there is an overabundance of excitation and seizures result.

19. On June 30, 2007, an SCN1A DNA Sequencing Clinical Diagnostic Report (the "2007 Report") issued by Athena, indicated that [redacted] possessed a DNA mutation in the SCN1A gene classified as a "Variant of unknown significance."

20. Specifically, Athena detected and identified "a transversion from thymine (T) to adenine (A) at nucleotide position 1237 at codon 413 resulting in the amino acid change of tyrosine (Y) to asparagine (N)" (hereinafter designated "1237T>A, Y413N" as it appears in the 2007 Report). Williams PR or Williams ID never saw the 2007 Report until September 29, 2014, as discussed below.

21. The glossary provided in the 2007 Report defines Variant of unknown significance as: "DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function."

22. Tragically for [redacted] and his mother, Williams ID, the 2007 Report by Athena, which classified his SCN1A DNA mutation as a "Variant of unknown significance," was incorrect. [redacted]'s specific DNA mutation (1237T>A, Y413N) in the SCN1A gene not only possessed the

characteristics expected of a disease causing alteration, but such mutation had also been reported, studied, and known in those persons expressing Dravet Syndrome. Yet, the test in question – designed, marketed, and employed to identify this well known mutation – failed to do so.

23. Because this test is specifically designed and marketed to identify mutations linked to Dravet Syndrome, doctors for [redacted] reasonably expected to receive unequivocal results. Given that Athena presented itself as an expert, with highly technical and specialized skill sets, [redacted]'s doctors reasonably expected the test to clearly confirm whether [redacted] possessed a DNA mutation linked to Dravet.

24. The Technical Results, of the 2007 Report, appear "methodologically accurate" and appear to correctly identify the transversion in question located on the correct SCN1A gene. However, the mutation simply is mislabeled. Doctors who seek the highly specialized testing at issue should be able to rely on the fact that such a test would correctly identify a well "Known disease-associated mutation" without regard to the speculation of the comments section. By not providing [redacted]'s doctors with the definitive answer that the mutation was known to be associated with Dravet Syndrome, which was the main reason for conducting the test in question, Defendants breached a duty of care owed to Williams PR and Williams ID by misleading the child's doctors.

25. Given the specific value of the test in question – to identify gene mutations linked to Dravet Syndrome – [redacted]'s doctors reasonably relied that the test in question would identify whether or not [redacted] possessed such a mutation, and if such a mutation were identified, then [redacted]'s doctors would have expected such a mutation to be labeled correctly – "Known disease-associated mutation."

26. Unfortunately, the test results definitively state that [redacted] possessed a mutation of "unknown significance," which in turn misinformed [redacted]'s doctors of the significance of the mutation in question. Doctors for [redacted] should have been able to rely on the classification of the variant type listed on the test's technical results without looking to boilerplate disclosures that speculate on the significance of mutations of purportedly "unknown significance."

27. A review of the SCN1A DNA Sequencing Diagnostic contained in the 2007 Report and the mistakes therein, indicates that Athena breached the standards of care set by Clinical Laboratory Improvement Amendments ("CLIA") – a federal certification process for laboratories that perform clinical diagnostic tests on human specimens in the United States – for a certified diagnostic laboratory performing high-complexity genetic testing. Specifically, Athena negligently failed to correctly classify the DNA missense mutation in the decedent's SCN1A gene. See 42 C.F.R 491.10 *et seq*.

28. The existence of two clinical publications establish the foundation necessary to link [redacted]'s DNA missense mutation to the DNA diagnostic variant of "Known disease-associated mutation," as listed in the 2007 Report. These variant classifications are based on criteria set forth and defined by Athena. Both of these publications, Berkovic et al., 2006 and Harken et al., 2007, were products of the laboratory that was granted a patent on the identification of SCN1A mutations and their utilization in the treatment of sodium-channel dysfunction disorders. This patent was licensed and utilized by Athena for SCN1A DNA clinical diagnostic testing in the United States (U.S. Patent # 7,078,515 - licensed to Athena by Bionomics Ltd. in September 2004).

29. Additionally, [redacted]'s mutation (1237T>A, Y413N) was specifically cited as an SCN1A DNA mutation that "disrupts the functioning of an assembled ion channel so as to produce an

epilepsy phenotype" in U.S. Patent #8,129,142 and #7,709,225, continuations-in-part of U.S. patent #7,078,515. That is, these patents originated in the same patent that Athena lists as exclusively licensed for SCN1A genetic testing. This patent was used for the development, validation and utilization of SCN1A DNA clinical diagnostic testing in the United States.

30. Moreover, because the process of identifying [redacted]'s particular mutation of the SCN1A gene was, at the time of the 2007 Report, subject to the above patents and Athena had the sole responsibility for creating and defining the possible "Variant Types" listed in the Technical Results, neither Williams PR nor Williams ID, nor their doctors had any meaningful opportunity for second opinions. Both Williams PR and Williams ID, as well as the doctors they employed, reasonably relied on the Technical Results and the Variant Type provided by the 2007 Report.

31. Further, the 2007 Report indicates that the Chief CLIA Laboratory Director for Athena, Batish, reviewed the laboratory results and submitted the erroneous clinical information of [redacted]. Moreover, Batish is one of the authors of the Harkin et al., 2007 publication referenced above, which identifies [redacted]'s mutation as one associated with Dravet. This scholarly paper was submitted and published prior to Athena's issuance of the 2007 Report. As such, Batish clearly knew, or should have known, that a mistake was apparent on the 2007 Report.

32. The errors cited above, violate the stated classification procedures of Athena's CLIA certification, including: CLIA regulation 42 C.F.R. §493.1291(a), "the laboratory must have an adequate system(s) in place to ensure test results are accurately and reliably sent from the point of data entry to final report destination, in a timely manner" and §493.1289(a) "the laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and when indicated, correct problems identified in the analytic systems." In addition, there is no date recorded for specimen collection date, violating §493.1283(a)(2), "the laboratory

must maintain an information or record system that includes the date and time of specimen receipt into the laboratory."

33. As a result of the various violations of CLIA and the errors in classification cited above, doctors for [redacted] continued in their mistaken original diagnosis that [redacted] suffered from a mitochondrial disorder and continued with treatments designed for same.

34. Reasonably relying on the erroneous 2007 Report issued by Athena, a diagnosis of Dravet Syndrome was rejected by Dr. Shoffner, Dr. Kendall, Dr. Livingston and Dr. Clarkson. As a result, [redacted] continued to be treated with increasing doses of multiple *sodium channel blocking medications* including Carbamazepine (Tegretol) and Lamotrigine (Lamictal) – a standard treatment for epileptic seizures *not caused by Dravet Syndrome (SMEI)*.

35. As a proximate cause of Athena's negligent laboratory practices and failure to accurately classify the identified DNA mutation according to Athena's own variant classification criteria listed in the 2007 Report, [redacted] lost his life on January 5, 2008 following a traumatic seizure.

36. The health records of [redacted] reflect treatment with *sodium channel blocking medications*, including Carbamazepine (Tegretol), Oxcarbazepine (Trileptal) and Lamotrigine (Lamictal), for treatment from onset of seizure presentation through the end of [redacted]'s life.

37. *Sodium channel blocking medications* have been reported in numerous publications, Horn et al., 1986, Wakai et al., 1996, Guerrini et al., 1998, to worsen seizures in those with Dravet Syndrome (SMEI). These publications include reference #11 on [redacted]'s 2007 SCN1A DNA Sequencing Clinical Diagnostic Report issued by Athena.

38. This causal connection between Athena's negligence and [redacted]'s ensuing death is further confirmed by the affidavit of Dr. Max Wiznitzer, M.D. (hereinafter "Dr. Wiznitzer"), a pediatric neurologist at the Rainbow Babies & Children's Hospital, board-certified by the

American Board of Pediatrics in Pediatrics and board-certified by the American Board of Psychiatry and Neurology both in Neurology, with special qualification in Child Neurology, and in Neurodevelopmental Disabilities. In his affidavit, Dr. Wiznitzer notes, "that if the ([redacted]'s) SMEI condition had been properly diagnosed and had [[redacted]] received appropriate care for the treatment and management of SMEI, [[redacted]] would not have suffered the fatal seizure on January 5, 2008." Dr. Wizniter's affidavit, and all of its attachments, are incorporated within this complaint and affixed hereto as Exhibit B, as if set forth herein verbatim.

39. Thus, Athena's violation of multiple CLIA federal regulatory standards posed immediate jeopardy to [redacted] (42 C.F.R. §493.2), "a situation in which immediate corrective action is necessary because the laboratory's noncompliance with one or more condition level requirements has already caused, is causing, or is likely to cause, at any time, serious injury or harm, or death, to individuals served by the laboratory."

40. The 2007 Report issued by Athena, breached the standard of care of a clinical diagnostic laboratory performing genetic testing by any one or all of the following:

a) its negligent failure to provide an accurate genetic confirmation of a Dravet Syndrome (SMEI),

b) failure to adhere to a post-analytic DNA variant classification system,

c) failure of timely notification of the SCN1A DNA mutation reclassification.

41. Such failures, as described in the foregoing, of each Athena agent listed above as having contributed to the laboratory results have deviated from the accepted standard of care protocol of any CLIA certified clinical diagnostic laboratory performing high-complexity genetic testing. This directly and proximately resulted in [redacted]'s doctors persisting in their erroneous diagnosis

and leading to dangerous and ineffective treatments of [redacted]. As such, this erroneous 2007 Report proximately caused the death of the young child [redacted].

42. Further, such failures, as described in the foregoing, of Athena's CLIA license holder Higgins have deviated from the accepted standard of care in the field of clinical diagnostic genetics, and proximately contributed to the erroneous 2007 Clinical Diagnostic Report. The clinical findings as reported lead to inappropriate treatments, thereby exacerbating the seizure disorder of and proximately contributing to the death of the child, [redacted].

43. Thereafter, in September 2014, at the request of Williams ID, Lola Kate Clarkson, M.D. (hereinafter "Dr. Clarkson") and Amy Dobson, M.S., CGC, Athena and Quest were contacted seeking a copy of the SCN1A DNA Sequencing Clinical Diagnostic Report for [redacted]. After significant delay, on January 30, 2015, Quest and Athena jointly produced a Revised Report (the "2015 Report"). This revised 2015 Report now correctly indicated that the SCN1A DNA mutation is a "Known disease-associated mutation," and not as initially classified a "Variant of unknown significance." Of note, this correction and proper reclassification was made *without* parental testing.

44. Both Athena and Quest jointly issued this purportedly "new" 2015 SCN1A DNA Sequencing Clinical Diagnostic and forwarded it to Dr. Clarkson as a facsimile imprinted with title "Quest Diagnostics" in pertinent part. However, this purportedly "new" 2015 Report does not cite any new publication references used in the re-classification of this mutation (1237T>A, Y413N). Such an omission violates CLIA regulation §493.1241(c)(8), "any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation."

45. Additionally, the laboratory results and submitted clinical information within the 2015 Report were purportedly authorized and reported by Nagan**,** ABMG, Director, Genetics, and Zhu, ABMG, Director, Genetics. Upon information and belief, both Nagan and Zhu left the employment of Athena before 2009 and as such could not have possibly authorized the issuance of the revised 2015 Report. This intentional misrepresentation violates CLIA regulation §493.1283(4) "the record system must include the records and dates of all specimen testing, including the identity of the personnel who performed the test(s)," which are necessary to assure proper identification and accurate reporting of test results. To issue the revised 2015 Report authorized by two geneticists that are no longer employed by Athena must have been done by Quest and Athena, in concert, with intent to circumvent or disobey CLIA's federal regulatory standards.

46. Moreover, both Defendants Athena and Quest violated CLIA regulation §493.1291(k) when neither issued a properly amended clinical diagnostic report when this DNA mutation (1237T>A, Y413N) was re-classified as a "Known disease-associated mutation." When errors in the reported test results are detected, "the laboratory must: (k)(1) Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors, (k)(2) Issue corrected reports promptly to the authorized person(s) ordering the test and, if applicable, the individual using the test results."

47. Furthermore, both Defendants Athena and Quest violated the Plaintiffs' right to access protected health information (CLIA regulation §493.1291(1), and the Health Insurance Portability and Accountability Act, regulation 45 CFR §164.524(c)(3)(ii)), when it refused to provide the completed test results to the Plaintiffs upon Williams ID's request in September 2014.

48. Moreover, such failures of the initial 2007 Report, as described in the foregoing, were known to all the Defendants for a considerable period of time prior to the issuance of the 2015 Report at Williams ID's request, through Dr. Clarkson. However, these defendants purposefully chose not to reveal this vital information to the Plaintiffs.

49. In failing to provide correct test results and the requisite updated results, Williams ID continued to believe she carried a mitochondrial mutation that might be passed along to any subsequent children she might bear.

50. Williams ID sought counseling and the companionship of other mothers with mitochondrial conditions in various support groups. Within these support groups Williams ID shared her story of loss and listened to the problems of others she believed to be similarly situated. Williams ID gathered what she reasonably believed to be important information about her mitochondrial condition and how such condition might affect future children.

51. Upon receiving the 2015 Report, Williams ID realized the true cause of the death of her son, and this created new psychological trauma whereby she relived the moment of the child's death and re-experienced the substantial and unparalleled pain of losing her only child.

FOR A FIRST CAUSE OF ACTION
(Negligence/Gross Negligence Resulting in Wrongful Death)

52. Each and every allegation set forth above is fully incorporated herein.

53. Williams PR is the duly appointed Personal Representative of the Estate of [redacted] and she brings this action on behalf of the Estate of [redacted] and for damages recoverable by the statutory beneficiaries of [redacted].

54. At the time when [redacted]'s initial 2007 Test was conducted, Athena had a duty to meet a reasonable standard of care in the course of providing CLIA-licensed high complexity clinical diagnostic genetic testing services to [redacted], in accordance with the standards of the National

Committee on Clinical Laboratory Standards ("NCCLS"). However, Athena breached those duties and standards of care in one or more of the following particulars:

a) failure to provide accurate genetic confirmation of Dravet Syndrome (SMEI) in the 2007 Report,

b) failure to adhere to post-analytic DNA variant classification system,

c) failure of timely notification of the SCN1A DNA mutation reclassification,

d) failure to follow the litany of CLIA federal regulatory standards as recited above.

55. Athena breached its duties to [redacted] in each of the aforementioned particulars. Such breaches were negligent, grossly negligent, careless, and/or reckless.

56. Athena, acting through various agents, Nagan, Zhu, Batish and Higgins, reviewed or had opportunity to review, the 2007 Report with the finding of a DNA mutation (1237T>A, Y413N) as plainly stated on the 2007 Report – all test results are reviewed, interpreted and reported by ABMG certified Clinical Molecular Geneticists. A simple review of such findings would have confirmed that [redacted]'s mutation demonstrated genetic characteristics consistent with Dravet Syndrome. At a minimum, the incorrect classification of [redacted]'s mutation as a "Variant of unknown significance" could not be supported by the finding of a missense "DNA mutation (1237T>A, Y413N)."

57. Moreover, Athena had a duty to correctly inform Williams PR of his SMEI condition; such failure prevented [redacted] from discovering more appropriate treatments available to mitigate [redacted]'s condition, and a duty to aid [redacted] in obtaining treatment to correct the condition, or otherwise mitigate the known consequences of Athena's erroneous diagnostic DNA variant classification. Such a breach of this duty was negligent, grossly negligent, careless, and/or reckless.

58. Further, Athena knew, or should have known, that results from their clinical diagnostic SCN1A genetic testing would have direct impact on [redacted]'s treatment and clinical management. Specifically, that a treating physician would likely continue to treat [redacted], or any other person with frequently occurring epileptic seizures *not caused by Dravet Syndrome (SMEI)*, with *sodium channel blocking medications* such as: Carbamazepine (Tegretol) and Lamotrigine (Lamictal), and that such a treatment would likely expose someone with Dravet Syndrome, such as [redacted], to a high risk of death, which in fact did occur.

59. The clinical findings of the 2007 Report, led to inappropriate medical treatments, thereby exacerbating [redacted]'s seizure disorder and proximately caused his death. [redacted] has suffered damages in an amount to be determined by the jury. As a direct and proximate cause of the aforementioned negligent, grossly negligent, careless, and/or reckless actions and/or omissions of Athena, acting by and through its agents, servants, and employees, as hereinabove more particularly set forth in paragraphs Forty (40) through Forty Three (43), [redacted] suffered a serious, severe, painful, debilitating, and fatal seizure resulting in his death on January 5, 2008.

60. [redacted]'s statutory beneficiaries, as represented by Williams PR, have suffered economic loss, severe emotional distress, anxiety, grief, and sorrow for which Williams PR is entitled to recover on behalf of the statutory beneficiaries actual and punitive damages (when allowable under law) pursuant to S.C. Code §§ 15-51-10 *et seq*. in an amount to be determined by the jury.

FOR A SECOND CAUSE OF ACTION
(Survivorship Action)

61. Each and every allegation set forth above is fully incorporated herein.

62. Athena, acting through its agents, Nagan, Zhu, Batish and Higgins, as well as those who might be uncovered through discovery, committed various acts and omissions as previously

outlined above, which constitutes negligence, gross-negligence, carelessness, recklessness and willfulness and wantonness.

63. As a direct and proximate result of the acts and/or omissions of Athena as listed above, [redacted], the decedent, sustained severe and permanent injuries, eventually leading to [redacted]'s death. Prior to his death, [redacted] suffered from numerous debilitating seizures, including the seizure that finally ended his life.

64. Williams PR is informed and believes that pursuant to S.C. Code § 15-5-90, [redacted]'s estate is entitled to a judgment against Athena for the damages, which [redacted] would be entitled had he survived the erroneous findings of the 2007 Report by Athena, both actual and punitive, for each additional seizure suffered by [redacted] including the seizure that ended the life of [redacted].

FOR A THIRD CAUSE OF ACTION
(Negligent Misrepresentation)

65. Each and every allegation set forth above is fully incorporated herein.

66. Upon information and belief, Defendants issued the 2007 Report to Plaintiff without regard to the consequences, which contained material information regarding the results of the genetic testing that included misrepresentations, errors, mistakes, or miscommunications which are known to be false, that directly resulted in damage to [redacted], as set forth above, and damage to Williams ID through the loss of a substantial period of her child bearing years based on the false belief she passed an uncharacterized mitochondrial disorder to her son [redacted], as well as severe emotional distress to Williams ID.

67. Upon information and belief, this negligent misrepresentation included the following:

a) The 2007 Report was a materially false representation made by these Defendants when they published the results that the mutation possessed by [redacted] was "a variant of unknown significance" instead of a "known disease-associated mutation" and distributed these results to [redacted]'s treating physicians;

b) These Defendants had a pecuniary interest in making the statement, due to the fact that through the course of their business, they perform these genetic tests for many patients;

c) These Defendants owed a duty of care to Plaintiffs and similarly situated individuals, to see that they communicated truthful information regarding the results of this testing;

d) The Defendants breached that duty by failing to exercise due care in reporting the results of the testing;

e) The Plaintiffs and [redacted]'s treating physicians were unaware of the falsity of this report and relied on the purported truth of this representation and had the right to rely on the representations made in the report;

f) As a direct result and proximate cause of the Defendants' false representation the Plaintiffs suffered injury and damages as set forth below.

68. As a direct and proximate result of the acts and/or omissions of all the Defendants, [redacted] sustained severe and permanent injuries, eventually leading to his death. [redacted]'s statutory beneficiaries, as represented by Williams PR, have suffered economic loss, severe emotional distress, anxiety, grief, and sorrow for which Williams PR is entitled to recover on behalf of the statutory beneficiaries actual and punitive damages.

69. As a direct and proximate result of the acts and/or omissions of all the Defendants, [redacted] sustained severe and permanent injuries, eventually leading to his death, and thereby proximately causing Plaintiff Williams ID the loss of comfort, services, companionship, and society of her son and child. Moreover, Williams ID lost a significant portion of her child bearing years and incurred medical expenses for the treatment of her severe emotional distress, the cost of the drugs that ultimately caused the demise of her child, the medical treatment that led to the death of her child, and the funeral expenses after the child passed. Williams ID is entitled to recover: the loss of her child bearing years, and the incurred medical expenses for the treatment of the child and her severe emotional distress.

FOR A FOURTH CAUSE OF ACTION
(Constructive Fraud)

70. Each and every allegation set forth above is fully incorporated herein.

71. Upon information and belief, Defendants issued the 2007 Report to [redacted]'s treating physicians which contained materially false information regarding the results of the genetic testing, that directly resulted in damage to [redacted], as set forth above, and damage to Williams ID through the loss of a substantial period of her child bearing years based on the false belief she passed an uncharacterized mitochondrial disorder to her son [redacted], as well as severe emotional distress to Williams ID.

72. Upon information and belief, this false representation included the following:

a) These Defendants issued a report to [redacted]'s treating physicians regarding the genetic testing results of [redacted] when they knew, or ought to have known, that the 2007 Report was a materially false representation; in that, they reported the mutation possessed by [redacted] was "a variant of unknown significance" instead of a "known disease-associated mutation";

b) The report was materially false and misleading;

c) These Defendants had the intent for Williams ID and [redacted]'s treating physicians to rely on the representation that they made and that she and her physicians would rely on and act upon the erroneous report;

d) Williams ID and [redacted]'s treating physicians were ignorant and uninformed of the material falsity of the assertions contained in the report;

e) Williams ID and [redacted]'s treating physicians relied upon the truth of the results published by Defendants in the report and had a right to rely on the truth of the statements made by Defendants who are entities that, in their normal course of business and in furtherance of their business, perform this type of genetic testing;

f) As a direct result and proximate cause of the Defendants' false representation and the Plaintiffs' and [redacted]'s treating physicians' reliance on the false representation, Plaintiffs have suffered injury and damages as set forth below.

73. As a direct and proximate result of the acts and/or omissions of all the Defendants, [redacted] sustained severe and permanent injuries, eventually leading to his death. [redacted]'s statutory beneficiaries, as represented by Williams PR, have suffered economic loss, severe emotional distress, anxiety, grief, and sorrow for which Williams PR is entitled to recover on behalf of the statutory beneficiaries actual and punitive damages.

74. As a direct and proximate result of the acts and/or omissions of all the Defendants, [redacted] sustained severe and permanent injuries, eventually leading to his death, and thereby proximately causing Plaintiff Williams ID the loss of comfort, services, companionship, and society of her son and child. Moreover, Williams ID lost a significant portion of her child bearing years and incurred medical expenses for the treatment of her severe emotional distress,

the cost of the drugs that ultimately caused the demise of her child, the medical treatment that led to the death of her child, and the funeral expenses after the child passed. Williams ID is entitled to recover: the loss of her child bearing years, and the incurred medical expenses for the treatment of the child and for her severe emotional distress.

FOR A FIFTH CAUSE OF ACTION
(Civil Conspiracy)

75. Each and every allegation set forth above is fully incorporated herein.

76. Upon information and belief, once the obvious misrepresentation and falsity in the 2007 Report was discovered, the decision to hide [redacted]'s actual diagnostic condition was a conscious one made exclusively to protect corporate assets of defendants Athena, ADI, and Quest. Both Athena and Quest jointly issued the revised 2015 Report on the SCN1A DNA Sequencing Diagnostic and forwarded this revised 2015 Report to Williams ID, through Dr. Clarkson.

77. At some point between the issuance of the 2007 Report and the issuance of the 2015 Report, two or more of the above named Defendants (Athena, ADI, and Quest) acting through their agents and/or executives conspired to intentionally withhold and cover-up the corrected information as reflected by the 2015 Report and as set forth in the "*Factual Background for Relief*."

78. Upon information and belief, Defendants recognized the significant risks these false reports posed to their respective financial assets and, in response, developed a plan to avoid responsibility for their respective acts by failing to disclose the false statement in the 2007 report to the Plaintiffs and by deliberate concealment of the false report.

79. The Defendants discovered the original false representation in the 2007 Report Technical

Results that misclassified the mutation at issue, and then engaged in deceitful conduct by intentionally concealing the initial false statement issued in the 2007 report. These intentional acts, in addition to the facts alleged in Plaintiffs' previous causes of action, were done willfully and in furtherance of the Defendants' conspiracy.

80. These Defendants lied, misrepresented, and actively concealed [redacted]'s actual genetic testing results in an attempt to protect corporate assets and hide their negligent misrepresentation and fraudulent malfeasance. This concealment not only harmed the Plaintiffs but violated regulatory standards as set forth under the CLIA. This conduct is both criminal, as a purposeful violation of CLIA regulation 42 C.F.R. § 493.1806(e), and shameful. Prior to 2015, the Defendants recognized that a substantial number of individuals were affected by similar erroneous SCN1A DNA Clinical Diagnostic Sequencing Reports. Defendants' intentional cover up and deception based upon concealing the known erroneous reporting prevented Williams ID from knowing the true nature of the DNA mutation at issue until the 2015 Revised Report was finally made available to Williams ID. These erroneous Clinical Diagnostic Reports can result in ill-informed health care choices, needless suffering, and death.

81. Defendants' conspiracy is also a violation of CLIA regulation §493.1291(k), as neither issued a properly amended clinical diagnostic report when this DNA mutation (1237T>A, Y413N) was re-classified as a "Known disease-associated mutation." When errors in the test results are detected, "the laboratory must: (k)(1) Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors, (k)(2) Issue corrected reports promptly to the authorized person(s) ordering the test and, if applicable, the individual using the test results."

82. Furthermore, both Defendants Athena and Quest violated the Plaintiffs' right to access

protected health information (CLIA regulation §493.1291(1), and the Health Insurance Portability and Accountability Act, regulation 45 CFR §164.524(c)(3)(ii)), when each refused to provide the completed test results to the Plaintiffs upon Williams PR's request in September 2014.

83. The revised 2015 Report does not cite any new publication references used in the re-classification of this mutation (1237T>A, Y413N). Such an error violates CLIA regulation §493.1241(c)(8), which states in part, "any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation."

84. The purportedly new laboratory results, as set forth within the 2015 Report, falsely state that both Nagan and Zhu authorized and signed-off on the issuance of the report. However, both agents left their employment with Athena before 2009 and, as such, could not have possibly authorized the issuance of the revised 2015 Report.

85. Such an intentional misrepresentation and intentional violation of CLIA federal regulatory standards is criminal, pursuant to § 493.1806(e), and civilly actionable due to the harm it has caused the Plaintiffs.

86. Since her discovery that [redacted] died from complications associated with Dravet Syndrome and the continued application of sodium channel blocking drugs, as opposed to other unknown genetic conditions, Williams ID has incurred special damages including the precise and ascertainable cost of a Whole Exome Sequencing, which included a mitochondrial DNA test. Mitochondrial DNA is separate from cellular DNA.

87. In June 2015, the Whole Exome Sequencing was undertaken by Williams ID when it

became apparent that she could not trust the results of the Defendants' reports, and was intended to confirm Williams ID's original mitochondrial diagnosis. Williams ID was compelled to determine the likelihood that she would pass such a condition to additional children that she might have. This test was wholly unnecessary given that [redacted] died from complications associated with Dravet Syndrome and would not have been undertaken by Williams ID had the corrected information not been suppressed by Defendants.

88. In addition to the cost of this additional genetic testing, until she received the results of the independent genetic testing conducted subsequent to the receipt of the 2015 Report, Williams ID continued to hold the belief that she might carry an inheritable mitochondrial disorder. As a result of the 2015 Report, and once she obtained confirmation that she did not carry an inheritable mitochondrial disorder, Williams ID sought and received additional extensive psychiatric counseling, including the prescription of different and/or increased medications, to deal with the real cause of her child's death, as well as the mental anguish and suffering associated with restricting herself from having more children out of fear of passing the same genetic mutation that she falsely believed she had.

FOR A FIFTH CAUSE OF ACTION
(Unfair Trade Practice Violations)

89. Each and every allegation set forth above is fully incorporated herein.

90. The Defendants sought to profit, and did profit, by performing SCN1A DNA testing on [redacted] and other clients. The Defendants also are among the world's leading providers of diagnostic testing on human tissue and offer services that range from routine blood tests, Pap testing, and white blood cell count, to such complex diagnostic testing as genetic and molecular testing.

91. The Defendants engaged in deceitful conduct by concealing the initial mistake issued in

the 2007 report. As more thoroughly set forth above, these Defendants lied, misrepresented, and actively concealed [redacted]'s actual genetic testing results in an attempt to protect corporate assets and hide their negligence and malfeasance. This concealment not only harmed the Plaintiffs but violated regulatory standards as set for under the CLIA. This deception prevented [redacted] and Williams ID from knowing the true nature of the DNA mutation at issue.

92. Left with the false belief that her son died of an uncharacterized mitochondrial disorder, Williams ID vainly sought to uncover what disorder she may have carried. Additionally, Williams ID was left with the false belief that this uncharacterized mitochondrial disorder might be inherited by any future children she might have. Williams ID spent a considerable amount of money and time in this fruitless endeavor since the specific DNA mutation (1237T>A, Y413N) on the SCN1A gene was known well to express Dravet Syndrome.

93. Further, Williams ID's false belief that her son died of an uncharacterized mitochondrial disorder and her seeming inability to obtain a clear cause of his death directly and proximately caused Williams ID to suffer severe emotional distress. Williams ID thereafter required the care and treatment of licensed medical providers at some significant personal expense.

94. Since her discovery that [redacted] died from complications associated with Dravet and the application of sodium channel blocking drugs, but not some other unknown genetic condition, Williams ID has incurred $31,065.82 to date in medical costs related to psychiatric care undertaken to help her cope with this discovery and the knowledge that [redacted]'s death was entirely preventable and proximately caused by the negligence of these Defendants.

95. The Defendants negligently, carelessly, recklessly, willfully and/or wantonly engaged in unfair or deceptive acts in the conduct of trade or commerce which are prohibited by S.C. Code Ann. § 39-5-20, *et seq.* (South Carolina Unfair Trade Practices Act).

96. The Defendants' unfair or deceptive acts are capable of repetition given the nature of Defendants' business and the vast number of people, both in South Carolina and around the country, who depend on the numerous diagnostic tests performed by these Defendants each year. Specifically, on November 11, 2004, in a "Chairman's Address to Bionomics Limited 2004 AMG," Bionomics suggested that as many as Two Hundred Thirty Thousand (230,000) children in the United States may be candidates for the same testing at issue here. The "Address" is attached and incorporated within this complaint and affixed hereto as Exhibit C, as if set forth herein verbatim. In September 2004, Bionomics granted a license to Athena Diagnostics for marketing of SMEI testing in the United States.

97. Additionally, Plaintiffs are aware of the case of [redacted], who sought the assistance of Athena in 2008. In 2008, Athena identified a mutation (2589+3A>T, IVS14+3A>T) in Claire's SCN1A gene. Athena classified this mutation as a "Variant of unknown significance." As with [redacted], this classification was incorrect since [redacted]'s mutation had been also identified in Harkin et al., 2007 as associated with Dravet. [redacted]'s mutation was reclassified in 2010 to be a "Known disease-associated mutation." Therefore, these acts are clearly capable of repetition and, as such, have an effect on the public and do concern the public interest.

98. As a direct and proximate result of the Defendants' unfair or deceptive acts, which are in violation of the South Carolina Unfair Trade Practices Act, Williams ID has suffered monetary damages, in an amount to be determined by the jury, in addition to treble damages and attorney's fees as authorized by statute.

WHEREFORE, Plaintiffs pray for judgment against the above named Defendants for actual and punitive damages in a reasonable amount for the costs of this action, including

reasonable attorney fees, for treble damages as may be assessed pursuant to South Carolina Unfair Trade Practices Act, S.C. Code Ann. § 39-5-20, *et seq.*, and for such other relief as the Court may deem just and proper.

Respectfully submitted,

s/Bradford W. Cranshaw
Bradford W. Cranshaw (Fed. Bar #9733)
Grier Cox and Cranshaw, LLC
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West Columbia, SC 29169
Phone: 803-731-0030
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ATTORNEYS FOR PLAINTIFF

June 2, 2016
Columbia, South Carolina

STATE OF SOUTH CAROLINA)
)
COUNTY OF RICHLAND)

In the Matter of the Estate for Christian Jacob Millare,

Amy Elizabeth Williams,
Personal Representative.

AFFIDAVIT OF ROBERT MULLAN COOK-DEEGAN, M.D.

Personally appeared before me, the undersigned notary public, Robert M. Cook-Deegan, M.D. who being first duly sworn deposes and states as follows:

1. I am a research professor in the Sanford School of Public Policy at Duke University, with secondary appointments in Internal Medicine (School of Medicine), and Biology (Trinity College of Arts & Sciences). I was the founding director for Genome Ethics, Law & Policy in Duke's Institute for Genome Sciences & Policy.

2. I have been consulted by attorneys from Grier, Cox & Cranshaw, LLC and Ervin & McGuire Law Firm, LLC, on behalf of their client Amy Elizabeth Williams (Personal Representative for the Estate of Christian Jacob Millare) to provide an expert opinion as to the SCN1A DNA sequencing diagnostic report performed on an extracted DNA sample from the late Christian Jacob Millare (August 23, 2005 - January 5, 2008) by Athena Diagnostics, Inc., a neurology division of Quest Diagnostics, Inc.

3. I am an independent academic researcher who came to the attention of the family and their legal counsel in part because of work performed at our Center for Public Genomics on clinical implications of gene patents.

4. I have received and reviewed the SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc. on June 30, 2007 (**Exhibit 1**), along with a second revised report issued January 30, 2015 (**Exhibit 2**). Additionally, I have received and reviewed the medical records pertaining to the decedent, Christian Jacob Millare.

Case History

5. On December 23, 2005, the medical record indicates that the decedent began suffering from febrile focal motor seizures following a routine 4th month vaccination. This condition developed into frequently occurring afebrile seizures of varying types, including tonic-clonic, atonic and absence seizures. These often reached status epilepticus. During his treatment, sodium channel blocking medications, including **Carbamazepine (Tegretol), Oxcarbazepine (Trileptal) and Lamotrigine (Lamictal)**, were prescribed.

6. On January 18, 2007, a whole blood sample was taken from the decedent and extracted DNA was provided to Athena Diagnostics, Inc. laboratory for a SCN1A DNA Sequencing Diagnostic Test. In a SCN1A DNA sequencing diagnostic report dated June 30, 2007 (**Exhibit 1**), Athena Diagnostics, Inc. indicated the decedent possessed a DNA mutation in the SCN1A gene classified as a "Variant of unknown significance."

7. A second revised SCN1A DNA sequencing diagnostic report was produced by Athena Diagnostics, Inc. on January 30, 2015 (**Exhibit 2**), which indicated that the SCN1A mutation that had been detected in the DNA of the decedent (initially classified as a "Variant of unknown significance") was re-classified as a "Known disease-associated mutation."

8. The medical records reflect the use of sodium channel blocking medications, including **Carbamazepine (Tegretol), Oxcarbazepine (Trileptal) and Lamotrigine (Lamictal)**, for treatment from onset of seizure presentation through the end of the child's life (**Exhibit 3**).

Errors in Original (June 30, 2007) SCN1A DNA Sequencing Diagnostic Report

9. It is apparent from the SCN1A DNA sequencing diagnostic report issued on June 30, 2007 (**Exhibit 1**) that Athena Diagnostics, Inc. breached the standard of care of a CLIA-certified diagnostic laboratory performing high complexity genetic testing by its negligent failure to correctly diagnose the DNA missense mutation in the decedent's SCN1A gene.

10. Specifically, the report erroneously made a misclassification of a DNA sequence variant: "A transversion for thymine (T) to adenine (A) at nucleotide position 1237 at codon 413 resulting in the amino acid change of tyrosine (Y) to asparagine (N)." By definition this should have been classified as "#3: Amino acid change of unknown significance" instead of "#4: Variant of unknown significance."

> **"3. Amino acid changes of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. The amino acid change is predicted based on simple interpretations of the genetic code. However, these same types of alterations may sometimes alter normal gene splicing and processing, and thereby cause more significant and unpredictable effects. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation."
>
> **"4. Variants of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. Typical examples include single nucleotide changes in the coding and noncoding regions of the gene that are sometimes labeled as "silent mutations" or "intronic polymorphisms." These types of alternations often have no effect, but may sometimes alter normal gene splicing and processing. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation."

11. The decedent's specific DNA mutation (1237T>A, Y413N) in the SCN1A gene not only possessed the characteristics expected of a disease causing alteration, but it had also been reported, studied, and known in patients expressing Dravet Syndrome, a severe form of epileptic encephalopathy also known as severe myoclonic epilepsy of infancy (SMEI).

(**Exhibit 4**)
Y413N = SMEI (Table 1 – Patient #9) [2006]
Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, Wirrell EC, Gill DS, Iona X, Mulley JC, Scheffer IE. De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. The Lancet Neurology. 2006;5(6):488-92. Epub 2006/05/23. doi: 10.1016/s1474-4422(06)70446-x. PubMed PMID: 16713920.

(**Exhibit 5**)
Y413N = SMEI (Supplement Table 2 – Patient #15) [2007]
Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A, Sutherland G, Berkovic SF, Mulley JC, Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain : A Journal of Neurology. 2007;130(Pt 3):843-52. Epub 2007/03/10. doi: 10.1093/brain/awm002. PubMed PMID: 17347258.

12. The existence of two clinical publications satisfies the criteria of variant classification "#1: Known disease-associated mutation."

    "**1. Known disease-associated mutations** (dominant) are documented in the literature to be associated with diseases inherited in a dominant manner. The individual is likely to be affected with, or predisposed to developing, a dominant genetic disease."

13. Both of these publications, Berkovic et al., 2006 & Harkin et al., 2007, were products of the laboratory that was granted the patent utilized by Athena Diagnostics, Inc. for SCN1A DNA sequencing in diagnosis (U.S. Patent # 7,078,515 - licensed to Athena Diagnostics, Inc. by Bionomics Limited in September 2004).

14. In addition, 1237T>A, Y413N was also cited as a SCN1A mutation that "disrupts the functioning of an assembled ion channel so as to produce an epilepsy phenotype" in U.S. Patent #8,129,142. This patent, filed in 2004, was one of several that the inventors obtained for the University of Adelaide and its spin-out, Bionomics Limited, for the development, validation and utilization of this SCN1A genetic test for SMEI diagnosis in the United States.

15. Furthermore, the Chief CLIA Laboratory Director that reviewed the laboratory results and submitted clinical information of the decedent, **Sat Dev Batish, Ph.D.**, is one of the authors of the Harkin et al., 2007 publication. That paper was submitted and published prior to the June 30, 2007 (**Exhibit 1**) SCN1A DNA sequencing diagnostic report on Christian Millare, issued by Athena Diagnostics, Inc.

16. The errors cited in numbers 9-13 violate the stated classification procedures of Athena Diagnostics, Inc. Clinical Laboratory Improvement Amendments (CLIA) certification (License # 22D0069726), including: §493.1291(a), "the laboratory must have an adequate system(s) in place to ensure test results are accurately and reliably sent from the point of data entry to final report destination, in a timely manner" and §493.1289(a) "the laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and when indicated, correct problems identified in the analytic systems." In addition, there is no date recorded for specimen collection date, violating §493.1283(a)(2), "the laboratory must maintain an information or record system that includes the date and time of specimen receipt into the laboratory."

Errors in Revised (January 30, 2015) SCN1A DNA Sequencing Diagnostic Report

17. The revised SCN1A DNA sequencing diagnostic report issued on January 30, 2015 (**Exhibit 2**) by Athena Diagnostics, Inc. does not cite any new publication references used in the re-classification of this mutation (1237T>A, Y413N). This error violates CLIA regulation §493.1241(c)(8), "any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation."

18. In addition, the laboratory results and submitted clinical information within this revised report were signed off by **Narasimhan Nagan, PhD**, ABMG, Director, Genetics, and **Hui Zhu, PhD**, ABMG, Director, Genetics; both of whom left Athena Diagnostics, Inc. before 2009. This error violates CLIA regulation §493.1283(4) "the record system must include the records and dates of all specimen testing, including the identity of the personnel who performed the test(s)," which are necessary to assure proper identification and accurate reporting of patient test results.

19. Moreover, Athena Diagnostics, Inc. violated CLIA regulation §493.1291(k) when it did not issue an amended report when this DNA mutation (1237T>A, Y413N) was re-classified as a "Known disease-associated mutation." "When errors in the reported patient test results are detected, the laboratory must: (k)(1) Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors. (k)(2) Issue corrected reports promptly to the authorized person(s) ordering the test and, if applicable, the individual using the test results."

20. Furthermore, Athena Diagnostics, Inc. violated the patient's right to access protected health information (CLIA regulation §493.1291(1), HIPAA rule 45 CFR §164.524(c)(3)(ii)), when it refused to provide the completed test results to the patient (or the patient's personal representative) upon request in September 2014.

Failure to Properly Diagnose, Resulting in Improper Treatment, Proximately Contributing to the Child's Death

21. Relying on the erroneous June 30, 2007 SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc., a diagnosis of Dravet Syndrome (SMEI) was rejected, and thus the decedent continued to be treated with increasing doses of multiple sodium channel blocking medications including **Carbamazepine (Tegretol) and Lamotrigine (Lamictal)**, a standard treatment for epileptic seizures not caused by Dravet Syndrome (SMEI). **(Exhibit 3)**

22. Sodium channel blocking medications, such as Lamotrigine (Lamictal) and Carbamazepine (Tegretol), have been reported in numerous publications to worsen seizures in patients with Dravet Syndrome/SMEI. These publications include reference #11 on the decedent's SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc (**Exhibit 1**). Furthermore, "Identifying anticonvulsant treatments to avoid" was also listed as an expectation of running an SCN1A genetic test (test code 535) by Athena Diagnostics, Inc. in 2007.

(Exhibit 6)
[1998]
Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia. 1998;39(5):508-12. Epub 1998/05/22. PubMed PMID: 9596203.

23. As a proximate result of Athena Diagnostics, Inc. negligent diagnosis and failure to accurately advise selection of appropriate therapy, the decedent lost his life on January 5, 2008 due to a traumatic seizure (**Exhibit 7**). Thus, Athena Diagnostics, Inc. violation of multiple CLIA regulations posed immediate jeopardy to the patient's health and safety (42 C.F.R. §493.2), "a situation in which immediate corrective action is necessary because the laboratory's noncompliance with one or more condition level requirements has already caused, is causing, or is likely to cause, at any time, serious injury or harm, or death, to individuals served by the laboratory".

Standard of Care Violation

24. It is my opinion to a reasonable degree of clinical genetic certainty that the SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc. on June 30, 2007 breached the standard of care of a diagnostic laboratory performing high complexity genetic testing by its negligent failure to provide accurate genetic confirmation of Dravet Syndrome (SMEI) diagnosis, failure to adhere to post-analytic DNA variant classification system, failure of timely notification of the SCN1A mutation reclassification, and failure to identify anticonvulsant treatments to avoid with SMEI diagnosis.

25. According to the foregoing, each Athena agent listed as having contributed to the laboratory results, **Sat Dev Batish, PhD**, FACMG, Chief Director, Genetics, **Narasimham Nagan, PhD**, ABMG, Director, Genetics, **Hui Zhu, PhD**, ABMG, Director, Genetics, have in my opinion to a reasonable degree of clinical genetic certainty, deviated from the accepted standard of care protocol of a CLIA-certified diagnostic laboratory performing high complexity genetic testing, and proximately contributed to the erroneous diagnosis as reported in **Exhibit 1**. Based on the clinical findings as reported, inappropriate medical treatments were employed, proximately contributing to the death of the child, Christian Jacob Millare.

26. According to the foregoing, Athena Diagnostics, Inc's Medical Director and CLIA license holder **Joseph J. Higgins, M.D.**, F.A.A.N., (CLIA # 22D0069726), has in my opinion, to a reasonable degree of medical certainty, deviated from the accepted standard of care for a medical practitioner in the field of clinical genetics, and proximately contributed to the erroneous diagnosis as reported in **Exhibit 1**. Further, it is my opinion that based on the clinical findings as reported, inappropriate medical treatments were employed, exacerbating the seizure disorder of and proximately contributing to the death of the child, Christian Jacob Millare.

27. I reserve the right to modify, amend or elaborate on the opinions stated herein as may be necessary if further information becomes available to me.

State: North Carolina
County: Durham

Sworn to before me this 04
Day of August, 2015.

Robert M. Cook-Deegan, M.D.

Notary Public – State of North Carolina
My Commission Expires: 12/8/2018

CATHERINE A. GULDNER
Notary Public, North Carolina
Durham County
My Commission Expires
December 08, 2018

athena diagnostics
200 Forest Street, 2nd Floor
Marlborough, MA 01752
(800) 394-4493 - (508) 756-2886

www.AthenaDiagnostics.com
Visit our website for more information on our neurodiagnostic testing services.

Diagnosis Service Report

Patient: Christian Millare
Date of Birth: 08/23/2005
Sex: M
Social Security Number:
Specimen Type: DNA
Test Category: Diagnostic (Symptomatic)
Test Requested: SCN1A DNA Sequencing Test

Requesting Physician:
Report to: John Shoffner, MD
Address: One Dunwoody Park
Suite 250
Atlanta, GA 30338
Additional Reports to:

Accession Number: 07025148
Family Number/Kindred Number:
Patient Number: 000000000
Specimen Collection Date: NO Date
Accession Date: 05/03/2007
Report Date: 06/30/2007

Interpretation

This individual possesses a DNA sequence variant or combination of variants in the *SCN1A* gene whose significance is unknown (missense variant of unknown significance). Testing of the biological parents is strongly recommended to resolve the uncertainty of these test results. Please refer to the Technical Results and Comments sections of this report for further information.

Technical Results

DNA Variant 1:	Transversion T > A
Nucleotide Position:	1237
Codon:	413
Amino Acid Change:	Tyrosine > Asparagine
Variant Type:	Variant of unknown significance (heterozygous)

No other abnormal DNA sequence variants were identified in the remainder of the coding sequence and intron/exon junction.

Comments

Most Significant result: Analysis of this individual's *SCN1A* gene identified a DNA sequence variant or combination of variants whose significance is unclear or unknown (variant(s) of unknown significance). Since these types of sequence variants are similar to those observed in both disease-associated mutations at other nucleotide positions and in benign polymorphisms, the nature of this variation precludes clear interpretation. These DNA sequence variants may or may not alter the functional aspects of the *SCN1A* gene and /or its protein product. While methodologically accurate, the results of this analysis cannot be definitively interpreted due to the absence of published studies correlating these variant(s) with clinical presentation and/or pathology. Therefore, based on this single analysis, it is not possible to conclude with any reasonable degree of clinical certainty at this time whether or not this variant is associated with the phenotype in question.

Possible outcomes– Although the clinical significance of this test result is not certain, several outcomes are possible:
Normal- the variant is a benign polymorphism that has not been previously detected or reported and it is very unlikely that this mutation is responsible for clinical symptoms or increased risk of disease.
Affected with less severe GEFS+ (generalized epilepsy with febrile seizures plus) - the variant is an unreported pathologic mutation that does not cause SMEI, but may be associated with less severe clinical symptoms.
Affected with SMEI- the variant is an unreported pathologic mutation and is therefore associated with the disease.

Other variants of less significance: This analysis may also have detected other types of sequence variants as listed in the Technical Results section, a common occurrence for an analysis of this scope. However, in the context of results reported, the presence of additional sequence variants is generally of reduced significance and does not modify the final interpretation of the test results. Benign polymorphisms, if identified, are considered normal and are not reported in the Technical Results section of this report, but are available upon request. Please

athena diagnostics
200 Forest Street, 2nd Floor
Marlborough, MA 01752
(800) 394-4493 - (508) 756-2686

Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

consult the Glossary for a detailed explanation of "DNA Variant Type" if indicated in the Technical Results section of this report.

Parental testing and other follow up recommendations: Testing of the biological parents is strongly recommended (for no additional charge) to help resolve the uncertainty of this sequence variant's pathogenicity and the uncertainty of the predicted phenotype. Missense mutations causing the severe phenotypes associated with SCN1A mutations (SMEI or SMEB) are usually (>90%) de novo, meaning that the mutation arose in the affected individual, and is not detected in the biological parents.10 Missense mutations associated with the milder phenotypes (GEFS+) are usually (>95%) inherited from one of the biological parents.10 Consultation with Athena's test counselor (1-800-394-4493) is recommended prior to parental testing.

Because SCN1A mutations can be inherited, this individual's family members may be at risk for possessing or inheriting these mutations. Careful reconciliation of this molecular data with this individual's clinical and family history is highly recommended. Athena recommends genetic counseling for this individual and his or her family members, and consideration of parental testing. Please contact Athena Client Services at 1-800-394-4493 or visit www.athenadiagnostics.com for further information on parental testing. The attached requisition forms will facilitate the submission and processing of parental specimens for this testing service.

Background Information: SCN1A encodes for the neuron voltage-gated sodium channel alpha 1 subunit protein. Mutations in th SCN1A gene have been associated with several overlapping epileps syndromes ranging from severe to mild phenotypes (SMEI, SMEB, an GEFS+).1-10 The severe phenotypes include SMEI, Severe Myoclon Epilepsy of Infancy or Dravet syndrome, and SMEB (SMEI borderline with some, but not all, of the classical features of SMEI. GEFS+ Generalized Epilepsy with Febrile Seizures Plus, encompasses a range o phenotypes from febrile seizures to mild generalized epilepsies, and less commonly, includes severe epileptic encephalopathies. SMEI has a poor prognosis, including developmental delay and refractory seizures. Furthermore, a confirmed diagnosis of SMEI may significantly guid treatment decisions.11

SCN1A mutations fall broadly into two groups.1-10 Truncation mutation that severely disrupt the gene are usually associated with sever phenotypes such as SMEI. Missense mutations are associated with a range of phenotypes from mild to severe. Most mutations that caus SMEI are de novo, or sporadic (arise in the affected individual rathe than being inherited)3,5,10 an inheritance pattern that can be confirmed b testing of parents. Familial mutations are usually associated with mil phenotypes but can be seen in SMEI. It is noteworthy that non-penetran mutation carriers and variable expression of affected carriers have bee observed in these syndromes in some families. This suggests tha modifying factors influence the expression of disease, and indicates tha the molecular analysis must be carefully reconciled with the clinic presentation and family history.

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Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Report to: John Shoffner, MD
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

Limitations of analysis: Mutations not detected by this analysis may include large deletions and large insertions. Furthermore, this test does not detect potential pathogenic mutations in the promoter, 5' flanking untranslated, 3' untranslated and non-sequenced intronic regions of the gene. Mutations in patients exhibiting mosaicism may not be detectable by automated sequencing technology.

Other Testing Available: Other epilepsy syndromes may appear clinically similar to those caused by mutations in the SCN1A gene. Although the course of these diseases may vary, patients with a progressive myoclonus epilepsy share some similar features to syndromes caused by SCN1A mutations including myoclonic and tonic-clonic seizures, ataxia, and abnormal neurophychological development. Athena offers testing for three types of progressive myoclonus epilepsy, EPM1, Lafora disease, and MERRF, that can further assist in the diagnosis of your patient's symptoms.

Methods

Analysis of the *SCN1A* gene was performed by PCR amplification of highly purified genomic DNA, followed by automated uni-directional DNA sequencing of the 26 exons of the *SCN1A* gene, including the highly conserved exon-intron splice junctions (e.g. GT....AG) between all 26 coding exons.[1] All abnormal sequence variants were confirmed by bi-directional sequencing or alternative sequencing chemistry. Studies conducted by Athena Diagnostics, Inc. indicate that mutations in this gene and in similar sequencing tests are detectable at an overall analytic sensitivity approaching 99%. All test results are reviewed, interpreted, and reported by ABMG certified Clinical Molecular Geneticists.

Nucleotides and amino acids are numbered following the internationally accepted Nomenclature set forth by the Ad Hoc Committee on Mutation Nomenclature. The initiator codon, Methionine, is designated as codon number 1 in the cDNA. The "A" of the "ATG" initiator codon is designated as nucleotide +1.

Abbreviations used:
SCN1A (neuronal voltage-gated sodium channel alpha 1 subunit); SMEI (severe myoclonic epilepsy of infancy); SMEB (borderline SMEI); GEFS + (generalized epilepsy with febrile seizures plus); DNA (deoxyribonucleic acid); PCR (polymerase chain reaction); A(Adenine); G(Guanine); C(Cytosine); T(Thymine).

This testing service is performed pursuant to a PCR license agreement with Roche Molecular Systems, Inc.

References

1. Escayg, A et al (2000) Nat Gen 24: 343-345
2. Wallace, RH et al (2001) Am J Hum Genet 68: 859-865
3. Claes, L et al (2001) Am J Hum Genet 68: 1327-1332
4. Wallace, RH et al (2003) Neurology 61: 765-769
5. Claes, L et al (2003) Hum Mut 21: 615-621
6. Fujiwara, T et al (2003) Brain 126: 531-546

Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Report to: John Shoffner, MD	Family Number/Control Number:
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

7. Fujiwara, T et al (2004) Epilepsia 45: 140-148
8. Kanai, K et al (2004) Neurology 63: 329-334
9. Coulemans, BPGM et al (2004) Pediatr Neurol 30: 236-243
10. Mulley, JC et al (pending) Hum Mut (Mutation update, submitted)
11. Guerrini R et al (1998) Epilepsia 39: 508-512

GLOSSARY

DNA sequence variants are deviations from the normal reference sequence of the gene(s) being analyzed. In dominant disorders, mutations must be found in only one allele of a gene to confirm the presence of the disease. However, it is not uncommon to have more than two types of DNA sequence variants detected in a gene. In addition, the clinical significance of individual variant types differs widely. The DNA variant types and additional terminology utilized in the report are explained below. (Consult the Technical Results and Comments section of this report to determine if any of the following apply to this individual.)

Variant Types:

1. Known disease-associated mutations (dominant) are documented in the literature to be associated with diseases inherited in a dominant manner. The individual is likely to be affected with, or predisposed to developing, a dominant genetic disease.

2. Predicted disease-associated mutations are expected to result in significant alteration of the structure and function of the protein encoded by the dominant gene. Typical examples include frame shift mutations, splicing mutations, nonsense mutations, and deletions or duplications of entire exons. Current literature indicates that DNA sequence variants of this type are associated with disease. However, due to the absence of established genotype-phenotype correlations for this specific DNA sequence variant, this result should be carefully reconciled with this individual's clinical and family history.

3. Amino acid changes of unknown significance are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. The amino acid change is predicted based on simple interpretations of the genetic code. However, these same types of alterations may sometimes alter normal gene splicing and processing, and thereby cause more significant and unpredictable effects. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

4. Variants of unknown significance are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. Typical examples include single nucleotide changes in the coding or non-

Diagnosis Service Report

Patient
Christian Millare

Date of Birth: 08/23/2005 | Sex: M | Social Security Number:

Specimen Type
DNA

Test Category
Diagnostic (Symptomatic)

Test Requested
SCN1A DNA Sequencing Test

Requesting Physician

Report to
John Shoffner, MD

Address
One Dunwoody Park

Suite 250

Atlanta, GA 30338

Additional Reports to:

Accession Number
07025148

Family Number/Kindred Number

Patient Number
000000000

Specimen Collection Date
NO Date

Accession Date
05/03/2007

Report Date
06/30/2007

coding regions of the gene that are sometimes labeled as "silent mutations" or "intronic polymorphisms." These types of alterations often have no effect, but may sometimes alter normal gene splicing and processing. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

5. Inconclusive test results are those unable to be interpreted as either negative or positive due to a technical problem in the assay, and thus can rule out neither the presence nor absence of abnormalities in these genes. Inconclusive results are typically resolved by analysis of a repeat specimen. There will be no charge for the repeat analysis. Please indicate "REPEAT SPECIMEN" along with the above Athena Accession Number on the requisition. If this test is part of a profile and a positive result was obtained for another gene, the submission of a repeat specimen may not be warranted.

6. Indeterminate test results, while methodologically accurate, are not clinically meaningful due to the lack of published clinical studies correlating test results in this specific category with clinical presentation and/or pathology. Due to the lack of published findings, these test results cannot be interpreted as either normal or abnormal. Indeterminate results are generally caused by test results that fall outside of the established interpretive criteria. Indeterminate test results are not resolved by analysis of a repeat specimen.

7. Benign polymorphisms are DNA sequence variants that have been shown to be present in unaffected control subjects, and are considered "benign" (non-pathogenic) sequence variants. If identified, these are considered normal variations and are not reported in the Technical Results section of this report, but are available upon request.

*** FINAL REPORT *** ver 1.0

This test was developed and its performance characteristics determined by Athena Diagnostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes and should not be regarded as investigational or for research only. Athena Diagnostics is licensed under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) to perform high complexity clinical testing. Athena Diagnostics has performed assay validation studies and has developed its laboratory protocols and operating procedures in consultation with experts in the field and in accordance with the standards of the National Committee on Clinical Laboratory Standards (NCCLS).

Laboratory results and submitted clinical information reviewed by,

Sat Dev Batish, PhD, FACMG
Chief Director, Genetics

Narasimhan Nagan, PhD, ABMG
Director, Genetics

Hui Zhu, PhD, ABMG
Director, Genetics

Laboratory oversight provided by Joseph J. Higgins, M.D., F.A.A.N., CLIA license holder, Athena Diagnostics (CLIA # 22D0069726)

Testing performed at:
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Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005 Sex: M Social Security Number:	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007
	Additional Reports to:	Report Date: 06/30/2007

Requisition for Parental Testing - Test Code 536

In order to provide a more comprehensive interpretation of this patient's SCN1A results, Athena Diagnostics is requesting samples from the biological parents of this patient. Athena will perform a target analysis on these samples for variant(s) identified in gene SCN1A only and use the findings to help interpret the patient's SCN1A result(s) at no additional charge. Please use this form as the requisition for parental testing on this patient. Enclose one completed form with each parent's sample and send to Athena. If you have any questions or require shipping kits, please contact Athena customer service at 1-800-394-4493,option 2.

Specimen Requirements: 1 lavendar tube (8.5ml) whole blood **Storage Conditions:** Refrigerate
Shipping Conditions: Room temperature, avoid freezing

Biological Mother Name: _____ Biological Father Name: _____

Date of Birth: _____ Date of Birth: _____

Address: _____ Address: _____

City,State,Zip: _____ City,State,Zip: _____

Phone: _____ Phone: _____

Specimen Collection Date: _____ Specimen Collection Date: _____

Indication for Testing: _____ UPIN: _____ Medical Practitioner Signature: _____

Testing Authorization and ICD-9 Code: I warrant that this test is either: 1) for the purpose of diagnosing or detecting an existing disease, illness, impairment, symptom or disorder, or 2) that if it is not for such purpose, I have obtained the appropriate prior written consent. This written consent was signed by the person who is the subject of the test (or if that person lacks capacity to consent, signed by the person authorized to consent for that person), and includes: a) a statement of the purpose and description of the test; b) a statement that prior to signing the consent form, the consenting person discussed with the medical practitioner ordering the test the reliability of positive or negative test results and the level of certainty that a positive test result for that disease or condition serves as a predictor of such disease; c) a statement that the consenting person was informed about the availability and importance of further testing, physician consultation and genetic counseling, and provided with written information identifying a genetic counselor or medical geneticist from whom the consenting person might obtain such counseling; d) a general description of each specific disease or condition tested for; and e) the person or persons to whom the test results may be disclosed as indicated above. v2.0 06/2014

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Diagnostic Service Report

Patient: Christian Millare	Requesting Physician:		Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Family Number/Referral Number:
Specimen Type: DNA	Report to: John Shoffner, MD		Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)	Address: One Dunwoody Park	Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338		Accession Date: 05/03/2007
	Additional Reports to:		Report Date: 06/30/2007

****REVISED REPORT # 1****

CAUTION, Revised Report, disregard previous report.

****See Attached Original Report**

The variant of unknown clinical significance previously reported in the *SCN1A* gene of this patient has been classified as a disease-causing mutation. Please see the Technical Results section below for more information.

Interpretation

POSITIVE

This individual possesses a DNA sequence variant in the *SCN1A* gene that is a previously reported **disease-associated mutation.**[1-10] This test result is consistent with a diagnosis of, or a predisposition to developing, the severe phenotypes associated with *SCN1A* mutations, SMEI or SMEB.[1-10] Please refer to the Technical Results and Comments sections of this report for further information.

Technical Results

DNA Variant 1:Transversion T > A

Nucleotide Position:	1237
Codon:	413
Amino Acid Change:	Tyrosine > Asparagine
Variant Type:	Disease-associated mutation (heterozygous)

No other abnormal DNA sequence variants were identified in the remainder of the coding sequence and intron/exon junction.

Comments

Most Significant result: Analysis of this individual's *SCN1A* gene identified a DNA sequence variant that has been reported in the literature to be associated with SMEI or SMEB, the severe phenotypes associated with *SCN1A* mutations.[1-10] **(disease-associated mutation).** This test result is consistent with a diagnosis of, or a predisposition to developing, SMEI or SMEB.[1-10]

Other variants of less significance: This analysis may also have detected other types of sequence variants as listed in the Technical Results section, a common occurrence for an analysis of this scope. However, in the context of results reported, the presence of additional sequence variants is generally of reduced significance and does not modify the final interpretation of the test results. Benign polymorphisms, if identified, are considered normal and are not reported in the Technical Results section of this report, but are available upon request. Please consult the Glossary for a detailed explanation of "DNA Variant Type" if indicated in the Technical Results section of this report.

Parental testing and other follow up recommendations: Testing of the biological parents (for no additional charge) may help identify whether this sequence variant is *de novo* or inherited. Because *SCN1A* mutations can be inherited, this individual's family members may be at risk for

Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Report to: John Shoffner, MD
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

possessing or inheriting these mutations. Careful reconciliation of this molecular data with this individual's clinical and family history is highly recommended. Athena recommends genetic counseling for this individual and his or her family members, and consideration of parental testing. Please contact Athena Client Services at 1-800-394-4493 or visit www.athenadiagnostics.com for further information on parental testing. The attached requisition forms will facilitate the submission and processing of parental specimens for this testing service.

Background Information: SCN1A encodes for the neuronal voltage-gated sodium channel alpha 1 subunit protein. Mutations in the SCN1A gene have been associated with several overlapping epilepsy syndromes ranging from severe to mild phenotypes (SMEI, SMEB, and GEFS+).1-10 The severe phenotypes include SMEI, Severe Myoclonic Epilepsy of Infancy or Dravet syndrome, and SMEB (SMEI borderline) with some, but not all, of the classical features of SMEI. GEFS+, Generalized Epilepsy with Febrile Seizures Plus, encompasses a range of phenotypes from febrile seizures to mild generalized epilepsies, and less commonly, includes severe epileptic encephalopathies. SMEI has a poor prognosis, including developmental delay and refractory seizures. Furthermore, **a confirmed diagnosis of SMEI may significantly guide treatment decisions.**11

SCN1A mutations fall broadly into two groups.1-10 Truncation mutations that severely disrupt the gene are usually associated with severe phenotypes such as SMEI. Missense mutations are associated with a range of phenotypes from mild to severe. Most mutations that cause SMEI are de novo, or sporadic (arise in the affected individual rather than being inherited)3,5,10 an inheritance pattern that can be confirmed by testing of parents. Familial mutations are usually associated with mild phenotypes but can be seen in SMEI. It is noteworthy that non-penetrant mutation carriers and variable expression of affected carriers have been observed in these syndromes in some families. This suggests that modifying factors influence the expression of disease, and indicates that the molecular analysis must be carefully reconciled with the clinical presentation and family history.

Limitations of analysis: Mutations not detected by this analysis may include large deletions and large insertions. Furthermore, this test does not detect potential pathogenic mutations in the promoter, 5' flanking untranslated, 3' untranslated and non-sequenced intronic regions of the gene. Mutations in patients exhibiting mosaicism may not be detectable by automated sequencing technology.

Methods

Analysis of the *SCN1A* gene was performed by PCR amplification of highly purified genomic DNA, followed by automated uni-directional DNA sequencing of the 26 exons of the *SCN1A* gene, including the highly conserved exon-intron splice junctions (e.g. GT....AG) between all 26 coding exons.1 All abnormal sequence variants were confirmed by bi-directional sequencing or alternative sequencing chemistry. Studies conducted by Athena Diagnostics, Inc. indicate that mutations in this gene and in similar sequencing tests are detectable at an overall analytic sensitivity approaching 99%. All test results are reviewed, interpreted,

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Diagnosis Service Report

Patient	Requesting Physician	Accession Number
Christian Millare		07025148
Date of Birth: 08/23/2005 Sex: M Social Security Number:	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007
	Additional Reports to:	Report Date: 06/30/2007

and reported by ABMG certified Clinical Molecular Geneticists.

Nucleotides and amino acids are numbered following the internationally accepted Nomenclature set forth by the Ad Hoc Committee on Mutation Nomenclature. The initiator codon, Methionine, is designated as codon number 1 in the cDNA. The "A" of the "ATG" initiator codon is designated as nucleotide +1.

Abbreviations used:
SCN1A (neuronal voltage-gated sodium channel alpha 1 subunit); SMEI (severe myoclonic epilepsy of infancy); SMEB (borderline SMEI); GEFS+ (generalized epilepsy with febrile seizures plus); DNA (deoxyribonucleic acid); PCR (polymerase chain reaction); A(Adenine); G(Guanine); C(Cytosine); T(Thymine).

This testing service is performed pursuant to a PCR license agreement with Roche Molecular Systems, Inc.

References

1. Escayg, A et al (2000) Nat Gen 24: 343-345
2. Wallace, RH et al (2001) Am J Hum Genet 68: 859-865
3. Claes, L et al (2001) Am J Hum Genet 68: 1327-1332
4. Wallace, RH et al (2003) Neurology 61: 765-769
5. Claes, L et al (2003) Hum Mut 21: 615-621
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7. Fujiwara, T et al (2004) Epilepsia 45: 140-148
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10. Mulley, JC et al (pending) Hum Mut (Mutation update, submitted)
11. Guerrini R et al (1998) Epilepsia 39: 508-512

GLOSSARY

DNA sequence variants are deviations from the normal reference sequence of the gene(s) being analyzed. In dominant disorders, mutations must be found in only one allele of a gene to confirm the presence of the disease. However, it is not uncommon to have more than two types of DNA sequence variants detected in a gene. In addition, the clinical significance of individual variant types differs widely. The DNA variant types and additional terminology utilized in the report are explained below. (Consult the Technical Results and Comments section of this report to determine if any of the following apply to this individual.)

Variant Types:

1. **Known disease-associated mutations** (dominant) are documented in the literature to be associated with diseases inherited in a dominant manner. The individual is likely to be affected with, or predisposed to developing, a dominant genetic disease.

2. **Predicted disease-associated mutations** are expected to result in significant alteration of the structure and function of the protein encoded

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Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Report to: John Shoffner, MD	Family Number/Funeral Number:
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

by the dominant gene. Typical examples include frame shift mutations, splicing mutations, nonsense mutations, and deletions or duplications of entire exons. Current literature indicates that DNA sequence variants of this type are associated with disease. However, due to the absence of established genotype-phenotype correlations for this specific DNA sequence variant, this result should be carefully reconciled with this individual's clinical and family history.

3. **Amino acid changes of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. The amino acid change is predicted based on simple interpretations of the genetic code. However, these same types of alterations may sometimes alter normal gene splicing and processing, and thereby cause more significant and unpredictable effects. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

4. **Variants of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. Typical examples include single nucleotide changes in the coding or non-coding regions of the gene that are sometimes labeled as "silent mutations" or "intronic polymorphisms." These types of alterations often have no effect, but may sometimes alter normal gene splicing and processing. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

5. **Inconclusive** test results are those unable to be interpreted as either negative or positive due to a technical problem in the assay, and thus can rule out neither the presence nor absence of abnormalities in these genes. Inconclusive results are typically resolved by analysis of a repeat specimen. There will be no charge for the repeat analysis. Please indicate "REPEAT SPECIMEN" along with the above Athena Accession Number on the requisition. If this test is part of a profile and a positive result was obtained for another gene, the submission of a repeat specimen may not be warranted.

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7. **Benign polymorphisms** are DNA sequence variants that have been

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Diagnostic Service Report

Patient: Christian Millare		Referring Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accessioning Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

shown to be present in unaffected control subjects, and are considered "benign" (non-pathogenic) sequence variants. If identified, these are considered normal variations and are not reported in the Technical Results section of this report, but are available upon request.

***** *REVISED REPORT* ***** ver 1.0

This test was developed and its performance characteristics determined by Athena Diagnostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes and should not be regarded as investigational or for research only. Athena Diagnostics is licensed under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) to perform high complexity clinical testing. Athena Diagnostics has performed assay validation studies and has developed its laboratory protocols and operating procedures in consultation with experts in the field and in accordance with the standards of the National Committee on Clinical Laboratory Standards (NCCLS).

Laboratory results and submitted clinical information reviewed by,

Sat Dev Batish, PhD, FACMG
Chief Director, Genetics

Narasimhan Nagan, PhD, ABMG
Director, Genetics

Hui Zhu, PhD, ABMG
Director, Genetics

Laboratory oversight provided by Joseph J. Higgins, M.D., F.A.A.N., CLIA license holder, Athena Diagnostics (CLIA # 22D0069726)

Testing performed at:
Athena Diagnostics Four Biotech Park 377 Plantation St Worcester MA 01605

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Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005 Sex: M Social Security Number:	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007
	Additional Reports to:	Report Date: 06/30/2007

Requisition for Parental Testing - Test Code 536

In order to provide a more comprehensive interpretation of this patient's SCN1A results, Athena Diagnostics is requesting samples from the biological parents of this patient. Athena will perform a target analysis on these samples for variant(s) identified in gene SCN1A only and use the findings to help interpret the patient's SCN1A result(s) **at no additional charge.** Please use this form as the requisition for parental testing on this patient. Enclose one completed form with each parent's sample and send to Athena. If you have any questions or require shipping kits, please contact Athena customer service at 1-800-394-4493,option 2.

Specimen Requirements: 1 lavendar tube (8.5ml) whole blood **Storage Conditions:** Refrigerate
Shipping Conditions: Room temperature, avoid freezing

Biological Mother Name: ______ Biological Father Name: ______

Date of Birth: ______ Date of Birth: ______

Address: ______ Address: ______

City,State,Zip: ______ City,State,Zip: ______

Phone: ______ Phone: ______

Specimen Collection Date: ______ Specimen Collection Date: ______

Indication for Testing: ______ UPIN: ______ Medical Practitioner Signature: ______

Testing Authorization and ICD-9 Code: I warrant that this test is either: 1) for the purpose of diagnosing or detecting an existing disease, illness, impairment, symptom or disorder, or 2) that if it is not for such purpose, I have obtained the appropriate prior written consent. This written consent was signed by the person who is the subject of the test (or if that person lacks capacity to consent, signed by the person authorized to consent for that person), and includes: a) a statement of the purpose and description of the test; b) a statement that prior to signing the consent form, the consenting person discussed with the medical practitioner ordering the test the reliability of positive or negative test results and the level of certainty that a positive test result for that disease or condition serves as a predictor of such disease; c) a statement that the consenting person was informed about the availability and importance of further testing, physician consultation and genetic counseling, and provided with written information identifying a genetic counselor or medical geneticist from whom the consenting person might obtain such counseling; d) a general description of each specific disease or condition tested for; and e) the person or persons to whom the test results may be disclosed as indicated above.

GRHealth
1120 15th Street
Augusta, Georgia 30912
(706) 721-0211

Patient Name:	MILLARE, CHRISTIAN J
MRN:	001384711
Birthdate:	8/23/2005
Account Number:	0024658486059
Visit Date:	2/28/2006
Discharge date:	3/3/2006
Patient Type:	Inpatient
Location:	4E

Discharge

Discharge Summary Authenticated By:

DISCHARGE SUMMARY
Medical College of Georgia
Hospital & Clinics

DISCHARGE SUMMARY

Name of Patient: MILLARE, CHRISTIAN J
Medical Record #: 00138-4711
Date Admitted: 02/28/2006
Date Discharged: 03/03/2006

REASON FOR ADMISSION:
Intractable seizure characterization.

HISTORY OF PRESENT ILLNESS:
Christian is a 6-month-old baby boy with a history of seizures since he was 5 months of age. He is currently taking Topiramate and oxcarbazepine for his seizures. His first seizure was in 12/05, and was described as right arm jerking for 15 minutes. He was then in a postictal state for about 2-1/2 hours. His second seizure was around the same time, but involved the left side of his body. This seizure lasted for 20 minutes and was associated with postictal vomiting and hemiparesis for 30 minutes. He had a third seizure that was described as right-sided jerking for about 15 minutes and was associated with postictal vomiting and hemiparesis. He had an EEG on 12/30/05 that showed bilateral background slowing, but no epileptiform activity. He also had a CT of the head without contrast that showed asymmetric dilatation of the left temporal horn. The seizures normally occur about two to three times per week.

PAST MEDICAL HISTORY:
He was born at 37-1/2 weeks by spontaneous vaginal delivery and had no perinatal or postnatal complications.

Past Surgical History:
No.

Medications:
1. Topamax 30 mg b.i.d.
2. Trileptal 2 ml b.i.d.

USC Pediatrics
Division of Pediatric Neurology
Tim Livingston, MD

9 Medical Park
Suite 200
Columbia, SC 29203

(803) 434-7961
(803) 434-7981(FAX)

FirstName Christian **LastName** Millare

Birthdate 8/23/2005

Date 7/2/2007 **Location** Office

Refering Physician/specialist Greenhouse, Debbie, MD

Complaint/History/Past History/ROS

Christian presents with his mom for follow up of seizures.
He has been having seizures about every 3-4 weeks. He has been more aggressive and hyperactive. He injured his grandfather's ear. He slaps and hits and kicks.
He has difficulty maintaining sleep.
He is making developmental progressive
Past History
no change
ROS
GENERAL: denies fever, weight loss, appetite changes
EYES: denies eye discharge, eye pain
RESPIRATORY:denies cough, dyspnea, snoring; + congestion
CARDIOVASCULAR: denies cyanosis
GI: denies vomiting, diarrhea, constipation, jaundice
GU: denies dysuria, polyuria
DERM: denies skin rash, desquamation
NEUROLOGIC: denies regression,
abnormal movements
PSYCHOLOGIC: + mood lability, insomnia
HEMATOLOGIC: denies easy bruising, bleeding, fatigue

CurrentMedications

carbamazepine 100 mg/5ml, 6 ml bid
Keppra 100 mg/ml, 6 ml bid
clonazepam 0.5 mg, 0.75 bid
carnitine, coq10, riboflaving

Allergies

NKDA

NeuroExam

skull: no evidence of cranial trauma
speech and language: poor comprehension and expression for age; some sylables expressed
mental status: awake and alert, good eye contact, playful
cranial nerves:
II-XII normal
motor: normal muscle strength, and bulk in proximal and distal muscle groups of the upper and lower extremities and torso.
no atrophy or fasciculations; diffuse hypotonia (no change)
involuntary movements: no chorea, athetosis, or myoclonus

coordination: moderate fine and gross motor incoordination (right greater than left)
cerebellar: no apparent ataxia or titubation
reflexes: 0-1/four biceps, triceps, brachioradialis, knee jerks, and heel jerks, plantar responses normal for age bilaterally
gait: non-ambulatory

general examination:
abdomen: no hepatosplenomegaly, nontender
skin: no rash, no neurocutaneous markers

Diagnoses

seizures
insomnia
aggression
mitochondrial disorder

Impression/Plan

I am concerned about his seizures and abilify. I discussed treatment options. We reviewed the risks of medication including, but not limited to, drowsiness, insomnia, appetite changes, ataxia, confusion, irritability, hepatopathy, anemia, bone marrow suppression, severe skin rash, Steven's-Johnson syndrome, nephro/urolithiasis and unexpected effects.
His mom agreed to the following plan.
-consider abilify
-taper Keppra
-carbamazepine level
-consider increasing carbamazepine
-f/u 4-6 weeks, sooner prn (mom agreed to call)
I spent greater than 50% of the visit face-to-face with the patient and family in counseling with regard to the above issues. Total time 32 min /Counseling time 20 min

dictated, but not read unless signed

Kristen Griffin, CPNP Tim Livingston, MD

Discharge Summary

MILLARE, CHRISTIAN - R013059682

* Final Report *

Result type:	Discharge Summary
Result date:	05 July 2007 13:14
Result status:	Auth (Verified)
Result title:	Discharge Summary
Performed by:	Palisin MD, Tenley E on 05 July 2007 13:14
Verified by:	Amrol MD, Jennifer G on 23 July 2007 12:45
Encounter info:	R0634900448, Richland, IPR-Inpatient, 12/15/06 - 12/18/06
Contributor system:	Softmed

* Final Report *

Discharge Summary (Verified)

Discharge Summary

TWO ATTENDINGS - DR. Mark Mcdonald and DR. Jennifer Amrol.

CONSULTS: Neurology.

PROCEDURES: Lumbar puncture.

DIAGNOSES:
1. Seizure disorder.
2. Respiratory distress with hypoxia, resolved.

HISTORY OF PRESENT ILLNESS: A 15-month-old white male with known seizure disorder of unknown etiology presented to the ED in status epilepticus. Per mom, patient was in usual state of health until that afternoon at daycare, where he had a 20-minute episode of seizure that required Diastat, with resolution of seizure. Mom picked patient up at daycare. When arrived at home, had another seizure and was given Diastat, but seizure did not resolve, so mom brought patient to ED for further care. Upon arrival, patient was given 6 mg Ativan, fosphenytoin 15 mg/kg and, phenobarbital 22 mg/kg. Patient continued to seize, so he was given rocuronium 0.1
mg/kg and the seizure stopped. Patient intubated and given 120-cc bolus normal saline, admitted to PICU for further care.

PAST MEDICAL HISTORY: Significant for first seizure at age 4 months, multiple hospitalizations, 2 PICU admissions, no intubation. His last

hospitalization was 12/10/06 for seizure, discharged next day. Otherwise, no hospitalizations. Status post adenoidectomy.
FAMILY HISTORY: Positive for seizure disorder.

SOCIAL HISTORY: Lives with mom, dad. No siblings. He attends daycare. No pets. No smokers. Developmentally delayed, 6 month motor skills, 8 month verbal skills. PT/OT/ST involved.

REVIEW OF SYSTEMS: Positive for rhinorrhea, congestion, cough. No vomiting, diarrhea, constipation, bleeding, rash, or trauma, otherwise negative.

PHYSICAL EXAMINATION:
VITAL SIGNS: Temp 102, heart rate 176, respiratory 18, blood pressure 103/42, 11 kg.
GENERAL APPEARANCE: The patient is sedated, responds to deep stimulation.
HEENT: Normocephalic, atraumatic. Anterior fontanelle not noted. Pupils
equal, round and reactive to light, 5-2. Oropharynx clear. ET tube is in
place. TMs with tubes. Clear nasal discharge noted.
NECK: No masses.
LUNGS: Clear to auscultation bilaterally. No wheezes, rales, or rhonchi.
PA diameter normal.
CARDIOVASCULAR: Regular rate and rhythm, no murmurs, rubs or gallops. Two-plus pulses, less than 2-second cap refill.
ABDOMEN: Soft, nondistended, no hepatosplenomegaly.
GU: Descended testes, fully in place.
EXTREMITIES: No edema.
NEUROLOGICAL: Postictal, sedated.
SKIN: Hemangioma mid abdomen.

LABORATORY DATA: White count 12.6, hemoglobin 11.7, platelets 353. Sodium 140, potassium 3.3, chloride 103, bicarb 23, BUN 10, creatinine 0.3, calcium 9.7, glucose 128.

HOSPITAL COURSE: Patient admitted in status epilepticus secondary to underlying seizure disorder. Was stable and patient was discussed with Dr. Livingston. Dilantin, Keppra, and Zonegran levels were checked. Recommendations per Neurology's dictated note were instituted. Patient

has resolution of activity after admission. Patient was discharged on Keppra, Klonopin, carbamazepine, and fosphenytoin. He was to follow up with Neurology as an outpatient. During his presentation to the ED, he was noted to be in respiratory distress with hypoxia and fever. LP was done and noted to be negative. Cultures were negative, believed secondary
to a viral illness. During his hospital course, had resolution of fevers,
with continued URI symptoms, which improved, and patient was weaned from O2 and off oxygen 24 hours prior to discharge.

DISCHARGE INSTRUCTIONS: Patient was to follow up with Dr. Greenhouse within 1-2 days of discharge and with Dr. Livingston, with Neurology, on 12/21/06 at 10 a.m. Return to regular activity as tolerated, with regular
diet. If symptoms return or worsen, temperature greater than 101, seizures that last longer than 5 minutes, a parent was to call MD or go to
the ED.

DISCHARGE MEDICATION:
1. Carbamazepine 80 mg by mouth twice daily.
2. Keppra 600 mg every 12 hours.
3. Klonopin 0.25 mg twice daily.
4. Albuterol nebulizers every 4 hours as needed.
5. Neo-Synephrine 2 drops 3 times daily times 1 more day.
6. Vitamin A D Ointment, apply as directed.
7. Diastat as needed for seizures.

DISCHARGE INSTRUCTIONS: Patient was discharged on Keppra, Klonopin, carbamazepine, and fosphenytoin.

Dictated by: Tenley E. Palisin, MD

TEP:hs
D: 04/14/2007 10:44A T:04/14/2007 11:04 A
JOB # 000620958 T Job #: 826571 Doc #: 1012917
Cc: Tenley E. Palisin, MD
Robin Stanfield, MD

07/30/2007 CUMULATIVE SUMMARY
22:03 PALMETTO HEALTH RICHLAND, COLUMBIA, SC PAGE1
EDWARD W. CATALANO, MD DIRECTOR OF PATHOLOGY

NAME:MILLARE,CHRISTIAN
ADMITTED:07/30/2007
HN:IDTL-654223508 LOC:IDTL AGE:23M SEX:M
ACCT:3333344444 DR:LIVINGSTON,TIM CODE:41842

========== PHYSICIAN COPY FOR DR: LIVINGSTON,TIM ==========

M60354 COLL: 07/30/2007 10:20 REC: 07/30/2007 10:26 PHYS: LIVINGSTON,TIM

TEGRETOL	9.3	[4.0-12.0] ug/mL

MILLARE,CHRISTIAN
07/30/2007 22:03

END OF REPORT

PAGE1
OUTPATIENT CUMULATIVE SUMMARY

These labs appear ok. TSL

USC Pediatrics
Division of Pediatric Neurology
Tim Livingston, MD

9 Medical Park
Suite 200
Columbia, SC 29203

(803) 434-7961
(803) 434-7981(FAX)

FirstName Christian **LastName** Millare

Birthdate 8/23/2005

Date 9/17/2007 **Location** Office

Refering Physician/specialist Greenhouse, Debbie, MD

Complaint/History/Past History/ROS

Christian presents with his dad for follow up of seizures.
He has had three seizures over the last 30 days. Some of these consist of prolonged drooling and unresponsiveness (10 minutes). Others have consisted of generalized jerking. He is developing well.
His speech is improving. His behavior fluctuates
Past History
no change
ROS
GENERAL: denies fever, weight loss, appetite changes
EYES: denies eye discharge, eye pain
ENT: + congestion, hearing loss, pharyngeal pain
RESPIRATORY:□denies cough, dyspnea, snoring
CARDIOVASCULAR: denies cyanosis
GI: denies vomiting, diarrhea, constipation, abdominal pain, jaundice
GU: denies dysuria, polyuria
DERM: denies skin rash, desquamation
NEUROLOGIC: denies regression
PSYCHOLOGIC: mild mood lability, insomnia
HEMATOLOGIC: denies easy bruising, bleeding, fatigue

CurrentMedications

singulair
lamictal 5 mg, 2 bid
carbamazepine 100 mg/5ml, 8 ml bid
clonazepam 0.5 mg, 0.75 bid
lamictal
carnitine, coq10, riboflavin

Allergies

NKDA

NeuroExam

skull: no evidence of cranial trauma
speech and language: poor comprehension and expression for age; some sylables expressed
mental status: awake and alert, good eye contact, playful
cranial nerves:
II-XII normal
motor: normal muscle strength, and bulk in proximal and distal muscle groups of the upper and lower extremities and torso,

no atrophy or fasciculations; diffuse hypotonia (no change)
involuntary movements: no chorea, athetosis, or myoclonus
coordination: moderate fine and gross motor incoordination (right greater than left)
cerebellar: no apparent ataxia or titubation
reflexes: 0-1/four biceps, triceps, brachioradialis, knee jerks, and heel jerks, plantar responses normal for age bilaterally
gait: non-ambulatory

general examination:
heart: RRR
abdomen: no hepatosplenomegaly, nontender
skin: no rash, no neurocutaneous markers

Diagnoses

refractory seizures
incoordination
mitochondrial disorder (complex I and III defect)

Impression/Plan

I remain concerned about his seizures. However, his development is improving substantially.
We reviewed the risks of medication including, but not limited to, drowsiness, insomnia, appetite changes, ataxia, confusion, irritability, hepatopathy, anemia, bone marrow suppression, severe skin rash, Steven's-Johnson syndrome, nephro/urolithiasis and unexpected effects.
His dad agreed to the following plan.
-cbc, cmp, carbamazepine
-titrate lamotrigine
-MRI this week (discussed risks of sedation including infection, bleeding, respiratory depression, seizures, exacerbation of mitochondrial disorder). There is no contraindication to sedation in my opinion.
-f/u 6-8 weeks
I spent greater than 50% of the visit face-to-face with the patient and family in counseling with regard to the above issues. Total time 28 min /Counseling time 16 min

dictated, but not read unless signed

Kristen Griffin, CPNP Tim Livingston, MD

Result type:	Emergency Dept
Result date:	25 October 2007 02:15
Result status:	Auth (Verified)
Result title:	Emergency Dept
Performed by:	Shenoy MD, Naren S on 25 October 2007 02:15
Verified by:	Eric A Brown, MD on 26 October 2007 15:44
Encounter info:	R0729702096, Richland, ERR-Emergency Room, 10/24/07 - 10/24/07
Contributor system:	Softmed

Emergency Dept

PALMETTO HEALTH RICHLAND
5 Richland Medical Park Drive
Columbia, SC 29203
(803)434-7000

PATIENT: Millare, Christian EMERGENCY
DEPARTMENT
MR #: 01-30-59-68-2
Page 2 of 2

DATE OF VISITATION: 10/24/2007

ACCOUNT NUMBER: 0729702096

Patient arrives by private transport.

ATTENDING PHYSICIAN: Eric A. Brown, MD

CHIEF COMPLAINT: Seizure.

HISTORY OF PRESENT ILLNESS: This is a 2-year-old white male with a known history of seizures presenting with his usual petit mal type seizure today. He seized for approximately 45 minutes which is longer than usual. Normally, mom gives him Diastat at home. Mostly, seizure goes on for 5 minutes, but she picked him up from school and did not have Diastat with her, so he continued to seize. He did not have any airway compromise while he was seizing. She says that recently he has been seizing every 3 days, but she has been keeping at home. She said today he just said hi to her and then fell on to the carpet. He did not hit his head. He seized for approximately 45 minutes, and the seizure stopped by the time he had got into the Emergency Department. He was postictal for a short while but then has been back to his normal active self since then. Mom says that he has recently seen Dr. Livingston in the last 3 weeks, and his Lamictal dose was increased. She

feels that has been causing problem with his sleep, making him sleep less, and she is wondering if that is why he has been having more frequent seizures. She also says that he has been having (_____) per his physical therapist and speech therapist that he has had decreased truncal strength, and he has been using less words over the last few months. He was diagnosed with a mitochondrial disorder within the last few months as well.

REVIEW OF SYSTEMS: As above, otherwise negative.

SOCIAL HISTORY: He lives with mom, dad. There are no pets. No smokers. He is in daycare.

PAST MEDICAL HISTORY: He does see Dr. Livingston. He is on Klonopin, Tegretol, Lamictal, Coenzyme Q-10, and Carnitor. He does have a mitochondrial defect that is managed by Dr. Livingston.

ALLERGIES: He has no known drug allergies.

IMMUNIZATIONS: Up to date.

PHYSICAL EXAMINATION:
VITAL SIGNS: Temperature is 98.6 rectally, pulse is 98, respiratory rate is 26, blood pressure is 104/64. His pulse ox is 100% on room air. He weighs 12.36 kg.
GENERAL APPEARANCE: He is in no apparent distress. He is nontoxic.
HEENT: He is normocephalic, atraumatic. Pupils are equally round and reactive to light. Mucous membranes are moist.
NECK: Soft, supple, no lymphadenopathy.
LUNGS: Clear to auscultation bilaterally. No wheezes, rales or rhonchi were appreciated.
CARDIOVASCULAR: Heart is regular rate and rhythm. No murmurs, rubs or gallops.
ABDOMEN: Soft, nontender, nondistended. No hepatosplenomegaly. He does have a small strawberry hemangioma on his abdomen.
EXTREMITIES: No clubbing, cyanosis or edema. Full range of motion.
NEUROLOGICAL: Appears to be intact; however, he does appear to be somewhat, mildly hypertonic on exam. He interacts and makes good eye contact, but he does not speak.

MEDICAL DECISION MAKING AND EMERGENCY DEPARTMENT COURSE: This is a child with a mitochondrial disorder and a known seizure disorder that is seen by Dr. Livingston and is well known to the Emergency Department and to the Pediatric Service here at the hospital. He did have his usual seizure. He did not have any airway compromise of any type. Mom just brought him in for precaution. We did check a set of electrolytes and a Tegretol level to make sure there was nothing else going on that would have triggered the seizure other than his usual seizure activity. His electrolytes were completely normal and his Tegretol level was 7.1 which was

also normal. We did speak with Dr. Livingston of Pediatric Neurology to see if we needed to increase medication dose. He recommended increasing the Lamictal, but after explaining to him that mom was concerned that was causing problems with sleep, he recommended that the patient's mother call him tomorrow morning to figure out (_____) go from here with the medication doses. Mom does have Diastat at home, and she felt like the child was well enough to go home as well. I do not feel any further workup was warranted at this time.

CLINICAL IMPRESSION: Seizure, status epilepticus.

DISPOSITION: The patient will be discharged home. Patient is to continue all of his home medications as previously prescribed by Dr. Livingston. Instructed mom that if patient continues to have any more seizures or any more status epilepticus or any airway compromise or any other concerns to return to the Emergency Department immediately; otherwise, call Dr. Livingston first thing in the morning to arrange for medication changes. Dr. Brown has seen the patient and agrees with the above.
Reviewed and agree with above note

Dictated by: Naren Shenoy, MD

NS:hs
D: 10/24/2007 8:06 P T: 10/25/2007 2:15 A
Job #: 000844961 T Job #: 756302 Doc #: 1103393
cc: Eric A. Brown, MD

Signature Line
Electronically Signed & Verified on 10/26/2007 15:44
by Eric A Brown, MD

Completed Action List:
* Perform by Shenoy MD, Naren S on 25 October 2007 02:15
* Sign by Eric A Brown, MD on 26 October 2007 15:44 Requested on 25 October 2007 02:53
* VERIFY by Eric A Brown, MD on 26 October 2007 15:44

USC Pediatrics, Division of Pediatric Neurology
Kristen P. Griffin, RN, CPNP

Name Christian Millare Birthdate 8-23-05 Date of visit 11-6-07
Physician Name Kilgore, MD Fax

History of present illness: Christian presents with his father for f/u. He has had increase in seizures lasting from 6-10 minutes, with 11 in October and one this month. These consist of with abnormal eye movement, then progressing to limbs. He has had aggression and agitation. Per mother, the therapists are reports worsening truncal control and speech regression. Mother is concerned that this is related to his condition or medication effect.s He had normal MRI 9/2007. He was seen in the ER lasting from 6-10 minutes, with 11 in October and one this month. These consist of with abnormal eye movement, then progressing to limbs. He has had aggression, fussy, and agitation / temper tantrums. Per mother, the therapists are reports worsening truncal control and speech regression. Mother is concerned that this is related to his condition or medication effects. He had normal MRI 9/2007. He was seen in the ER for prolonged seizure.

Past Medical History: No change.

Review of Systems: Insomnia. Denies fever, weight loss, appetite changes. visual disturbance, hearing loss, pharyngeal pain. cough, dyspnea, exercise intolerance, cyanosis vomiting, diarrhea, constipation, abdominal pain, dysuria. skin rash, fatigue.
Current Medications: Clonazepam 0.5mg, ½ tab BID, Carbamazepine 8ml BID Lamictal 25mg, 1.5 am and .5 hs, MVI **Allergies** NKDA

Neurologic exam: Skull: Normocephalic without evidence of trauma. Mental Status: Awake, alert. Fussy during exam. Cries. Cranial Nerves: II – XII intact. EOM intact. PERRL. Motor: Diffuse hypotonia. Muscle strength normal in upper and lower extremities. Moderate fine and gross coordination for age. Involuntary movements: No chorea, athetosis, myoclonus. Gait: Incoordinated, but walks well without assistance.

General exam: Vital signs: Weight 15.5kg Observation: No acute distress. HEENT: No nasal discharge. Oropharynx clear. Lungs: Clear to auscultation. No shortness of breath. Heart: Regular rate and rhythm. Abdomen: Nontender. Extremities: No asymmetry, clubbing, cyanosis, or edema. Skin: No rash, neurocutaneous markers.

Diagnoses: 1. refractory seizures 2. hypotonia

Impression/Plan I consulted Dr. Livingston with this f/u appt. We discussed MRI findings and the risk of error with any medical testing. We discussed that mother could bring raw data of MRI to PRMH or MCG for review. We discussed seizures, sleep, and medications in relation to his symptoms. We discussed medication changes and consideration of discontinuing Lamictal. We do not recommend stopping seizure medication due to risk of withdrawal seizures. We discussed use of clonidine and we do not recommend this due to cardiac risks. We discussed long term effect on bone health and mitochondrial disorder. Mother agrees to taper Lamictal over 6 weeks (written instructions given for this). Change to carbamazepine chewable tablets. F/u 6 weeks, sooner if necessary. Total time spent 45 minutes, > 50% of this time was counseling.

Kristen P. Griffin, RN, CPNP
Pediatric Nurse Practitioner

I have personally seen and examined this patient, and I agree with the above assessment and plan.

Timothy Livingston, MD
Director, Division of Pediatric Neurology

De-novo mutations of the sodium channel gene *SCN1A* in alleged vaccine encephalopathy: a retrospective study

Samuel F Berkovic, Louise Harkin, Jacinta M McMahon, James T Pelekanos, Sameer M Zuberi, Elaine C Wirrell, Deepak S Gill, Xenia Iona, John C Mulley, Ingrid E Scheffer

Lancet Neurol 2006; 5: 488–92
Published Online April 20, 2006
DOI:10.1016/S1474-4422(06)70446-X

See Reflection and Reaction page 465

Epilepsy Research Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg West, Victoria, Australia (S F Berkovic MD, J M McMahon BSc, J T Pelekanos MBBS, I E Scheffer MBBS); Department of Genetic Medicine, Women's and Children's Hospital, Adelaide, Australia (L Harkin MSc, X Iona DipBiomedSci, J C Mulley PhD); Department of Paediatrics, University of Adelaide, Australia (L Harkin); Fraser of Allander Neurosciences Unit, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK (S M Zuberi FRCP); Department of Pediatrics and Neurosciences, University of Calgary, Calgary, Alberta, Canada (E C Wirrell MD); TY Nelson Department of Neurology, The Children's Hospital at Westmead, Westmead, NSW, Australia (D S Gill FRACP); School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, Australia (J C Mulley); and Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Victoria, Australia (I E Scheffer)

Correspondence to: Prof Samuel F Berkovic, Epilepsy Research Centre, Austin Health, West Heidelberg, Victoria 3081, Australia
s.berkovic@unimelb.edu.au

Summary

Background Vaccination, particularly for pertussis, has been implicated as a direct cause of an encephalopathy with refractory seizures and intellectual impairment. We postulated that cases of so-called vaccine encephalopathy could have mutations in the neuronal sodium channel α1 subunit gene (*SCN1A*) because of a clinical resemblance to severe myoclonic epilepsy of infancy (SMEI) for which such mutations have been identified.

Methods We retrospectively studied 14 patients with alleged vaccine encephalopathy in whom the first seizure occurred within 72 h of vaccination. We reviewed the relation to vaccination from source records and assessed the specific epilepsy phenotype. Mutations in *SCN1A* were identified by PCR amplification and denaturing high performance liquid chromatography analysis, with subsequent sequencing. Parental DNA was examined to ascertain the origin of the mutation.

Findings *SCN1A* mutations were identified in 11 of 14 patients with alleged vaccine encephalopathy; a diagnosis of a specific epilepsy syndrome was made in all 14 cases. Five mutations predicted truncation of the protein and six were missense in conserved regions of the molecule. In all nine cases where parental DNA was available the mutations arose de novo. Clinical-molecular correlation showed mutations in eight of eight cases with phenotypes of SMEI, in three of four cases with borderline SMEI, but not in two cases with Lennox-Gastaut syndrome.

Interpretation Cases of alleged vaccine encephalopathy could in fact be a genetically determined epileptic encephalopathy that arose de novo. These findings have important clinical implications for diagnosis and management of encephalopathy and, if confirmed in other cohorts, major societal implications for the general acceptance of vaccination.

Introduction

The sudden occurrence of seizures and developmental regression after vaccination in previously healthy infants led to the implication of a causal link, especially with pertussis vaccination.[1,2] Extensive debate ensued, but subsequent epidemiological studies did not lend support to the view of a causal association between immunisation and permanent brain damage.[3–8] In individual cases, however, the perception of causality can be difficult to challenge, especially if no alternative cause is identified, and has led to successful litigation. Public interest in this issue is high with a vocal minority urging avoidance of vaccination,[9] with the grave consequence of a potential resurgence of preventable serious childhood illnesses.[10] This issue is difficult to clarify largely because the diagnostic features of vaccine encephalopathy have never been defined. Reported cases have an apparent temporal relation to vaccination (varying from <1 day to 14 days) and typically have multiple seizure types with developmental arrest or regression.[2,3,7,8,11–16]

There are various causes of seizures and developmental regression in infancy, some of which have been previously misdiagnosed as vaccine encephalopathy.[17] A particular epilepsy syndrome, severe myoclonic epilepsy of infancy (SMEI), has become increasingly recognised. SMEI begins in the first year of life in previously healthy children. Hemiclonic seizures, which may be long lasting, are characteristic and can be associated with fever. Myoclonic, absence, tonic–clonic, and partial seizures also occur. The epilepsy is refractory and developmental regression ensues.[18,19] The syndrome is associated with more than 100 different mutations in the neuronal sodium channel α1 subunit gene *SCN1A*. Most cases of SMEI have such mutations, although the exact percentage is still debated. Around half the mutations truncate the protein and about 95% are de novo.[20–27]

We noted a similarity between the clinical pattern of SMEI and alleged cases of vaccine encephalopathy. Thus, we postulated that *SCN1A* mutations might underlie such cases where the physician or family believed that vaccination was causal. This finding would imply that the encephalopathy was not fundamentally caused by vaccination, but was due to a genetically determined, age-specific, epileptic encephalopathy.

Methods

Patients

This retrospective study of post-vaccination cases was nested within a larger study of 96 patients with unexplained encephalopathies and seizures beginning in the first year of life. We recruited participants from child neurologists around Australia and New Zealand during 2002 and 2003 for whom clinical details and DNA were obtainable and other causes of epileptic encephalopathies (perinatal,

post-traumatic, post-infectious, metabolic, and structural, etc) were excluded by appropriate metabolic and imaging studies. A few referrals were also accepted from outside Australasia. The study was approved by the Human Research Ethics Committee of Austin Health. Written informed consent was obtained from parents, guardians, or the appropriate government authority.

Cases were systematically classified on the basis of an exhaustive review of medical records from child neurologists, paediatricians, hospitals, and other treating doctors. Source records from initial medical presentations were sought to determine the precise onset details relative to vaccination. No specific neurological phenotype has been described for vaccine encephalopathy, so all cases were coded as vaccine encephalopathy when a relation to vaccination had been previously claimed and our review showed that the first seizure occurred within 72 h of vaccination. The time interval has no agreed definition, but on the basis of the published work we selected the time frame of documented seizure onset within 72 h of vaccination.[3,5,10,12,14,15]

All patients had epileptic encephalopathy (refractory seizures and developmental slowing); febrile seizures and other benign epilepsies were excluded. Epileptic seizures and epilepsy syndrome were diagnosed according to the International League Against Epilepsy classifications.[19,28] For this study, SMEI was diagnosed if all the following characteristics were present: onset in the first year with hemiclonic or generalised seizures; previous normal development; evolution of myoclonic seizures and generalised spike-wave discharges; and subsequent neurological deterioration. In Lennox-Gastaut syndrome, tonic seizures, atypical absences, and slow spike-wave on EEG were regarded as characteristic. Lennox-Gastaut syndrome can evolve from West syndrome with infantile spasms and hypsarrhythmia. The term borderline SMEI (SMEB), introduced by Japanese authors,[18,26] was used for cases without key features of SMEI (eg, lack of generalised spike-wave discharges, lack of myoclonus, few or atypical seizure types).

Procedures

After clinical classification of the epilepsy syndrome, molecular analysis was done on genomic DNA extracted from patients' venous blood samples. All 26 exons of *SCN1A* were PCR amplified with flanking intronic primers and standard PCR conditions (primers available on request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature before being analysed by denaturing high-performance liquid chromatography (dHPLC) on the WAVE 3500HT instrument (Transgenomic, NE, USA). Amplicons showing altered dHPLC chromatogram patterns compared with normal control DNA were sequenced from independent PCR products in both directions on an ABI 3700 sequencer (Applied Biosystems, CA, USA). Numbering of each mutation was taken from the start codon ATG of the full length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where a mutation was identified, the parents' DNA (if available) was checked for the mutation by direct sequencing.

Sequence changes were identified as mutations rather than as normal polymorphisms if they were not reported as common variants and they resulted in the generation of stop codons or deletions or, for missense mutations, if they resulted in a non-conservative amino-acid change and arose de novo, if parental DNA was available. Specific missense mutations were further validated by excluding them with dHPLC screening from a panel of anonymous Australian blood donors used as the control population.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

14 patients were identified for whom vaccination had been judged as causative of the epileptic encephalopathy and our review confirmed seizure onset within 72 h of vaccination. The patients were aged 2·5–47 years at the time of study (mean 12 years [SD 11]). They were 2–11 months old (5·4 months [2·6]) at the onset of the illness, which followed vaccination by 1–48 h (22 h [15 h]). The vaccines included pertussis in all cases (table).

The first seizure was described as hemiclonic (n=5), generalised clonic or tonic–clonic (n=6), infantile spasms (n=1), tonic (n=1), and unclassified (n=1). The first seizure was definitely associated with fever (>38°C) in five patients, six were afebrile, and in three the temperature was not recorded. Status epilepticus (seizures lasting ≥30 min) occurred at presentation in six cases. All cases had severe epilepsy with multiple seizure types and intellectual disability. Our review of the subsequent clinical course led to diagnosis of SMEI in eight patients, SMEB in four, and Lennox-Gastaut syndrome in two. In the two patients with Lennox-Gastaut syndrome, spasms and hypsarrhythmia occurred early, representing the known evolution from West syndrome. MRI showed no focal lesions and no evidence of destructive or inflammatory processes; scans in all cases were either normal (n=8) or showed varying degrees of diffuse atrophy and delayed myelination (n=6).

Molecular genetic analysis showed heterozygous mutations of *SCN1A* in 11 of 14 cases. These mutations were predicted to lead to truncation of the protein in five cases (three frameshift and two non-sense mutations); the other six were missense mutations (figure).[29,30] A display of evolutionary conservation of the residues where the mutations were found is shown in the webfigure. None of the six missense mutations were identifed in the blood donor control population; a

See Online for webfigure

	Age at study (years)	Age at onset (months)	Seizure onset post vaccination (h)	Vaccine type	First seizure			Later seizures	Epilepsy syndrome	*SCN1A* mutation	De-novo mutation
					Febrile	Status epilepticus	Seizure type				
1	17·5	8	24	2nd TA	N	N	Hemiclonic	Ab, At, H, T, GTCS, SE	SMEI	Frameshift C1354fsX1359	Y
2	2·5	2·5	24	1st TA	Y	N	Hemiclonic	Ab, At, H, M, T, CPS	SMEI	Missense R946H	Y
3	5	3	5	3rd TA	Y	Y	Hemiclonic	H, F, GTCS, SE	SMEB	Frameshift K1077fsX1079	Y
4	4·5	7	48	3rd TA	N	Y	Hemiclonic	Ab, At, H, M, GTCS,	SMEI	Nonsense R1407X	Y
5	4	6	12	3rd TA	N	Y	GC	Ab, F, M, GTCS, SE	SMEI	Missense R1645Q	Y
6	12	3	24	1st TA	Y	Y	GCS	H, M, GCS, GTCS, SE	SMEI	Missense E1238D	Unknown
7	6·5	2	9	1st TA	N	N	GC	Ab, H, M, T, GTCS, SE,	SMEI	Frameshift N1509fsX1511	Y
8	13·5	6	6	3rd TA	N	Y	GTCS	F, M, SGTCS, SE	SMEB	Missense C1396G	Y
9	4·5	7	24	3rd PV	Y	Y	Hemiclonic	H, M, SGTCS, SE,	SMEI	Missense Y413N	Y
10	47	6	24	1st TA	Unknown	Unknown	Unknown	At, F, M, GTCS, SE,	SMEB	Nonsense W384X	Unknown
11	8	4	36	2nd TA	Unknown	N	GCS	F, M, GTCS, SE,	SMEI	Missense F403L	Y
12	16·5	11	24	3rd TA	Y	N	GTCS	Ab, At, M, Sp, GTCS	SMEB	None detected	NA
13	13·5	7	1	3rd TA	Unknown	N	Spasms	Sp, T, At, M, GTCS, SE,	LGS	None detected	NA
14	14·5	2·5	48	1st TA	N	N	Tonic	Sp, T, F, M, GTCS, SE	LGS	None detected	NA

TA=triple antigen (diptheria, pertussis, tetanus); PV=pentavalent vaccine (diptheria, pertussis, tetanus, inactivated polio, and haemophilus); GTCS=generalised tonic-clonic seizures; GCS=generalised clonic seizures; GC=generalised convulsion (uncertain if tonic-clonic or clonic); M=myoclonic seizures; Ab=absences; At=atonic; SE=status epilepticus; CPS=complex partial seizures; H=hemiclonic; SGTCS=secondarily generalised tonic-clonic seizures; F=focal seizures; Sp=spasms; T=tonic, SMEI=severe myoclonic epilepsy of infancy, SMEB=borderland SMEI, LGS= Lennox-Gastaut syndrome, NA=not applicable

Table: **Clinical characteristics of 14 patients with alleged vaccine encephalopathy**

minimum of 130 and maximum of 149 control samples were successfully screened for each mutation. In nine of the 11 patients with *SCN1A* mutations for whom samples from both parents were available, the mutations were absent in parental DNA and thus arose de novo. In patient six, parental DNA was not available. In patient ten, the mother was tested and did not have the mutation and the father was deceased. This patient had a deceased brother who was also said to have seizures beginning after vaccination, but medical records were destroyed and this could not be verified. Correlation of the clinically diagnosed phenotype with the molecular analyses showed that the sodium channel mutations were confined to the cases diagnosed as SMEI (eight of eight cases) or SMEB (three of four cases) and were absent in patients who had Lennox-Gastaut syndrome.

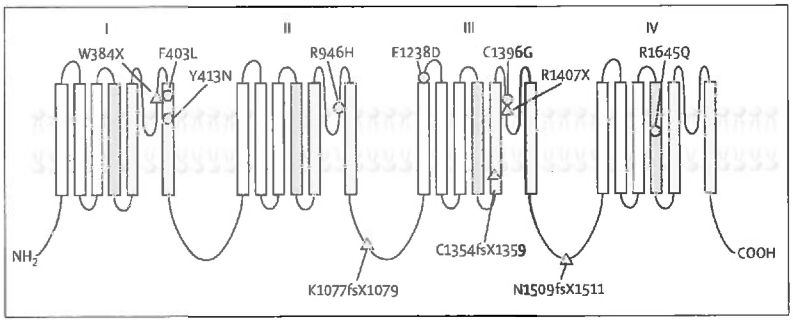

Figure: **Schematic representation of the proposed structure of SCN1A protein**
The protein comprises four homologous domains (I–IV), each with six transmembrane segments. Segments five and six (blue) form the ion channel pore and segment four (grey) is the voltage sensor.[7] The relative location of the six missense mutations (green circles) and five mutations possibly causing protein truncation (pink triangles) in the 11 cases with alleged vaccine encephalopathy are shown. The missense mutations predominantly occurred in the exons coding for the pore forming segments, as previously described in SMEI.[7]

Discussion

In this retrospective cohort of unexplained encephalopathies in the first year of life, 14 patients were judged by clinicians and families to have a vaccine encephalopathy and had documented seizure onset within 72 h of vaccination. With careful electroclinical analysis, we established that the clinical syndrome was SMEI in eight patients and the related syndrome SMEB in four. Eleven patients were shown to have mutations in the sodium channel gene *SCN1A*, which is now a well established finding in SMEI.[20–27] Two of the mutations have been reported before in association with SMEI or SMEB (R946H, R1407X) after nomenclature were standardised to the full length isoform given in Genbank accession number AB093548.[21,26,27] The mutations led to truncation of the protein in five cases, consistent with previous reports of truncation mutations being responsible for about half of SMEI cases with *SCN1A* mutations.[7] The other six patients had missense mutations. All are likely to be pathogenic as they have not been reported in control populations nor were they found in our controls, and the observed missense mutations affect highly conserved amino-acid sites (data not shown), are in regions where SMEI mutations have been previously described,[10] and arose de novo in all cases where both parents were tested.

There is no satisfactory case definition of the specific neurological phenotype in vaccine encephalopathy; indeed, even the temporal relation to immunisation is loose with cases described with onset of symptoms from less than 1 day to 14 days post vaccination.[2,4,5,8,11–16] Although we showed that SMEI or SMEB were important phenotypes in vaccine encephalopathy we were surprised

that no less than 12 of 14 patients were diagnosed as such with careful phenotypic analysis. We do not know if this finding is representative of cases in other centres, but previous reports of seizures in SMEI being associated with vaccination as well as fever lend support to our findings.[5,11,12] The multiple seizure types in SMEI and SMEB can make diagnosis difficult for observers unfamiliar with these disorders; patients can be judged as having an unclassified form of epilepsy and intellectual disability. The discovery of *SCN1A* mutations has led to improved awareness and diagnosis of these severe infantile encephalopathies.[11]

Scientific and medicolegal controversy of vaccine encephalopathy has spanned seven decades.[1,4] We suspect that the nature of cases has changed because of increasingly sophisticated clinical and neurological diagnoses and investigations. Some patients had coma at onset whereas others had seizures with subsequent regression. In the early research, detailed analysis led to the conclusion that some alleged cases were probably due to heterogeneous causes, including viral encephalitis and Reye's syndrome.[17] The molecular delineation of genetic encephalopathies with phenotypes of SMEI and SMEB now seems to be another major piece in the heterogeneous diagnostic puzzle of alleged vaccine encephalopathies.

The genetically determined epilepsy syndromes of SMEI and SMEB typically arise in association with de novo *SCN1A* mutations, presumably due to mutations in the gametes or in the very early post-fertilisation period.[5,11,12,16,17] In alleged vaccine encephalopathy the assumption of vaccination as a cause has been reinforced by the absence of a family history of severe epilepsy. Now, the molecular findings could explain the nature of the encephalopathy and the usual lack of family history since around 95% of mutations in SMEI occur de novo.[20–27]

SMEI often begins with febrile seizures and fever is frequently associated with seizures early in the clinical course. In the presence of *SCN1A* mutations, vaccination can still be argued to be a trigger for the encephalopathy, perhaps via fever or an immune mechanism. Our experimental design does not address this issue, but the role of vaccination as a significant trigger for the encephalopathy is unlikely for several reasons. First, although vaccination might trigger seizures as shown by the increased risk of febrile seizures on the day of triple antigen or MMR vaccination, there is no evidence of long-term adverse outcomes.[6–8] Second, less than half our patients had documented fever with their first seizure, which indicates that fever is not essential. Third, our neuroimaging data showed no evidence of an inflammatory or destructive process. Finally, truncation and missense mutations reported in conserved parts of *SCN1A* have not been found in many hundreds of healthy patients.[20,22,23,25,26] Thus, individuals with such mutations seem to develop SMEI or SMEB whether or not they are immunised in the first year of life. We do not think that avoiding vaccination, as a potential trigger, would prevent onset of this devastating disorder in patients who already harbour the *SCN1A* mutation.

The mechanism by which *SCN1A* mutations cause SMEI is unknown. Few causative mutations have so far been subjected to functional analysis, and the results are inconsistent; however, these mutations are presumed to cause abnormal neuronal excitability.[14,15] Studies of less severe mutations of *SCN1A* that cause milder phenotypes have also produced conflicting results dependent on the techniques and the model system investigated.[16,17] Definitive data from neuronal systems have yet to emerge. Moreover, because many of the mutations associated with SMEI cause truncation of the protein, these proteins are unlikely to be expressed at the cell surface; thus poorly understood changes to sodium-channel density, stoichiometry, and function might all contribute to the phenotypes observed. Further study in neuronal systems, and ideally whole animal models, is needed to clarify the complex functional effects of *SCN1A* mutations.

We did not find a molecular explanation for three patients with alleged vaccine encephalopathy. These could be chance associations of vaccination with other causes leading to the onset of encephalopathies. Other cases could be due to large deletions or undiscovered mutations in non-translated parts of the *SCN1A* gene or perhaps due to rare mutations in related genes, such as *GABRG2*[38] and *SCN2A*.[39]

Although epidemiological studies have cast doubts on the hypothesis of vaccination as a cause of encephalopathy,[4,7] families, the medical profession, and society remain difficult to reassure of the lack of causality in individual patients in whom vaccination and onset of encephalopathy were coincidental. For individual cases, this problem is particularly significant in the legal setting. For society, fear of adverse consequences of vaccination is a major factor in suboptimum immunisation rates. The identification of a genetic cause of encephalopathy in a particular child should finally put to rest the case for vaccination being the primary cause. Confirmation of our findings by others would be of value in determining their generalisability and the broad societal implications.

Cases of vaccine encephalopathy should be carefully assessed clinically for characterisitcs of SMEI or SMEB, and testing for *SCN1A* mutations should be considered. Correct diagnosis will reassure the family as to the true cause, remove the blame of having vaccinated the child, direct appropriate treatment, and allow realistic planning for prognosis. Specific treatment regimens for seizures in SMEI are emerging with controlled data showing the effectiveness of stiripentol,[40] and uncontrolled open studies suggesting avoidance of lamotrigine[41] and probable benefit of topiramate.[42] Medical and societal energies that have focused on the alleged association with vaccination need to be redirected towards the care of these severely handicapped individuals and towards novel approaches to treat and ultimately prevent these encephalopathies.

Contributors

SFB developed the hypothesis and wrote the first draft. Analysis of clinical data was done principally by IES, JMM, JTP, and SFB, and also by SMZ, ECW, and DSG. Molecular analysis was undertaken by LH, XI, and JCM. All authors critically revised the first draft and approved the final manuscript.

Conflicts of interest

SFB, IES, and JCM have received research support and honoraria from Bionomics Ltd. Bionomics Ltd has licensed a diagnostic test for *SCN1A* mutations.

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The spectrum of *SCNIA*-related infantile epileptic encephalopathies

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The relationship between severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) and the related syndrome SMEI-borderland (SMEB) with mutations in the sodium channel alpha I subunit gene *SCNIA* is well established. To explore the phenotypic variability associated with *SCNIA* mutations, 188 patients with a range of epileptic encephalopathies were examined for *SCNIA* sequence variations by denaturing high performance liquid chromatography and sequencing. All patients had seizure onset within the first 2 years of life. A higher proportion of mutations were identified in patients with SMEI (52/66; 79%) compared to patients with SMEB (25/36; 69%). By studying a broader spectrum of infantile epileptic encephalopathies, we identified mutations in other syndromes including cryptogenic generalized epilepsy (24%) and cryptogenic focal epilepsy (22%). Within the latter group, a distinctive subgroup designated as severe infantile multifocal epilepsy had *SCNIA* mutations in three of five cases. This phenotype is characterized by early onset multifocal seizures and later cognitive decline. Knowledge of an expanded spectrum of epileptic encephalopathies associated with *SCNIA* mutations allows earlier diagnostic confirmation for children with these devastating disorders.

Keywords: *SCNIA*; SMEI; SMEB; epileptic encephalopathy; channelopathies

Abbreviations: dHPLC = denaturing high performance liquid chromatography; GEFS+ = generalized epilepsy with febrile seizures plus; ICEGTC = intractable childhood epilepsy with generalized tonic clonic seizures; LGS = LennoxGastaut syndrome; MAE = Myoclonicastatic epilepsy; PCR = polymerase chain reaction; SCNIA = sodium channel alpha I subunit gene; SMEB = SMEI-borderland; SMEB-M = SMEI-borderland-myoclonic seizures; SMEB-O = SMEI-borderland more than one feature; SMEB-SW = SMEI-borderland-spike wave; SMEI = severe myoclonic epilepsy of infancy

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Introduction

SCN1A, the gene encoding the sodium channel alpha 1 subunit, has emerged as the most important of the epilepsy genes currently known (Mulley *et al.*, 2005). *SCN1A* mutations underlie more than 70% of patients with the epileptic encephalopathy severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) (Dravet *et al.*, 1982; Claes *et al.*, 2001; Mulley *et al.*, 2005). More than 170 documented mutations are associated with SMEI and the related syndrome of borderland SMEI, known as SMEB. Truncation mutations account for nearly 50% of mutations found in SMEI, with the remainder comprising missense, splice site and deletion mutations (Mulley *et al.*, 2005). These mutations affect many domains of the gene with suggested clustering of missense mutations occurring in the N- and C-termini and the S5–S6 pore-forming regions of the protein (Kanai *et al.*, 2004). Recently intragenic and whole gene deletions have been identified in a few cases of SMEI without truncation, missense or splice-site mutations (Madia *et al.*, 2006; Mulley *et al.*, 2006; Suls *et al.*, 2006).

Approximately 95% of *SCN1A* mutations in SMEI patients arise *de novo*. The remaining cases have familial mutations with milder phenotypes in other family members often consistent with the generalized epilepsy with febrile seizures plus (GEFS+) spectrum (Scheffer and Berkovic, 1997; Escayg *et al.*, 2000; Singh *et al.*, 2001; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Scheffer, 2003; Kimura *et al.*, 2005). Recently, germline and somatic *SCN1A* mutational mosaicism has been reported in unaffected parents (or parents mildly affected with febrile seizures) where their children have SMEI or SMEB (Depienne *et al.*, 2006; Gennaro *et al.*, 2006; Marini *et al.*, 2006; Morimoto *et al.*, 2006).

SMEI or Dravet syndrome is a distinctive syndrome with seizure onset in the first year of life, typically beginning with prolonged febrile hemiclonic seizures or generalized tonic–clonic seizures (Dravet, 1978; Dravet *et al.*, 1982, 2005). The disorder evolves with other seizure types such as myoclonic, focal, absence and atonic seizures developing between 1 and 4 years of age. Development is normal in the first year of life followed by developmental slowing and regression. Pyramidal signs and ataxia may evolve. Cognitive outcome is usually poor and seizures remain refractory for those who survive to adulthood (Jansen *et al.*, 2006).

The phenotypic spectrum of patients with *SCN1A* mutations has been extended beyond SMEI. The related syndrome SMEB (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004) refers to children who lack several of the key features of SMEI such as myoclonic seizures or generalized spike-wave activity (Sugama *et al.*, 1987; Dravet *et al.*, 2005). In two studies, 26% (7/27) and 88% (15/17) of SMEB patients were found to have *SCN1A* mutations respectively (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004). As with SMEI, these mutations are spread throughout the gene with a mixture of types of mutation including truncation, missense and splice-site changes (Mulley *et al.*, 2005). A subgroup of SMEB has been variously described as intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC, originally called high voltage slow waves grand mal by Japanese authors) or Severe idiopathic generalized epilepsy of infancy with generalized tonic–clonic seizures. These infants have generalized tonic–clonic seizures beginning in the first year of life without the evolution of other seizure types and they follow a similarly unfavourable developmental course to children with SMEI (Fujiwara *et al.*, 1992; Kanazawa, 1992, 2001; Sugama *et al.*, 1987; Doose *et al.*, 1998). In one series, 7/10 ICEGTC patients had missense mutations in *SCN1A* (Fujiwara *et al.*, 2003); truncation, missense and splice-site mutations were reported in 3/18 patients described as severe idiopathic generalized epilepsy of infancy (Ebach *et al.*, 2005). We reported the only case so far of West syndrome with an *SCN1A* mutation (Wallace *et al.*, 2003).

Given the overlapping yet heterogeneous clinical features of these epilepsy syndromes, we postulated that *SCN1A* mutations may be associated with other phenotypes. Here we studied unselected patients with severe epileptic encephalopathies (including SMEI) with onset primarily during the first year of life.

Material and methods

Clinical methods

Patients with epileptic encephalopathies of unknown cause were referred by paediatric neurologists and neurologists from Australia and around the world. Epileptic encephalopathies are defined as disorders in which there is a temporal relationship between deterioration in cognitive, sensory and motor function and epileptic activity comprising frequent seizures and/or extremely frequent 'interictal' paroxysmal activity (Nabbout and Dulac, 2003). Cases were only included where magnetic resonance imaging was normal or showed non-specific features without a definite aetiology. A subset of 14 patients, included in this study, with so-called 'vaccine encephalopathy' has been published previously (Berkovic *et al.*, 2006).

Electroclinical data were obtained on all patients with specific emphasis on early seizure history including age of onset, occurrence of status epilepticus, presence of fever sensitivity, clinical photic sensitivity and evolution of other seizure types. A detailed early developmental history was obtained with attention to acquisition of early milestones, timing of plateau or regression of development and current functioning. Other important details included general and neurological examination, family history of seizure disorders and results of EEG, video-EEG monitoring and neuroimaging studies. Results of other available investigations such as chromosomal analysis were also obtained.

SMEI was defined according to the following criteria: onset in the first year of life of convulsive seizures which were hemiclonic or generalized; myoclonic seizures; other seizure types which could include focal seizures, absence seizures, atonic seizures, tonic

seizures; normal development in the first year of life with subsequent slowing including plateauing or regression; generalized spike-wave activity and either normal MRI or non-specific findings.

SMEB was divided into subgroups based on the absence of specific features that are regarded as required for the diagnosis of SMEI. SMEB-M referred to patients who did not have myoclonic seizures but otherwise satisfied SMEI criteria. SMEB-SW defined patients who had all the SMEI criteria but had never had generalized spike-wave activity documented on EEG. SMEB-O referred to patients who had more than one feature that was not in keeping with SMEI; examples include absence of generalized spike-wave activity recorded on EEG, a normal developmental outcome and absence of myoclonic seizures. SMEB included cases with ICEGTC where they followed the same course but only had convulsive seizures.

Cryptogenic generalized epilepsy (CGE) denoted individuals who have multiple seizure types, generalized sharp and slow activity and intellectual disability with no known aetiology. Lennox–Gastaut syndrome (LGS) defined patients with tonic seizures and slow generalized spike-wave activity and abnormal development (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Beaumanoir and Blume, 2005). Myoclonic–astatic epilepsy (MAE) referred to individuals with myoclonic–astatic seizures and other generalized seizure types with generalized spike-wave activity and variable developmental outcome (Doose *et al.*, 1970; Guerrini *et al.*, 2005).

A further subgroup was called cryptogenic focal epilepsy where an individual had focal seizures and uni- or multifocal EEG epileptiform patterns. These individuals showed a variable degree of intellectual disability and usually had normal neuroimaging. Several individuals were included with abnormal neuroimaging that did not account for the clinical presentation such as hydrocephalus, bilateral periventricular leucomalacia, etc.

Other syndromes were defined according to the ILAE classification (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Patients were called 'unclassified' if we had insufficient evidence to make a syndrome diagnosis or the patient did not fit into a recognized syndrome despite detailed evaluation.

The Austin Health Human Research Ethics Committee approved this study. Informed consent was obtained from the parents or guardians of minors and from adult subjects of normal intellect. In the case of adults with intellectual disability, legal consent was obtained from the appropriate government authority or legal guardian.

Molecular analysis

Molecular analysis was carried out on genomic DNA extracted from venous blood. All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR) using flanking intronic primers and standard PCR conditions (primers available upon request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature to form heteroduplex products which were analysed by denaturing high performance liquid chromatography (dHPLC) on the Transgenomic WAVE 3500HT instrument (dHPLC conditions available upon request). Amplicons showing altered dHPLC chromatogram patterns were sequenced in both directions from independent PCR products, on an ABI 3700 sequencer. The final subset of patients (43) was screened by direct sequencing of PCR products (without prior dHPLC screening) by Athena Diagnostics under diagnostic conditions. The numbering for each mutation is taken from the start codon ATG of the full-length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where an *SCN1A* mutation was detected, the appropriate amplicon from parental DNA (where available) was tested by DNA sequencing to distinguish between *de novo* and familial variants. Mutations or rare variants were distinguished from coding single nucleotide polymorphisms which have previously been reported (Escayg *et al.*, 2001).

Results

Clinical diagnoses

One hundred and eighty-eight patients were recruited from Australia (110), Canada (27), United Kingdom (23), New Zealand (20), Israel (4), USA (3) and Denmark (1) with seizure onset in the first 2 years of life. These included 14 cases who were negative for *SCN1A* mutations on single-stranded conformation analysis in our previous study (Wallace *et al.*, 2003); the eight positive cases and two, who were negative on sequencing, are not included in the data presented here.

Our total cohort contained 66 with SMEI, 36 with SMEB including the various subcategories, 25 with cryptogenic generalized epilepsy, 18 with cryptogenic focal epilepsy, 10 with MAE and 12 with LGS. The remaining cases had a range of other syndromes or were unable to be classified (Table 1).

Table 1 *SCN1A* mutations in patients with epileptic encephalopathies

	Total	*SCN1A* mutation
SMEI	66	52
SMEB	36	25
SMEB-O	16	10
SMEB-SW	14	11
SMEB-M	4	3
ICEGTC	2	1
Cryptogenic generalized epilepsy	25	6
Cryptogenic focal epilepsy	18	4
Myoclonic–astatic epilepsy	10	2
Lennox–Gastaut syndrome	12	1
West syndrome	5	
Idiopathic spasms	1	
Early myoclonic encephalopathy	1	
Progressive myoclonic epilepsy	1	
Alternating hemiplegia of childhood	1	
Unclassified	12	
Total	188	90

SMEI, severe myoclonic epilepsy of infancy; SMEB-SW, SMEI borderland without generalized spike wave; SMEB-M, SMEI borderland without myoclonic seizures; SMEB-O, SMEI borderland lacking more than one feature of SMEI; ICEGTC, intractable childhood epilepsy with generalized tonic–clonic seizures.

Molecular analysis

Of the 188 patients examined, 90 (48%) had *SCN1A* mutations. Ninety-four sequence variants were identified in the 90 mutation positive patients as four children each had two changes. In each child, the putative pathogenic variant was distinguished from the likely non-pathogenic variant; the latter was not included in further analyses (see later and Supplementary Table). The majority of mutations are novel (72/90, 80%), reinforcing the mutational heterogeneity characteristic of *SCN1A*. Of the 90 cases, DNA was available from 76 sets of parents and 73/76 (96%) were *de novo* mutations.

Amino acid alignments of the missense mutations show that they affect conserved domains of the protein in other human alpha channels (SCN2A, SCN3A and SCN8A), chimpanzee, rat, mouse, *Fugu* and *Drosophila* consistent with their interpretation as pathogenic mutations (Supplementary Fig. S1). Moreover, the probability that these missense mutations are pathogenic mutations is supported by their *de novo* origin (in 34/37 cases where parents have been examined) and previously published observations in SMEI.

SMEI

Fifty-two of the 66 (79%) of patients with SMEI had *SCN1A* mutations (Table 1). Forty-four percent (23/52) of the SMEI-related mutations were non-sense or frameshift mutations resulting in protein truncation, 39% (20/52) were missense mutations and the remaining 17% (9/52) were intronic splice donor or splice acceptor site changes. These mutations were spread throughout the gene with the majority of missense mutations (14/20, 70%) localized to the transmembrane regions of the protein, in particular the S5–S6 loop of domain II that forms part of the ion channel pore (Supplementary Table, Fig. 1A). In contrast, 57% (13/23) of truncation mutations were positioned in the intracellular loops of the protein (Supplementary Table, Fig. 1A).

Parental DNA was available for testing for 42/52 SMEI patients who were mutation positive. Analysis of the DNA from these parents confirmed that all 42 mutations were *de novo*.

There were four patients with two sequence variants that posed challenges in clinico-molecular interpretation (Supplementary Table). Patient 2 had two *SCN1A* sequence variants: one was a *de novo* missense change (Y84C) affecting a highly conserved amino acid site (Supplementary Fig. S1) and the second was a splice acceptor site change found to have a maternal origin. The mother was unaffected; the maternal grandfather had a history of convulsions until 7 years but was negative for the splice acceptor site change. There was no further seizure history within this family suggesting that the change within the splice site was probably a benign variant. Therefore this variant was not considered in the determination of mutation frequencies.

Patients 6, 32 and 38 also had two sequence variants detected but parental DNA was unavailable in order to ascertain which variant was *de novo* and thus the likely pathogenic mutation (Supplementary Table). Patient 6 had two intronic mutations detected, both potentially pathogenic. In the absence of parental DNA we can only assume that one is likely to be pathogenic. The intron IVS3-13T→A change was chosen as the most likely variant to affect splicing since it is within the consensus C/T run in the splice acceptor site. Patient 32 had both a missense (E1238D) and an intronic donor splice site mutation. Since the missense change affected a highly conserved amino acid site (Supplementary Fig. S1), this was considered to be the true mutation. Patient 38 had a truncation mutation (R1525X) also seen in Patient 39 with SMEI and in a previous study (Supplementary Table) (Kearney *et al.*, 2006). Patient 38 also had a missense change not as highly conserved as most missense mutations (Supplementary Fig. S1) so the truncation mutation was considered the likely pathogenic mutation. The second variant found in each case has not been included in the mutational analyses.

Simultaneous double mutation in the same patient is a theoretical possibility, as is a *de novo* mutation adversely interacting with a pre-existing rare variant. However, in the absence of definitive evidence from other SMEI cases and

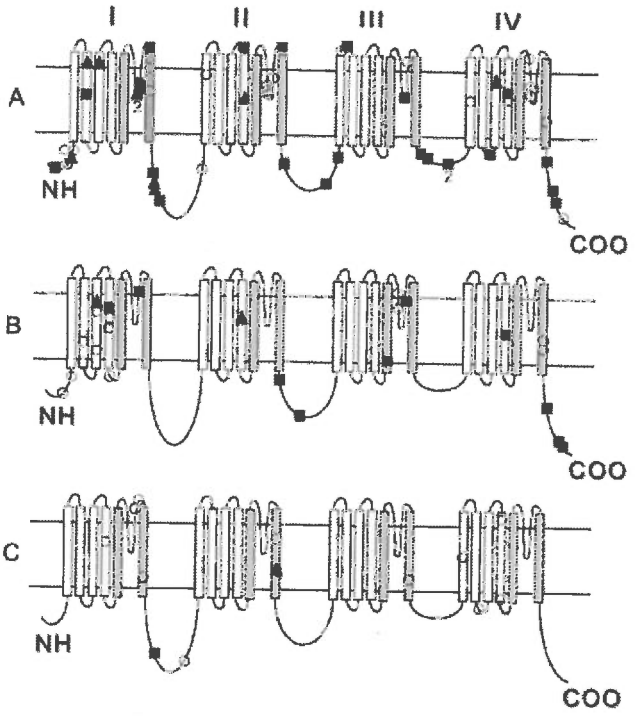

Fig. 1 Schematic representation of mutations in *SCNIA* in patients with (**A**) SMEI, (**B**) SMEB and (**C**) other phenotypes. Refer to Supplementary Table for details. The SCNIA protein consists of four domains designated I–IV, each contains six transmembrane segments designated S1–S6. ■ = truncation, ○ = missense, ▲ = splice-site mutations.

absence of parental DNA to establish *de novo* origin, the most parsimonious explanation is that of a single mutational event unless proven otherwise.

SMEI—borderland

SCN1A mutations were identified in 25/36 (69%) patients with SMEB including all subcategories (Table 1). Over half of these changes were missense mutations (13/25) with 40% (10/25) being truncation mutations; the remaining two were splice-site mutations. The mutations were spread throughout the gene with the majority (18/25, 72%) localized to the transmembrane domain regions. Missense mutations were clustered in the S2–S4 transmembrane segments of domain I (Fig. 1B).

Analysis of parental DNA from 22/25 patients with mutations confirmed 95% (21/22) were *de novo*. Patient 63 had a paternally inherited mutation (A239T). The proband's father had febrile seizures plus (Scheffer and Berkovic, 1997) and the paternal grandmother had unclassified seizures. Both individuals were found to carry the A239T change, which when taken together with the amino acid conservation of this residue (Supplementary Fig. S1), reinforces the status of this variant as a true pathogenic mutation of *SCN1A*. The family had a bilineal family history of seizures as the proband's mother had febrile seizures and did not carry the *SCN1A* mutation (Supplementary Fig. S2).

Cryptogenic generalized epilepsy

Of the 25 patients with cryptogenic generalized epilepsy, six (24%) had mutations. None of the mutations have been previously reported, however the T226M in Patient 78 was also seen in Patient 61 within this cohort with SMEB-O (Supplementary Table). Four mutations arose *de novo*; parental DNA was unavailable for one patient and for Patient 82 the mutation (M973V) was found in her unaffected father. There was no family history of seizures but the amino acid conservation at this site is reasonably strong (Supplementary Fig. S1) providing circumstantial evidence that it is a true mutation. If so, then it must be non-penetrant in the father or else function as a susceptibility allele acting in tandem with other unidentified susceptibility genes responsible for the phenotype in the proband.

The six cases with mutations had heterogeneous phenotypes with onset between 1.5 and 12 months (Table 2). Two had a phenotype with onset in the first 2 months of life and abnormal early development but other features were similar to SMEI. Patient 80 fixed and followed and smiled by 6 weeks when seizures began. Development slowed from 6 weeks: he sat late, walked at 18 months and development stagnated from 2 years. He died at 13 years. Patient 78 had seizure onset at 8 weeks, smiled at 3 months, never sat or acquired words.

The other four cases presented a mixed picture, but generalized spike wave and focal discharges were usually

Table 2 Clinical features of *SCNIA* mutation positive patients with diagnosis other than SMEI and SMEB

Patient	Age at study (years)	Seizure onset (months)	Seizure types	Intellect	Neurological signs	Epilepsy classification	*SCNIA* mutation	Inheritance
78	5	2	GTCS, H, MJ, F, NCS	Severe ID	Increased tone, later generalized hypotonia	CGE	T226M	De novo
79	23	5.5	FS, GTCS	Mild-moderate ID	None	CGE	A395P	De novo
80	14	1.5	GTCS, H, At, MJ, F, SE	ID	None	CGE	V422E	De novo
81	14	12	FS, GTCS, aAb, MJ, SE	ID	Ataxia, intermittent movement disorder	CGE	S626G	ND
82	35	9	FS, GTCS, MJ, F-SG	Low average	None	CGE	M973V	Paternal
83	3	6	FS, GTCS, MJ	Normal	None	CGE	IVSI5+IG→T	*De novo*
84	16	7	FS, GTCS, MJ, F, NCS	Mild ID	Mild generalized spasticity	CFE (SIMFE)	F575fsX622	*De novo*
85	5	4.5	F, H, SE	ID	None	CFE (SIMFE)	FI543S	Maternal
86	20	5	GTCS, MJ, F, T	Moderate ID	Ataxia, mild left hemiparesis	CFE (SIMFE)	RI596C	*De novo*
87	5	18	FS, F-SG, SE	Normal	None	CFE	RI657H	*De novo*
88	21	0.75	IS, At, aAb, T, SE, NCS	ID	Mild right hemiparesis	LGS	RI636Q	*De novo*
89	11	4	FS, GTCS, MJ, At, T, H	ID	None	MAE	R393C	*De novo*
90	12	13	FS, F, MA, MJ	Moderate ID	None	MAE	GI480V	*De novo*

FS, febrile seizures; aAb, atypical absence seizures; At, atonic seizures; F, focal seizures (not hemiclonic/unilateral); GTCS, generalized tonic–clonic seizures; H, hemiclonic; IS, infantile spasms; MA, myoclonic–astatic; MJ, myoclonic jerks; NCS, non-convulsive status epilepticus; SE, status epilepticus; SG, secondary generalization; T, tonic seizures; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; LGS, Lennox–Gastaut syndrome; MAE, myoclonic–astatic epilepsy; SIMFE, severe infantile multifocal epilepsy; ID, intellectual disability; ND, not done.

seen. The severity of the seizures varied with some only having generalized tonic–clonic seizures, which settled by adult life.

Cryptogenic focal epilepsy

Of 18 patients with cryptogenic focal epilepsy within this cohort of infantile epileptic encephalopathies, four (22%) had mutations (Table 1). Five cases presented with severe infantile multifocal epilepsy with developmental delay and are described later. Three had mutations: two (Patients 84 and 86) arose *de novo* (F575fsX622, R1596C) and one was maternally inherited. The latter (Patient 85) had a putative mutation (F1543S) that was highly conserved (Supplementary Fig. S1) and was carried by her unaffected mother, and may represent a susceptibility allele.

One (Patient 87) had recurrent febrile status epilepticus with onset at 18 months (Table 2). Twenty-four episodes of status epilepticus occurred, some with focal features with variable lateralization. MRI was normal. The patient died at 5 years due to complications of status epilepticus. He had a *de novo* missense *SCN1A* mutation (R1657H).

Severe infantile multifocal epilepsy

Five cases had this phenotype with seizure onset at a mean of 4 months. Of those with *SCN1A* mutations (Patients 84, 85 and 86, Table 2), onset occurred at mean of 5.5 months (4.5, 5 and 7 months) compared with 6- and 8-week onsets in the other two cases. Each child had multiple types of focal seizures with varying semiology. EEG studies showed abundant multifocal epileptiform activity typically with no (or exceptional) generalized or bilaterally synchronous discharges. The three patients with mutations had MRI brain studies; two showed mild atrophy. The remaining two had CT brain scans; one showed mild right sided atrophy.

Developmental delay became evident in all cases. In the two cases that were mutation negative, seizures began at 6 and 8 weeks concurrent with the recognition that developmental delay was present. In the three cases with *SCN1A* mutations, early development was normal with developmental slowing noted at the ages of 16 months, 3–4 years and 6 years even though seizure onset occurred at 4.5, 5 and 7 months, respectively (Table 2). Developmental outcome was poor with intellectual disability ranging from mild (one case, mutation positive: Patient 85), moderate (three cases, two had mutations: Patients 84 and 86) to severe (one case).

Other phenotypes

De novo SCN1A mutations were identified in 2/10 patients with MAE (Patients 89 and 90) and 1/12 patients with LGS (Patient 88) (Tables 1 and 2). No mutations were identified in patients with West syndrome, idiopathic spasms, early myoclonic encephalopathy, progressive myoclonic epilepsy, alternating hemiplegia of childhood or the 12 cases that could not be classified.

Discussion

The sodium channel alpha 1 subunit gene, *SCN1A*, is currently the most clinically relevant epilepsy gene. Mutations in *SCN1A* are an important cause of SMEI and SMEB and its subset ICEGTC (Claes *et al.*, 2001; Mulley *et al.*, 2005). Recently we showed that so-called 'vaccine encephalopathy' should be regarded as SMEI/SMEB on clinical and molecular grounds (Berkovic *et al.*, 2006). Whilst *SCN1A* was originally associated with a small proportion of patients with the mild phenotypes characteristically seen in the GEFS+ syndrome (Escayg *et al.*, 2000; Mulley *et al.*, 2005), mutations within this gene have been identified far more often in patients with more severe forms of epilepsy. This study examines epileptic encephalopathies beginning early in life and expands the phenotypic spectrum of *SCN1A* defects beyond that previously recognized, to now include patients with cryptogenic generalized epilepsy and cryptogenic focal epilepsy.

The majority of mutations identified in the 90 children in this study were novel (72/90, 80%), whereas 18 (20%) had been previously published (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Wallace et al., 2003; Fukuma *et al.*, 2004; Mulley *et al.*, 2005; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006). This expanded list of mutations, taken together with those reviewed by Mulley *et al.* (2005), provides an essential mutational database for use as an interpretative aid for diagnostic laboratories offering *SCN1A* mutation testing. Unlike some disorders where mutations are largely concentrated in 'hot spots', the mutations within *SCN1A* are widely distributed throughout the gene.

Parental DNA was available in 84% (76/90) of cases of which 96% (73/76) arose *de novo* and 4% (3/76) were familial. Familial *SCN1A* mutations have been previously reported in around 5% of SMEI where family members have mild GEFS+ phenotypes, as we observed here (Supplementary Fig. S2) (Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Mulley *et al.*, 2005). In these probands, it is likely that their disorder has a multifactorial basis where *SCN1A* is a major but not the sole contributing gene. This would explain the marked disparity in phenotypic severity between the proband and their relatives. This model could explain probands 63, 82 and 85 where the parent was unaffected or had a mild phenotype. It is worth noting that these probands had a range of phenotypes including cryptogenic generalized and cryptogenic focal epilepsies.

Given the current state of knowledge, the majority of *SCN1A* mutations remain novel. This creates a challenge in determining whether new variants are pathogenic or not. Where the variant is *de novo* or results in truncation of the protein, then the likelihood of it being pathogenic is

extremely high; 79 (88%) of our 90 positive cases fitted these criteria. In cases with missense changes, where DNA from parents is unavailable, or where an unaffected transmitting parent is identified, the case for pathogenicity rests on circumstantial evidence provided by evolutionary conservation of protein structure. Definitive functional studies are rarely available for this particular ion channel. In the four cases with two rare *SCN1A* variants, one of the two was assessed as more likely to be relevant to the observed phenotype. In cases where an unaffected transmitting parent is identified, these changes may be incidental benign variants, incompletely penetrant pathogenic variants or represent a susceptibility allele that contributes to the phenotype in a polygenic manner. Another alternative is that the variant has a major effect on the proband, whereas the transmitting parent has unrecognized protective molecular mechanisms.

SMEI

This study reinforces the high frequency of *SCN1A* mutations in patients with SMEI. The initial report described mutations in 7/7 cases (Claes *et al.*, 2001). Subsequently, large series from a number of centres have reported mutations in 61–87% cases consistent with our finding of 79% reported here (Ohmori *et al.*, 2002, 2003; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Fukuma *et al.*, 2004). Lower mutation rates of 35% (33/93) and 33% (55/169) have been reported (Nabbout *et al.*, 2003*a*; Suls *et al.*, 2006) and of 33% (8/24) by our laboratory (Wallace *et al.*, 2003). The latter study used single-strand conformation analysis for mutation detection, a rapid screening technology less sensitive than dHPLC used here. DHPLC has >96% sensitivity and specificity (Xiao and Oefner, 2001). Additional direct sequencing was performed in five cases (two negative). Fourteen of the remaining negative SMEI cases from our study were tested by dHPLC (3 cases) or direct sequencing (11 cases) here. Eight mutations were identified (two by dHPLC and six by sequencing), bringing the mutation rate to 16/24 (66%) for those cases reported in our original study (Wallace *et al.*, 2003). Of our *SCN1A* mutation negative SMEI cases on dHPLC, 2 of 13 (15%) were subsequently found to have whole exon deletions detected by multiple ligase-dependent probe amplification (Mulley *et al.*, 2006). Other SMEI cases lacking point mutations have been shown to have microdeletions including the *SCN1A* gene (Madia *et al.*, 2006; Suls *et al.*, 2006).

SMEB

We found 69% of our SMEB cases had *SCN1A* mutations. This figure is higher than the 26% reported by Fukuma *et al.* (2004) and more in keeping with the 88% mutation rate of Ohmori *et al.* (2003). The majority of SMEB mutations detected in this study were novel changes (17/25, 68%), with eight mutations being previously reported in patients with SMEI (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Wallace *et al.*, 2003; Fukuma *et al.*, 2004; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006).

SMEB is distinguished from SMEI by the absence of specific features. The question of whether myoclonic seizures are an essential component of a SMEI phenotype remains controversial (Ogino *et al.*, 1988; Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Ohmori *et al.*, 2003; Fukuma *et al.*, 2004; Dravet *et al.*, 2005). Dravet and colleagues observed that myoclonic seizures may be segmental or occur immediately prior to convulsive seizures and they postulate that subtle myoclonus may be missed (Dravet *et al.*, 2005). Our data suggest that myoclonic seizures are not obligatory as three of four patients with an SMEI phenotype lacking only obvious myoclonic seizures (SMEB-M) carried a *SCN1A* mutation. Similarly, generalized spike-wave activity is considered the EEG hallmark of SMEI, but we found that 11/14 (79%) of our patients with a SMEI picture without demonstrated generalized spike-wave activity (SMEB-SW) had mutations.

Our findings in SMEB have important implications for the 'lumpers and splitters' debate. Whilst Ohmori and co-workers (2003) found a higher mutation rate in SMEB (88%) than SMEI (72%), our larger study shows the reverse. Moreover, three mutations are associated with both SMEI and SMEB (Patients 4 and 54, 8 and 59, 48 and 74) in this study. Similarly, eight cases have a mutation previously associated with the alternate phenotype (Supplementary Table). The recent ILAE classification proposal suggests the new name of Dravet syndrome for SMEI (Engel, 2001). In terms of clinical utility, we suggest that it may be more helpful to conceptualize SMEI and SMEB as a spectrum and incorporate both under the eponym of Dravet syndrome. This would also resolve the inaccuracy in terminology arising from the absence of myoclonic seizures in some cases of SMEI despite 'myoclonic' being part of the syndrome's name.

SCN1A mutations in SMEI and SMEB

Our data show similar results to those previously summarized in our review of *SCN1A* mutations (Mulley *et al.*, 2005), with mutations comprising 43% (33/77) truncation and 43% (33/77) missense changes. The proportion of missense (39% versus 52%) and truncation (44% versus 40%) mutations is similar in SMEI and SMEB. Our new data fail to fully confirm previous observations of a predilection for missense mutations to occur in the ion channel pore region (Kanai *et al.*, 2004), with only 15/33 (46%) in this region. Previous studies suggested clustering of missense mutations in SMEI in the S5–S5 loops of domain I and II (Mulley *et al.*, 2005) but here, clustering in domain I was not seen (Fig. 1A).

No consistent pattern of clustering has emerged in SMEB although 18/25 mutations were located in the transmembrane domains (Fig. 1B). Here, clustering of mutations was noted in the S2–S4 transmembrane segments of domain I, in contrast to patterns seen previously where clustering in domain II was observed (Mulley *et al.*, 2005). More data are required in order to establish if a true pattern of clustering exists.

Broader phenotypes of *SCN1A* mutations (Table 3)

The specific generalized epilepsy syndromes of MAE and LGS had a low yield of mutations with 2/10 and 1/12 positive cases respectively confirming that *SCN1A* is rarely associated with these syndromes (Wallace *et al.*, 2001; Nabbout *et al.*, 2003*b*; Ebach *et al.*, 2005). The nosological boundaries between these disorders, SMEI, SMEB and other cryptogenic generalized epilepsies are blurred. Indeed, in the large group of patients with cryptogenic generalized epilepsy of early onset where a more specific syndromal diagnosis could not be reached, 6/25 had *SCN1A* mutations. Two patients had a phenotype with features similar to SMEI but had onset in early infancy with abnormal early development and a more severe course. Others had heterogeneous phenotypes of generalized epilepsy with intellectual disability including those previously recognized in GEFS+ families (Scheffer and Berkovic, 1997; Singh *et al.*, 1999).

In patients classified as cryptogenic focal epilepsy, we identified a clinical subgroup who presented with a devastating multifocal epileptic encephalopathy. Of the five cases, three had *SCN1A* mutations. We designated this group severe infantile multifocal epilepsy (SIMFE) as onset is in the first year of life and multiple seizure types occur, with the most prominent being focal seizures. Multiple types of focal seizures occur including complex partial seizures of temporal lobe origin and hemiclonic seizures. Video-EEG telemetry showed that the variation in seizure semiology was not due to seizure spread patterns. Focal myoclonus may occur or even be brought out by specific anti-epileptic drugs known to exacerbate myoclonic seizures, such as vigabatrin. Patients may also have convulsive or non-convulsive status epilepticus, tonic seizures with focal features and tonic–clonic seizures. Interictal EEGs show abundant multifocal epileptiform discharges. These individuals do not have generalized spike-wave activity on EEG. Their MRI brain scans are normal or show non-specific features. They usually have normal early development followed by cognitive decline, with the refractory seizure disorder culminating in intellectual disability. Abnormal neurological signs such as ataxia and spasticity may evolve. The factor that distinguishes these children from SMEI is the absence of generalized absence and myoclonic seizures, generalized spike-wave activity on EEG, and that their cognitive decline may be later than the second year of life. These children had a severe, progressive and hitherto puzzling phenotype, where extensive investigations had been performed searching for an aetiology such as muscle biopsy, lumbar puncture and liver biopsy.

Similar cases are described in the literature by many authors (Noriega-Sanchez and Markand, 1976; Markand, 1977; Blume, 1978; Malik *et al.*, 1989; Ohtsuka *et al.*, 1990, 2000; Burnstine *et al.*, 1991; Ohtahara *et al.*, 1995; Nabbout and Dulac, 2003; Yamatogi and Ohtahara, 2003). Some clinicians regard this phenotype as being the later evolution of a 'burnt out' symptomatic generalized epilepsy, but these patients never have the EEG signature of generalized spike-wave activity. The phenotype could also be regarded as part of 'severe epilepsy with multiple independent spike foci' described by Ohtahara and colleagues where generalized minor seizures are also emphasized (Ohtsuka *et al.*, 1990; Ohtahara *et al.*, 1995; Yamatogi and Ohtahara, 2003, 2006). This group incorporates a heterogeneous array of causes

Table 3 Epileptic encephalopathies with *SCN1A* mutations

	SMEI ($n = 66$)	SMEB ($n = 36$)	CGE ($n = 25$)	CFE ($n = 13$)	SIMFE ($n = 5$)
Average age seizure onset (months)	5.5	6	9.5	8	4
Clinical features					
Hemiclonic and/or generalized convulsions	Always	Always	Often	Often	Often
Myoclonic seizures	Always	Often	Often	Occasional	Often
Other focal seizures	Often	Often	Occasional	Always	Always
Other generalized seizures	Often	Often	Often	Occasional	Rare
EEG					
Generalized spike wave	Always	Occasional	Often	Rare	No
Multifocal epileptiform activity	Occasional	Occasional	Occasional	Occasional	Always
SCN1A mutations	52 (79%)	25 (69%)	6 (24%)	1 (8%)	3 (60%)
Truncation	23	10	–	–	1
Missense	20	13	5	1	2
Splice site	9	2	1	–	–

SMEI, severe myoclonic epilepsy of infancy; SMEB, SMEI borderland; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; SIMFE, severe infantile multifocal epilepsy.

such as tuberous sclerosis and birth asphyxia. In contrast, SIMFE encompasses those patients hitherto without a known cause, with 3/5 found to have mutations of *SCN1A*. SIMFE is an important group of patients with a devastating epileptic encephalopathy who are presently difficult to classify. The discovery of *SCN1A* mutations as the basis of their disorder avoids further potentially invasive investigations for alternative causes and assists in targeting therapy. For example, anti-epileptic drugs that exacerbate myoclonic seizures, such as vigabatrin and tiagabine, should be avoided.

This extensive study of the role of *SCN1A* mutations in epileptic encephalopathies beginning in the first year of life has, not surprisingly, expanded the phenotypic spectrum. Disorders are initially identified in a 'pure cohort' with a specific group of essential features. As the molecular basis is determined, phenotype–genotype correlation results in broadening of the phenotypic spectrum to include milder cases or seemingly unrelated disorders. This is just beginning to be possible in epileptology, as *SCN1A* is the first gene shown to have a role in epilepsies previously regarded as cryptogenic. An important finding is that children with an epileptic encephalopathy with multifocal features in the setting of normal MRI may have *SCN1A* mutations as may children with cryptogenic generalized epilepsy. A strong indicator for *SCN1A* analysis is an epileptic encephalopathy with seizure onset before 1 year of age, even if cognitive decline does not occur for several years thereafter. The social and economic benefit in making a definitive diagnosis in children with epileptic encephalopathies cannot be underestimated. Neurologists continue to perform investigations looking for an aetiology in children with cryptogenic encephalopathies such that establishing a definitive molecular diagnosis is cost-effective. More importantly, families are very grateful for a specific diagnosis especially with the treatment and genetic counselling implications that a *SCN1A* mutation carries.

Supplementary material

Supplementary material is available at *Brain* Online.

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TABLE 2: *SCN1A* mutations in patients with epileptic encephalopathies

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
1	SMEI	M	4.5	Exon 1	c.41delT	F14fsX91	N-terminal	*De novo*	Novel
2	SMEI	F	4	Exon 1	c.251A>G	Y84C	N-terminal	*De novo*	Novel
				Intron 9	*c.1378-1G>A*	*IVS9-1G>A*	*DI-DII linker*	*Maternal*	*Novel - non pathogenic*
3	SMEI	F	7	Intron 1	c.265-1G>A	IVS1-1G>A	N-terminal	*De novo*	Novel
4	SMEI	F	6	Exon 2	c.301C>T	R101W	N-terminal	*De novo*	Novel - this study [SMEB-SW]
5	SMEI	M	5	Exon 2	c.302G>A	R101Q	N-terminal	*De novo*	Fukuma et al., 2004 [SMEB]
6	SMEI	F	4	Intron 3	c.474-13T>A	IVS3-13T>A	DIS2	ND	Novel
				Intron 4	*c.602+5G>A*	*IVS4+5G>A*	*DIS3*	*ND*	*Novel - non pathogenic*
7	SMEI	F	5.5	Exon 4	c.495_496insGTGAATC	T166fsX170	DIS2	*De novo*	Novel
8*	SMEI	F	5	Intron 4	c.602+1G>A	IVS4+1G>A	DIS3	*De novo*	Fujiwara et al., 2003 [SMEI] This study [SMEB]
9	SMEI	F	3	Intron 7	c.1028+1G>T	IVS7+1G>T	DIS5-S6 loop	*De novo*	Novel - this study [SMEI]
10	SMEI	M	3	Intron 7	c.1028+1G>T	IVS7+1G>T	DIS5-S6 loop	*De novo*	Novel - this study [SMEI]
11	SMEI	F	2.25	Exon 8	c.1048_1049delAT	M350fsX355	DIS5-S6 loop	*De novo*	Novel
12*	SMEI		6	Exon 8	c.1055_1056delTG	V352fsX355	DIS5-S6 loop	*De novo*	Novel
13*	SMEI	M	4	Exon 9	c.1197C>A	Y399X	DIS5-S6 loop	*De novo*	Novel
14	SMEI	F	4	Exon 9	c.1207T>C	F403L	DIS6	*De novo*	Novel
15	SMEI	F	7	Exon 9	c.1237T>A	Y413N	DIS6	*De novo*	Novel
16	SMEI	F	3.5	Exon 10	c.1639_1640delAA	K547fsX569	DI-DII linker	ND	Novel
17*	SMEI	F	3	Intron 10	c.1662+2T>C	IVS10+2T>C	DI-DII linker	*De novo*	Novel
18*	SMEI	M	9.5	Intron 10	c.1663-1G>C	IVS10-1G>C	DI-DII linker	*De novo*	Novel
19	SMEI	F	5	Exon 11	c.1687delC	L563fsX622	DI-DII linker	*De novo*	Novel
20	SMEI	M	4	Exon 11	c.2021A>G	D674G (long isoform)	DI-DII linker	*De novo*	Novel
21	SMEI	M	6	Exon 13	c.2348T>C	L783P	DIIS1	*De novo*	Novel
22*	SMEI	F	8	Exon 14	c.2562delA	G854fsX876	DIIS3-S4 loop	*De novo*	Novel
23*	SMEI	F	4	Intron 14	c.2589+3A>T	IVS14+3A>T	DIIS4	*De novo*	Novel
24	SMEI	M	7	Exon 15	c.2831T>A	V944E	DIIS5-S6 loop	*De novo*	Novel
25*	SMEI	F	6	Exon 15	c.2833T>C	F945L	DIIS5-S6 loop	ND	Novel

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
26	SMEI	M	2.5	Exon 15	c.2837G>A	R946H	DIIS5-S6 loop	*De novo*	Fukuma et al., 2004 [SMEB]
27*	SMEI	F	4	Exon 15	c.2849G>A	G950E	DIIS5-S6 loop	*De novo*	Novel
28	SMEI	F	6	Exon 15	c.2893C>T	Q965X	DIIS5-S6 loop	ND	Novel
29	SMEI	M	3.5	Exon 16	c.3096delA	E1032fsX1045	DII-DIII linker	*De novo*	Novel
30	SMEI	M	7	Exon 17	c.3462delT	G1154fsX1163	DII-DIII linker	*De novo*	Novel
31	SMEI	F	5	Exon 18	c.3561_3562delAA	Q1187fsX1215	DII-DIII linker	*De novo*	Novel
32	SMEI	M	3	Exon 19	c.3714A>C	E1238D	DIIIS1-S2 loop	ND	Novel
				Intron 14	*c.2589+1G>C*	*IVS14+1G>C*	*DIIS4*	*ND*	*Novel - non pathogenic*
33	SMEI	F	7	Exon 19	c.3733C>T	R1245X	DIIIS1-S2 loop	ND	Nabbout et al., 2003 [2x SMEI]
34	SMEI	F	7	Exon 21	c.4219C>T	R1407X	DIIIS5-S6 loop	*De novo*	Sugawara et al., 2002 [SMEI]
									Fukuma et al., 2004 [SMEI]
35	SMEI		6	Exon 22	c.4321G>C	A1441P	DIIIS5-S6 loop	*De novo*	Novel
36	SMEI	M	2	Exon 24	c.4526delA	N1509fsX1511	DIII-DIV linker	*De novo*	Novel
37*	SMEI	F	4.5	Exon 24	c.4547C>A	S1516X	DIII-DIV linker	*De novo*	Sugawara et al., 2002 [2x SMEI]
38*	SMEI	F	8.5	Exon 24	c.4573C>T	R1525X	DIII-DIV linker	ND	Kearney et al., 2006 [SMEI]
									This study [SMEI]
				Exon 11	*c.1811G>A*	*R604H*	*DI-DII linker*	*ND*	*Novel - non pathogenic*
39	SMEI	F	5	Exon 24	c.4573C>T	R1525X	DIII-DIV linker	ND	Kearney et al., 2006 [SMEI]
									This study [SMEI]
40	SMEI	F	8	Exon 25	c.4633A>G	I1545V	DIVS1	*De novo*	Novel
41	SMEI	M	4	Exon 25	c.4794T>A	Y1598X	DIVS2-S3 loop	*De novo*	Novel
42	SMEI	M	9	Intron 25	c.4853-14T>G	IVS25-14T>G	DIVS3	*De novo*	Novel
43	SMEI	M	4	Exon 26	c.4933C>T	R1645X	DIVS4	*De novo*	Fukuma et al., 2004 [SMEI]
44	SMEI	F	6	Exon 26	c.4934G>A	R1645Q	DIVS4	*De novo*	Novel
45	SMEI	M	3	Exon 26	c.5119T>G	F1707V	DIVS5-S6 loop	ND	Novel
46	SMEI	M	4	Exon 26	c.5162C>G	T1721R	DIVS5-S6 loop	*De novo*	Novel
47*	SMEI	M	9	Exon 26	c.5176T>C	W1726R	DIVS5-S6 loop	*De novo*	Novel
48	SMEI	F	4	Exon 26	c.5347G>A	A1783T	DIVS6	*De novo*	Novel - this study [SMEB-SW]
49	SMEI	F	6	Exon 26	c.5436G>A	W1812X	C-terminal	*De novo*	Novel
50*	SMEI	F	4	Exon 26	c.5656C>T	R1886X	C-terminal	*De novo*	Mancardi et al., 2006 [SMEI]

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
51*	SMEI	M	6	Exon 26	c.5710C>T	Q1904X	C-terminal	*De novo*	Novel
52	SMEI	M	5	Exon 26	c.5765T>C	I1922T	C-terminal	ND	Novel
53	SMEB-O	M	3	Exon 1	c.235G>C	D79H	N-terminal	*De novo*	Novel
54	SMEB-SW	F	8	Exon 2	c.301C>T	R101W	N-terminal	*De novo*	Novel - this study [SMEI]
55	SMEB-SW	M	7	Exon 4	c.512T>A	I171K	DIS2	*De novo*	Novel
56	SMEB-O	F	6	Exon 4	c.523G>A	A175T	DIS2	*De novo*	Novel
57	SMEB-O	F	3	Exon 4	c.580G>A	D194N	DIS3	ND	Mancardi et al., 2006 [SMEI]
58	SMEB-SW	F	7	Exon 4	c.596C>G	T199R	DIS3	*De novo*	Novel
59*	SMEB-O	M	4	Intron 4	c.602+1G>A	IVS4+1G>A	DIS3	*De novo*	Fujiwara et al., 2003 [SMEI] Marini et al. 2006 [SMEI] This study [SMEI]
60	SMEB-O	F	6	Exon 5	c.664C>T	R222X	DIS4	*De novo*	Claes et al., 2001 [SMEI] Nabbout et al., 2003 [2x SMEI]
61	SMEB-O	F	6.5	Exon 5	c.677C>T	T226M	DIS4	*De novo*	Novel - this study [CGE]
62	SMEB-SW	M	6	Exon 5	c.680T>G	I227S	DIS4	ND	Nabbout et al., 2003 [3x SMEI]
63	SMEB-SW	M	6	Exon 6	c.715G>A	A239T	DIS4-S5 loop	Paternal	Novel
64	SMEB-O	F	6	Exon 8	c.1152G>A	W384X	DIS5-S6 loop	ND	Novel
65	SMEB-SW	M	7	Intron 14	c.2589+2T>A	IVS14+2T>A	DIIS4	*De novo*	Novel
66	SMEB-SW	M	5.5	Exon 16	c.3022G>T	E1008X	DII-DIII linker	*De novo*	Novel
67	SMEB-O	F	3	Exon 16	c.3231delA	K1077fsX1079	DII-DIII linker	*De novo*	Novel
68	SMEB-M	F	5	Exon 21	c.4062delT	C1354fsX1359	DIIIS5	*De novo*	Novel
69*	SMEB-M	M	9	Exon 21	c.4168G>A	V1390M	DIIIS5-S6 loop	*De novo*	Ohmori et al., 2002 [SMEI]
70	SMEB-SW	F	6	Exon 21	c.4186T>G	C1396G	DIIIS5-S6 loop	*De novo*	Novel
71	SMEB-SW	F	6.5	Exon 21	c.4279C>T	Q1427X	DIIIS5-S6 loop	*De novo*	Novel
72	SMEB-M	F	4.5	Exon 26	c.4949_4950insT	I1650fsX1672	DIVS4	*De novo*	Novel
73	SMEB-O	F	4	Exon 26	c.5339T>C	M1780T	DIVS6	*De novo*	Nabbout et al., 2003 [SMEI]
74	SMEB-SW	F	10	Exon 26	c.5347G>A	A1783T	DIVS6	*De novo*	Novel - this study [SMEI]
75*	SMEB-SW	F	4	Exon 26	c.5536_5539delAAAC	K1846fsX1856	C-terminal	*De novo*	Claes et al., 2001 [SMEI] Wallace et al., 2003 [SMEI] Kearney et al., 2006 [SMEI]

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
76	SMEB-O	F	3	Exon 26	c.5674C>T	R1892X	C-terminal	*De novo*	Sugawara et al., 2002 [SMEI] Fukuma et al., 2004 [2x SMEI]
77	ICEGTC	F	7	Exon 26	c.5741_5742delAA	Q1914fsX1943	C-terminal	*De novo*	Novel
78	CGE	M	2	Exon 5	c.677C>T	T226M	DIS4	*De novo*	Novel - this study [SMEB]
79	CGE	M	5.5	Exon 9	c.1183G>C	A395P	DIS5-S6 loop	*De novo*	Novel
80	CGE	M	1.5	Exon 9	c.1265T>A	V422E	DIS6	*De novo*	Novel
81	CGE	M	12	Exon 11	c.1876A>G	S626G	DI-DII linker	ND	Novel
82	CGE	F	9	Exon 15	c.2917A>G	M973V	DIIS6	Paternal	Novel
83	CGE	F	6	Intron 15	c.2946+1G>T	IVS15+1G>T	DIIS6	*De novo*	Novel
84	CFE (SIMFE)	F	7	Exon 11	c.1724delT	F575fsX622	DI-DII linker	*De novo*	Novel
85	CFE (SIMFE)	F	4.5	Exon 25	c.4628T>C	F1543S	DIVS1	Maternal	Novel
86*	CFE (SIMFE)	F	5	Exon 25	c.4786C>T	R1596C	DIVS2-S3 loop	*De novo*	Novel
87*	CFE	M	18	Exon 26	c.4970G>A	R1657H	DIVS4	*De novo*	Novel
88	LGS	M	0.75	Exon 26	c.4907G>A	R1636Q	DIVS4	*De novo*	Novel
89*	MAE	M	4	Exon 9	c.1177C>T	R393C	DIS5-S6 loop	*De novo*	Novel
90	MAE	M	13	Exon 24	c.4439G>T	G1480V	DIIIS6	*De novo*	Novel

Novel mutations refer to those not previously reported, note that some novel mutations are recurrent in this cohort

* Patients sequenced by Athena Diagnostics

Mutations in italics were not considered pathogenic, see text for explanation, and are not included in figure 1.

SMEI = Severe Myoclonic Epilepsy of Infancy, SMEB-SW = SMEI Borderland without generalised spike wave, SMEB-M = SMEI Borderland without myoclonic seizures, SMEB-O = SMEI Borderland lacking more than 1 feature of SMEI, ICEGTC = Intractable Childhood Epilepsy with Generalised Tonic Clonic seizures, CGE = Cryptogenic Generalised Epilepsy,

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Lamotrigine and Seizure Aggravation in Severe Myoclonic Epilepsy

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Summary: *Purpose:* In severe myoclonic epilepsy of infancy (SME), multiple drug-resistant focal and generalized seizure types occur. Lamotrigine (LTG), found effective in many generalized and partial seizures, has been little used in severe childhood epilepsy syndromes with multiple seizure types. We studied the effects of LTG in SME.

Methods: Twenty-one patients with SME, aged 2–18 years, were treated with LTG, 20 in add-on and one in monotherapy. LTG was started at 0.2–2.5 mg/kg/day and increased to 2.5–12.5 mg/kg/day. For each seizure type, excluding atypical absences, >50% variations compared with the 2 months preceding LTG were considered indicators of response, also taking into account the degree of disability each seizure type produced.

Results: LTG induced worsening in 17 (80%) patients, no change in three, and improvement in one. There was >50% increase in convulsive seizures in eight (40%) of 20 patients. Myoclonic seizures worsened in six (33%) of 18 patients. Of five patients improving in at least one seizure type, four had concomitant worsening of more invalidating seizures. Clear-cut worsening appeared within 3 months in most patients but was insidious in some. LTG was suspended in 19 patients after 15 days–5 years (mean, 14 months) with consequent improvement in 18.

Conclusions: The pronounced seizure deterioration during LTG treatment was not attributable to the natural course of the disease and could be a direct effect of therapeutic LTG doses. LTG treatment seems inappropriate in SME. **Key Words:** Lamotrigine—Severe myoclonic epilepsy—Seizure worsening.

Severe myoclonic epilepsy (SME) in infants (1) is one of the most disabling epileptic syndromes. Seizures begin during the first year of life in previously normal children as generalized or unilateral attacks, facilitated by fever, and often occurring in the form of status epilepticus. Such seizures are followed later, between ages 1 and 4 years, by myoclonus, atypical absences, and complex partial seizures, accompanied in some children by clinical photosensitivity. Often coinciding with the onset of myoclonus, there is a slowing in psychomotor development, patients being variably mentally retarded from school age on. All seizure types are extremely resistant to drug treatment. Although SME is diagnosed only in ~1% of patients with epilepsy (2), the management of these patients is particularly time demanding and costly, as they undergo multiple periods of hospitalization and antiepileptic drug (AED) trials in which almost all combinations of available drugs are tried.

Lamotrigine (LTG) has proven to be effective in the treatment of many generalized and partial seizure types in both adults and children (3–5). Although LTG is currently used in various childhood epilepsy syndromes (4, 6,7), conclusive data on its efficacy in severe epilepsies with multiple seizure types are scanty. Add-on LTG management of Lennox–Gastaut syndrome and nonspecific forms of symptomatic generalized epilepsies has been evaluated, with several open studies (7–10) and one controlled study (11). Results appear to be favorable, as reflected in reduction of atonic, tonic, and atypical absence seizures. No data on the efficacy of LTG in SME are available to date.

After preliminary observations of seizure aggravation in four patients (12), we examined the results of LTG treatment in 21 patients with SME, the majority of whom had been treated with add-on in the framework of prospective studies including children affected by different types of severe epilepsies.

PATIENTS AND METHODS

Data were collected in three centers. Twenty-one patients with SME, aged 2–18 years (mean, 9 years 1

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month) were treated with LTG, 20 in add-on and one in monotherapy. All patients had poorly controlled epilepsy. Nineteen had two or more types of seizures, and two had one seizure type only (Table 1). All patients had been treated with various antiepileptic drugs (AEDs), singly or in various combinations, which had failed to achieve seizure control. LTG was added to the preexisting drug regimen, starting with a daily dosage of 0.2–2.5 mg/kg (the smaller starting dosages were used to minimize the risk of skin rashes, especially in VPA-treated patients) and increased to a maximum dose of 2.5–10 mg/kg/day according to type of comedication and clinical response. A twice-daily dosing regimen was used in all patients. AEDs used at the start of LTG treatment are shown in Table 1. Concurrent AEDs were adjusted depending on patients' response, but on account of the lack of positive clinical response, in most patients previous medication could not be tapered. One patient (patient 12) was enrolled in a prospective trial of LTG monotherapy that was continued for 1 year. Sixteen patients were included in prospective studies of add-on LTG treatment. Data for the remaining four patients were collected retrospectively. All patients underwent EEG during wakefulness and sleep while receiving LTG treatment. However, only in 12 of the 21 patients was one EEG performed within the 2 months preceding LTG treatment, and another, designed to be comparable, during treatment. Throughout the period of LTG treatment, patients were seen regularly every 1–3 months in the clinic for evaluation of clinical efficacy of the drugs and any adverse effects, through general questioning of parents and clinical examination. Seizures were recorded daily in calendars by the patients' relatives. Treatment was continued for as long as it was thought either to improve seizures or to have beneficial effects on patient well-being. LTG was discontinued by reducing the maintenance dose

TABLE 1. *Main clinical findings of 21 patients with severe myoclonic epilepsy and effects of LTG treatment*

Pat No. sex	Age at beginning of LTG	Age at follow-up	Seizure types before LTG	Associated AEDs	Max LTG dose (mg/kg/day	Improved seizures	Worsened seizures	Appearance of new seizures	Duration of LTG treatment	Follow-up after LTG discontinuation	Overall LTG effect
1/F	6 yr 5 mo.	7 yr 6 mo	CP, AA, UCl, Myocl	VPA, CZP	5	No	UCl	No	7 mo	6 mo	Aggravation
2/M	2 yr 8 mo	5 yr 7 m	GCl, SP, CP, SG	VPA, CLB	4.2	No	GCl	No	4 mo	2 yr 6 mo	Aggravation
3/F	2 yr	5 yr	GCl, Myocl, T	VPA, CZP, PGB	5	No	Myocl	No	4 mo	2 yr 8 mo	Aggravation
4/M	4 yr 2 mo	6 yr 3 mo	UCl, Cl, AA SP, Myocl	VPA	4.1	SP, Myocl	No	GTC	5 mo	1 yr 8 mo	Aggravation
5/M	5 yr 10 mo	7 yr 3 mo	GTC, AA, Myocl	VPA, CZP	10	No	Myocl	No	5 mo	1 yr	Aggravation
6/F	3 yr 10 mo	5 yr	SP, Myocl, AA, GTC	VPA, CZP	2.5	No	GTC	No	1 yr 2 mo	3 mo	Aggravation
7/F	10 yr 10 mo	12 yr	SP, SG, AA, GCl, GTC, T, Myocl	VPA, ESM, CZP	3.3	No	GCl	No	2 mo	1 yr	Aggravation
8/F	5 yr	7 yr	UCl, SG, AA, Myocl	GVG, CZP	10	No	GTC	No	7 mo	1 yr 7 mo	Aggravation
9/M	12 yr	15 yr	GTC, Myocl, AA	VPA, CZP	5	GTC, Myocl	No	Myocl-at.	1 yr 1 mo	2 yr	Tremor-Aggravation
10/F	3 yr 7 mo	5 yr 4 mo	GTC, AA, CP, Myocl	PB, CZP	7.5	No	Myocl	No	7 mo	1 yr 2 mo	Aggravation
11/M	9 yr 6 mo	11 yr	AA, Myocl, GCl, CP	VPA, CLB, PB, GVG	3	No	CP	No	0.5 mo	1 yr 6 mo	Aggravation
12/M	18 yr	22 yr	Myocl	No	5.5	No	Myocl	No	1 yr	3 yr	Aggravation
13/M	3 yr	4 yr 4 mo	Myocl, GTC	VPA, ESM	5	No	GTC	No	5 mo	1 yr	Aggravation
14/F	4 yr 9 mo	13 yr	GCl	PHT, CLB	5	No	No	No	1 yr 3 mo	7 yr	No change
15/M	10 yr 2 mo	12 yr	GCl, Myocl	VPA, GVG	4	No	No	No	1 yr	10 mo	No change
16/F	4 yr	6 yr 6 mo	GCl, GTC, Myocl	VPA, CLB, PGB	5.3	No	GCl	No	8 mo	1 yr 10 mo	Aggravation
17/M	4 yr 7 mo	7 yr 6 mo	GTC, Myocl, Myocl Ab	VPA, CZP	2.5	GTC	Myocl	Myocl status	2 yr 2 mo	9 mo	Aggravation
18/F	9 yr 10 mo	17 yr	GTC, CP, GCl, Myocl, SP	VPA, CLB	7.4	Cl	Myocl	No	5 yr	—	Aggravation
19/F	2 yr 3 mo	4 yr 6 mo	GTC, Myocl, GCl, AA, Unil	VPA, CZP, PGB	4	No	Unil	No	3 mo	2 yr	Aggravation
20/F	12 yr 3 mo	14 yr 6 mo	UCl, GTC	GVG, CZP, CLB	4.4	UCl, GTC	No	No	2 yr 3 mo	Still taking LTG	Improvement
21/F	5 yr	10 yr 5 mo	AA, Myocl, GTC, Myocl status	PB, CZP	12.5	No	No	No	5 yr 4 mo	Still taking LTG	No change

F, female, M, male, AA, atypical absences; at, atonic, Cl, clonic; CP, complex partial; GCl, generalized clonic; GTC, generalized tonic–clonic; Myocl, myoclonic; Myocl Ab, myoclonic absences; SG, secondarily generalized; SP, simple partial; T, tonic; UCl, unilateral clonic; Unil, unilateral; CLB, clobazam; CZP, clonazepam, ESM, ethosuximide; GVG, vigabatrin; LTG, lamotrigine; PGB, progabide; PHT, phenytoin; PB, phenobarbial; VPA, valproate.

by 25 mg/week or to 50% for 2 weeks and then to 25% for a further 2 weeks.

To assess the effects of LGT, seizure frequency was determined for the 2 months preceding LTG introduction and was compared with the frequency during LTG treatment. Variations >50% were taken as indicators of improvement or worsening. Because epilepsy syndromes with multiple seizure types may show different degrees of disability and varying response to treatment, we assessed the efficacy of LTG relative to each seizure type, excluding atypical absences, and expressed a global impression of seizure-related disability. In general, convulsive seizures were considered more severe than myoclonic seizures, which in turn were more severe than complex partial seizures. To estimate seizure-related disability, we also took the specific seizure characteristics of each patient into account, because extensive intra- and interindividual variation was observed in any given category. For example, prolonged clonic seizures followed by protracted postseizure sedation were found to be far more disabling than brief tonic–clonic seizures occurring during sleep, although both are convulsive seizures. Because clinical detection of subtle seizures such as atypical absences is arbitrary, especially in mentally retarded children, for the latter, we merely requested parents to provide us a global impression of the modification in total quantity of absences.

RESULTS

Results are summarizd in Table 1. LTG treatment produced worsening of the epileptic syndrome in 17 (80%) patients, resulted in no substantial change in three and in improvement in one. Table 2 shows the cumulative effect on the main seizure types. There was appearance of new seizure types in three patients. Analysis of the distribution of the various drugs administered as comedication in patients who showed no seizure exacerbation compared with those with worsening suggested that the aggravation was not attributable to a particular association with other AEDs.

TABLE 2. *Cumulative effects of LTG on the main seizure types (21 patients)*

Main seizure types		Worsening >50%	Improvement >50%	No change
Myoclonic	18	6	2	10
Absence	12	0	0	12
Gen Clonic	10	3	1	6
Gen TC	12	3	3	6
CP	5	1	0	4
SP	5	0	1	4
UCI	5	2	1	2

Gen, generalized; TC, tonic–clonic; CP, complex partial; SP, simple partial, UCI, unilateral clonic.

The most important finding was the >50% increase in the frequency of convulsive seizures (unilateral, generalized clonic, and generalized tonic–clonic) in eight (40%) of 20 patients. Myoclonic seizures also worsened in six (33%) of 18 patients, in one of whom a first episode of myoclonic status occurred after LTG administration. Although an improvement in at least one seizure type was found in five patients (patients 4, 9, 17, 18, and 20), four of them were considered to have had no improvement because there was a concomitant worsening of more disabling types of seizures (patients 4, 9, 17, and 18).

In most patients, seizure worsening appeared between 15 days and 3 months after the start of LTG treatment, occurring in three of them during titration. In some patients, however, worsening was slow and insidious, becoming evident over several months.

LTG was suspended in 19 patients after 15 days to 5 years (mean, 14 months) from its introduction. In 18 patients, seizures improved with reversal to levels preceding LTG treatment. In 14 of them, including the one receiving LTG monotherapy (patient 12), improvement occurred on LTG discontinuation, without introducing further medication, whereas in the remaining five, LTG was replaced by another drug. After LTG discontinuation, patient 12 was maintained drug free for 1 year because the parents were skeptical about the usefulness of any treatment. Mean duration of follow-up after cessation of LTG treatment was 21 months (range, 1 month–7 years). The parents of four children who had improved or unchanged seizures claimed to have noticed an improvement in their child's behavior and alertness (better contact, less irritable). For this reason, treatment was continued in some of the unchanged patients (14, 15, and 21) at their parents' request, even after a lack of efficacy on seizures had been observed.

Of the 12 patients who underwent EEG in the 2 months before and during LTG therapy, three (patients 6, 7, and 17) showed increase in interictal paroxysmal EEG abnormalities, two (patients 1 and 16), a moderate slowing in background EEG activity; six (patients 8–13) showed no change, and one, an improvement, with marked reduction of interictal paroxysmal activity (patient 20).

DISCUSSION

Although pediatric experience with LTG is still limited, this drug has been reported to be effective in controlling generalized and partial seizures in childhood and to be of particular value in the management of absence seizures (7).

The poor results we observed in SME, with seizure worsening in 80% of patients, have no easy explanation. Severe childhood epileptic syndromes are particularly

prone to AED-induced seizure aggravation (13,14), through mechanisms that are as yet poorly understood. Although the majority of reports referred to West and Lennox–Gastaut syndrome, aggravation of generalized convulsive seizures in SME also has been reported with CBZ treatment (15,16).

Exacerbation of seizure frequency has been reported in some studies involving LTG in children (4,7), although no mention was made as to whether this adverse effect involved a particular seizure type or a specific syndrome. However, in these studies, the number of patients who worsened did not exceed 11%, a rate of worsening no higher than that observed in patients with drug-resistant seizures after addition of placebo (17).

Although periods of spontaneous improvement or worsening are quite commonly observed in severe epilepsies (18), especially in children, the rate of deterioration we recorded during LTG treatment was too pronounced to be attributed to the natural course of the disease in most patients. On the other hand, deterioration could not be ascribed to concurrent tapering of previous medication or to a particular combination of LTG with other drugs. It appears, therefore, that deterioration of convulsive and myoclonic seizures in SME should be related to a direct effect of LTG administered at therapeutic doses.

Although in three patients, severe worsening occurred during LTG titration, increase in seizures was insidious rather than showing the sudden onset that generally occurs after inappropriate drug choice or paradoxic reaction (13,14). This may have been due to the slow increase in LTG dose we adopted at the beginning of treatment.

The few available data on the effect of LTG in epilepsies with predominant myoclonic seizures have suggested that lack of efficacy or worsening are found in a substantial proportion of both adult and pediatric patients (7,19,20). However, in these studies, the type of myoclonic epilepsy is infrequently specified. On the other hand, LTG can be effective in myoclonus associated with typical 3-Hz spike-and-wave discharges of myoclonic astatic epilepsy, juvenile myoclonic epilepsy, and myoclonic absences (21,22).

A characteristic of SME is the multiplicity of seizure types observed, with multiple brain areas appearing able to generate seizures. In this syndrome, it has been suggested that myoclonic seizures would seem to be more an expression of local than of generalized epileptogenesis (23). Yet the known efficacy of LTG in convulsive seizures (24) makes the deterioration we observed in this type of seizure particularly surprising. SME is a neurobiologically unitary syndrome, as testified by its cryptogenicity, low convulsive threshold, homogeneous clinical presentation, and strong family history of epilepsy (25–64%; 25–29) with several affected siblings including monozygotic twins (1,30,31). Multifocal microdysgenesis of cerebral and cerebellar cortex, observed by Renier and Renkawek (23) in one autopsied case, could be the neuropathologic substrate for this syndrome. Although such structural changes need further confirmation, they could account for the high epileptogenicity.

LTG is reported to be effective in the treatment of both typical and atypical absence seizures (9,32,33). Even though these reports have not been supported by quantification of seizures with EEG monitoring, the amount of clinical evidence gathered so far seems to indicate that LTG is a powerful drug against absence seizures of epilepsies with abundant spike–wave activity. In SME, generalized spike and wave activity, although present in almost all patients, is scanty. Atypical absences were described by the parents of our patients as showing no substantial change in frequency with LTG. However, because assessment of atypical absences in our study was performed without prolonged EEG monitoring, we do not believe our experience can provide any reliable information concerning the effects of LTG on this type of seizure in SME.

Although results of medical treatment are in general disappointing in SME (34), VPA and BZDs are preferable to other drugs. In these patients, PHT offers no obvious advantage and may produce more severe side effects than PB (1,2). CBZ may worsen myoclonus and atypical absence seizures (35,36). ESM may be helpful in reducing myoclonus. Among the new AEDs, VGB may lead to interesting results, reducing convulsive seizures, but only in older patients in whom myoclonus is no longer a prominent symptom (37).

The poor results obtained, with a marked tendency toward seizure aggravation, suggest that use of LTG is inappropriate in SME.

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2008-000025

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

* Amended *

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

DATE OF AUTOPSY: 1/6/2008 09:30

REQUESTOR: GARY M WATTS

PROSECTOR: CLAY A. NICHOLS MD

FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

CAUSE OF DEATH: **SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).**

CAN:krh 3-13-08

Electronically Signed Out By Clay A Nichols MD

2008-000025

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

NAME: MILLARE, CHRISTIAN
DOB: 8/23/2005 (Age: 2)
SEX: M
HOSP #: 999A08010
DATE OF DEATH: 1/5/2008
PROVISIONAL REPORTED: 1/7/2008

AUTOPSY #: A08-10
RACE: Caucasian

DATE OF AUTOPSY: 1/6/2008

REQUESTOR: GARY M WATTS
PROSECTOR: CLAY A. NICHOLS MD

PROVISIONAL ANATOMICAL DIAGNOSIS

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

IV. TOXICOLOGY PENDING.

CAUSE OF DEATH SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).

CAN:jh01/07/07

Electronically Signed Out By CLAY A. NICHOLS MD

TOXICOLOGY (SLED): No blood ethanol is identified. Carbamazepine is identified in blood at a level of 1.6 mg/L.

CAN:krh 3-13-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

Amendments

Amended: 3/13/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 1/8/2008

Amended: 4/10/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 3/14/2008

2008-000025

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

* Amended *

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

DATE OF AUTOPSY: 1/6/2008 09:30

REQUESTOR: GARY M WATTS

PROSECTOR: CLAY A. NICHOLS MD

FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

CAUSE OF DEATH: SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).

CAN:krh 3-13-08

Electronically Signed Out By Clay A Nichols MD

TOXICOLOGY (SLED): No blood ethanol is identified. Carbamazepine is identified in blood at a level of 1.6 mg/L.

CAN:krh 3-13-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

Amendments

Amended: 3/13/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 1/8/2008

2008-UCCO25

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

DATE OF AUTOPSY: 1/6/2008 09:30

REQUESTOR: GARY M WATTS

PROSECTOR: CLAY A. NICHOLS MD

FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

IV. TOXICOLOGY PENDING.

CAUSE OF DEATH SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).

CAN:krh 1-8-08

Electronically Signed Out By Clay A Nichols MD

CLINICAL SUMMARY:

This 2-year-old white male was found unresponsive at his residence on the morning of January 5, 2008. The deceased was found half on and half off of his bed and without evidence of trauma. He was transported to a local emergency room where resuscitative efforts were unsuccessful and death was declared at 9:49 a.m. The deceased has a past history of a seizure disorder caused by a mitochondrial disorder (type not specified). There is no suspicion of foul play from the scene investigation. The history is provided by David Burns of the Richland County Coroner's Office. The Richland County Coroner's Office authorized the autopsy.

CAN:jh01/07/08

GROSS ANATOMICAL DESCRIPTION:

EXTERNAL DESCRIPTION: This is the body of a well-developed, well-nourished white male child appearing compatible with the stated age. The height is 37 inches and the weight is estimated at ~ 40 pounds. The rigor is full and generalized. Purple lividity is posterior and fixed. The body is identified by the coroner's office. The head is covered by light brown hair. The head and face are normally formed and atraumatic. The pupils are equal at 0.2 inch each. The irides are blue. No petechial hemorrhage is identified. There is no evidence of trauma to the nose, mouth or ears. The neck is free of trauma or masses. The chest, abdomen and posterior aspects are appropriately developed and free of trauma. The genitalia are those of a normal male child. There is no evidence of trauma or discharge to the genitals or anus. The upper and lower extremities are appropriately developed and free of trauma.

TOTAL BODY X-RAYS do not identify any evidence of fracture.

EVIDENCE OF MEDICAL INTERVENTION: An endotracheal tube is in place. EKG monitor pads and pacing pads are on the anterior chest. Multiple needle puncture marks are on the arms and ankle. An interosseous catheter is in the right shin.

INTERNAL DESCRIPTION: The body is opened with a standard Y-shaped incision revealing all organs in their normal anatomic relationship. Dissecting through the soft tissues of the chest and neck reveals no evidence of trauma or contusion. Removal of the chest plate reveals unremarkable bilateral chest cavities. Opening the abdominal cavity reveals all organs in their normal anatomic relationship and no evidence of excess fluid accumulation.

CARDIOVASCULAR: The heart weighs 60 grams. The pericardial surface is smooth without adhesions. There is a normal amount of epicardial fat within a normal distribution. The coronary arteries are of normal distribution and demonstrate no atherosclerotic vascular disease. Opening the heart reveals normally formed heart valves without evidence of vegetations, incompetence or stenosis. The papillary muscles are well formed. The myocardium is red brown without evidence of hemorrhage or fibrosis. The vessels arise from the heart in a normal fashion.

RESPIRATORY: There is no obstruction of the upper airway. The larynx, epiglottis and trachea are within normal limits. The trachea divides into the major bronchi in a normal fashion. The right lung weighs 70 grams and the left lung weighs 50 grams. The bronchi divide in a normal fashion through the lung. Examination of the pulmonary vasculature reveals no evidence of embolus or fibrosis of the perivascular bed. There is no evidence of an infectious process of the lungs.

LIVER AND BILIARY: The liver weighs 660 grams. The capsular surface is smooth and glistening. Sectioning the liver reveals a uniform red brown parenchyma without evidence of fibrosis, cirrhosis or neoplasia. The cut surfaces of the liver are smooth. The hepatic vasculature is within normal limits and the biliary tree is grossly unremarkable. The gallbladder demonstrates mild autolysis. Cholelithiasis is not present.

RENAL AND URINARY: The right kidney weighs 50 grams, the left kidney weighs 30 grams. Both

have a normal amount of perirenal fat. The capsules of the kidneys strip easily away revealing smooth cortical surfaces. The cortices and medullae are within normal limits and the corticomedullary junctions are distinct. The renal papillae are well-developed without blunting or necrosis. The renal pelves drain freely to the ureters which are unobstructed to the bladder.

GASTROINTESTINAL: The esophagus is covered with a steel gray mucosa without evidence of ulceration or varices. The esophagogastric junction is distinct without evidence of tearing, ulceration or dysplasia. The stomach has normal rugal folds and autolyzed mucosa without evidence of ulceration or thickening. The stomach contains approximately two ounces of partially digested food. The lower gastrointestinal tract is intact and otherwise unremarkable. An appendix is present.

SPLEEN: The spleen is covered with steel gray slightly wrinkled capsule. The spleen weighs 90 grams. Sectioning the spleen reveals a mulberry red interior with a normal distribution of white and red pulp.

PANCREAS: The pancreas is unremarkable in size, weight or configuration. There is no evidence of peripancreatic cyst, pseudocyst, hemorrhage, calcification or fibrosis.

LYMPHATICS: Unremarkable.

ENDOCRINE: The bilobed thyroid is deep, red brown and within normal limits. There is no evidence of abnormality in the pituitary. The adrenals demonstrate normal cortices and medullae without evidence of neoplasm.

BRAIN: The scalp is reflected revealing no evidence of trauma or contusion. There is no evidence of a skull fracture. Removal of the calvarium reveals no evidence of a subarachnoid, subdural or epidural hemorrhage. The sulci and gyri are normally formed. The cerebral vessels are of normal distribution and without evidence of berry aneurysm formation. Dissecting of the brain reveals a normal distribution of white and gray matter without evidence of hemorrhage or infarction. There is no evidence of a disease process in the mid brain, cerebellum or the cerebrum. The brain weighs 1300 grams.

NECK ORGANS: A bite mark is identified on the right lateral tongue. The hyoid bone is intact. There is no evidence of laryngeal trauma.

TOXICOLOGY: Blood is obtained. Portions of liver, brain, kidney and pulled head hair are retained. A DNA card is obtained. All tissues not retained for diagnostic purposes are returned to the body.

CASSETTE SUMMARY:

1. Heart.
2. Left lung, kidney and adrenal.
3. Right lung, kidney and adrenal.
4. Liver, spleen, pancreas and thyroid.
5. Brain, thymus, skeletal muscle and bit mark right lateral tongue.

CAN:jh01/07/08

MICROSCOPIC DESCRIPTION:

Examination of sample tissues demonstrates varying degrees of congestion and autolysis and are otherwise noncontributory.

TOXICOLOGY: This report is being issued without benefit of a final toxicology report. When a report becomes available, an addendum will be issued.

SUMMARY & COMMENT: This unfortunate child as a result of a seizure due to complications of mitochondrial disorder. The pertinent anatomic findings are summarized under "Final Anatomic Diagnoses".

CAN:krh 1-8-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

STATE OF SOUTH CAROLINA)
)
COUNTY OF RICHLAND)

In the Matter of the Estate for Christian Jacob Millare,

Amy Elizabeth Williams,
Personal Representative.

AFFIDAVIT OF
MAX WIZNITZER, M.D.

Personally appeared before me, the undersigned notary public, Max Wiznitzer, M.D. who being first duly sworn deposes and states as follows:

1. I am a pediatric neurologist at the Rainbow Babies & Children's Hospital, board-certified by the American Board of Pediatrics in Pediatrics and board-certified by the American Board of Psychiatry and Neurology both in Neurology, with special qualification in Child Neurology, and in Neurodevelopmental Disabilities. In clinical practice, I commonly treat patients with seizure disorders, including Dravet Syndrome. In addition, I am a Professor of Pediatrics & Neurology at Case Western Reserve University.

2. I have received and reviewed the SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc. on June 30, 2007 (Exhibit 1), along with a second revised report issued on January 30, 2015 (Exhibit 2). Additionally, I have received and reviewed the pertinent medical records pertaining to the decedent, Christian Jacob Millare.

The Decedent Suffered From SMEI:

3. Christian Millare's clinical history is consistent with the diagnosis of severe myoclonic epilepsy of infancy (SMEI or Dravet's syndrome) and clearly details the presence of the clinical evolution of his seizures.

4. In agreement with the revised SCN1A diagnostic report, issued by Athena Diagnostics, Inc. on January 30, 2015, the variant 1237T>A, Y413N is a disease-causing mutation, causally related to a clinical diagnosis of SMEI.

5. Specifically, the decedent's DNA mutation (1237T>A, Y413N) in the SCN1A gene not only possessed the characteristics expected of a disease-causing alteration, but it had also been correlated with a clinical presentation of SMEI in the medical literature.

(Exhibit 3)
Y413N = SMEI (Table 1 – Patient #9) [2006]
Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, Wirrell EC, Gill DS, Iona X, Mulley JC, Scheffer IE. De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a

retrospective study. The Lancet Neurology. 2006;5(6):488-92. Epub 2006/05/23. doi: 10.1016/s1474-4422(06)70446-x. PubMed PMID: 16713920.

(Exhibit 4)
Y413N = SMEI (Supplement Table 2 – Patient #15) [2007]
Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A, Sutherland G, Berkovic SF, Mulley JC, Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain : A Journal of Neurology. 2007;130(Pt 3):843-52. Epub 2007/03/10. doi: 10.1093/brain/awm002. PubMed PMID: 17347258.

6. It is my opinion, to a reasonable degree of medical certainty, the decedent suffered from SMEI secondary to a mutation in his SCN1A gene. This was the sole cause of his epilepsy condition.

Negligent Failure to Properly Diagnose SMEI And Treat Appropriately:

7. An individual with Dravet Syndrome has a significant chance of surviving into adulthood.

8. Sodium channel blocking medications, such as Lamotrigine (Lamictal) and Carbamazepine (Tegretol), have been reported in publications to worsen seizures (pronounced seizure deterioration not attributable to the natural course of the disease) in patients with Dravet Syndrome/SMEI. Specifically noting, that "once a diagnosis of SME (SMEI) has been established, CBZ (Carbamazepine) should not be administered." These publications include reference #11 on the decedent's SCN1A DNA sequencing diagnostic report issued by Athena Diagnostics, Inc (Exhibit 1).

(Exhibit 5)
[1998]
Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia. 1998;39(5):508-12. Epub 1998/05/22. PubMed PMID: 9596203.

(Exhibit 6)
[1986]
Horn CS, Ater SB, Hurst DL. Carbamazepine-exacerbated epilepsy in children and adolescents. PediatrNeurol 1986;2:340-5.
PubMed PMID: 3508708.

(Exhibit 7)
[1996]
Wakai S, It0 N, Sucoka H, Kawamoto Y, Hayasa H, Chiba S. Severe myoclonic epilepsy in infancy and carbamazepine. Eur J Pediatr 1996;155:724.
PubMed PMID: 8839737

9. The decedent's medical records reflect treatment with increasing doses of sodium channel blocking medications, including Carbamazepine (Tegretol), through the end of the child's life. The seizure patterns of the decedent, while being treated with sodium

channel blocking medications, were consistent with the conclusions detailed in Exhibits 5, 6 and 7.

10. The decedent lost his life on January 5, 2008; the cause of death was a seizure (Exhibit 8).

11. It is my opinion, to a reasonable degree of medical certainty, that if the decedent's SMEI condition had been properly diagnosed and had he received appropriate care for the treatment and management of SMEI, he would not have suffered the fatal seizure on January 5, 2008.

Sworn to before me this 23
Day of Feb, 2016.

Max Wiznitzer, M.D.

Notary Public – State of Ohio
My Commission Expires: 3-27-2017

2/23/16

ERICA MUNIAK, NOTARY PUBLIC
STATE OF OHIO
COMM. EXP. MAR. 27, 2017

athena diagnostics
200 Forest Street, 2nd Floor
Marlborough, MA 01752
(800) 394-4493 - (508) 756-2885

Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:	Accession Number: 07025148	
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000	
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date	
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007	
	Additional Reports to:	Report Date: 06/30/2007	

Interpretation

This individual possesses a DNA sequence variant or combination of variants in the *SCN1A* gene whose significance is unknown (missense variant of unknown significance). Testing of the biological parents is strongly recommended to resolve the uncertainty of these test results. Please refer to the Technical Results and Comments sections of this report for further information.

Technical Results

DNA Variant 1:	Transversion T > A
Nucleotide Position:	1237
Codon:	413
Amino Acid Change:	Tyrosine > Asparagine
Variant Type:	Variant of unknown significance (heterozygous)

No other abnormal DNA sequence variants were identified in the remainder of the coding sequence and intron/exon junction.

Comments

Most Significant result: Analysis of this individual's *SCN1A* gene identified a DNA sequence variant or combination of variants whose significance is unclear or unknown (variant(s) of unknown significance). Since these types of sequence variants are similar to those observed in both disease-associated mutations at other nucleotide positions and in benign polymorphisms, the nature of this variation precludes clear interpretation. These DNA sequence variants may or may not alter the functional aspects of the *SCN1A* gene and /or its protein product. While methodologically accurate, the results of this analysis cannot be definitively interpreted due to the absence of published studies correlating these variant(s) with clinical presentation and/or pathology. Therefore, based on this single analysis, it is not possible to conclude with any reasonable degree of clinical certainty at this time whether or not this variant is associated with the phenotype in question.

Possible outcomes– Although the clinical significance of this test result is not certain, several outcomes are possible:
Normal- the variant is a benign polymorphism that has not been previously detected or reported and it is very unlikely that this mutation is responsible for clinical symptoms or increased risk of disease.
Affected with less severe GEFS+ (generalized epilepsy with febrile seizures plus) - the variant is an unreported pathologic mutation that does not cause SMEI, but may be associated with less severe clinical symptoms.
Affected with SMEI- the variant is an unreported pathologic mutation and is therefore associated with the disease.

Other variants of less significance: This analysis may also have detected other types of sequence variants as listed in the Technical Results section, a common occurrence for an analysis of this scope. However, in the context of results reported, the presence of additional sequence variants is generally of reduced significance and does not modify the final interpretation of the test results. Benign polymorphisms, if identified, are considered normal and are not reported in the Technical Results section of this report, but are available upon request. Please

athena diagnostics
200 Forest Street, 2nd Floor
Marlborough, MA 01752
(800) 394-4493 - (508) 756-2885

Diagnosis Service Report

Patient: Christian Millare		Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Report to: John Shoffner, MD
Specimen Type: DNA		Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)		Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test		Atlanta, GA 30338	Accession Date: 05/03/2007
		Additional Reports to:	Report Date: 06/30/2007

consult the Glossary for a detailed explanation of "DNA Variant Type" if indicated in the Technical Results section of this report.

Parental testing and other follow up recommendations: Testing of the biological parents is strongly recommended (for no additional charge) to help resolve the uncertainty of this sequence variant's pathogenicity and the uncertainty of the predicted phenotype. Missense mutations causing the severe phenotypes associated with SCN1A mutations (SMEI or SMEB) are usually (>90%) de novo, meaning that the mutation arose in the affected individual, and is not detected in the biological parents.10 Missense mutations associated with the milder phenotypes (GEFS+) are usually (>95%) inherited from one of the biological parents.10 Consultation with Athena's test counselor (1-800-394-4493) is recommended prior to parental testing.

Because SCN1A mutations can be inherited, this individual's family members may be at risk for possessing or inheriting these mutations. Careful reconciliation of this molecular data with this individual's clinical and family history is highly recommended. Athena recommends genetic counseling for this individual and his or her family members, and consideration of parental testing. Please contact Athena Client Services at 1-800-394-4493 or visit www.athenadiagnostics.com for further information on parental testing. The attached requisition forms will facilitate the submission and processing of parental specimens for this testing service.

Background Information: SCN1A encodes for the neuron voltage-gated sodium channel alpha 1 subunit protein. Mutations in th SCN1A gene have been associated with several overlapping epilepsy syndromes ranging from severe to mild phenotypes (SMEI, SMEB, an GEFS+).[1-10] The severe phenotypes include SMEI, Severe Myoclonic Epilepsy of Infancy or Dravet syndrome, and SMEB (SMEI borderline with some, but not all, of the classical features of SMEI. GEFS+ Generalized Epilepsy with Febrile Seizures Plus, encompasses a range o phenotypes from febrile seizures to mild generalized epilepsies, and less commonly, includes severe epileptic encephalopathies. SMEI has a poor prognosis, including developmental delay and refractory seizures. Furthermore, a confirmed diagnosis of SMEI may significantly guid treatment decisions.[11]

SCN1A mutations fall broadly into two groups.[1-10] Truncation mutations that severely disrupt the gene are usually associated with sever phenotypes such as SMEI. Missense mutations are associated with a range of phenotypes from mild to severe. Most mutations that caus SMEI are de novo, or sporadic (arise in the affected individual rathe than being inherited)[3,5,10] an inheritance pattern that can be confirmed b testing of parents. Familial mutations are usually associated with mil phenotypes but can be seen in SMEI. It is noteworthy that non-penetran mutation carriers and variable expression of affected carriers have bee observed in these syndromes in some families. This suggests tha modifying factors influence the expression of disease, and indicates tha the molecular analysis must be carefully reconciled with the clinic presentation and family history.

Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:
Date of Birth: 08/23/2005	Sex: M
Social Security Number:	Report to: John Shoffner, MD
Specimen Type: DNA	Address: One Dunwoody Park
Test Category: Diagnostic (Symptomatic)	Suite 250
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	Additional Reports to:
Accession Number: 07025148	
Family Number/Kindred Number:	
Patient Number: 000000000	
Specimen Collection Date: NO Date	
Accession Date: 05/03/2007	
Report Date: 06/30/2007	

Limitations of analysis: Mutations not detected by this analysis may include large deletions and large insertions. Furthermore, this test does not detect potential pathogenic mutations in the promoter, 5' flanking untranslated, 3' untranslated and non-sequenced intronic regions of the gene. Mutations in patients exhibiting mosaicism may not be detectable by automated sequencing technology.

Other Testing Available: Other epilepsy syndromes may appear clinically similar to those caused by mutations in the SCN1A gene. Although the course of these diseases may vary, patients with a progressive myoclonus epilepsy share some similar features to syndromes caused by SCN1A mutations including myoclonic and tonic-clonic seizures, ataxia, and abnormal neurophychological development. Athena offers testing for three types of progressive myoclonus epilepsy, EPM1, Lafora disease, and MERRF, that can further assist in the diagnosis of your patient's symptoms.

Methods

Analysis of the *SCN1A* gene was performed by PCR amplification of highly purified genomic DNA, followed by automated uni-directional DNA sequencing of the 26 exons of the *SCN1A* gene, including the highly conserved exon-intron splice junctions (e.g. GT....AG) between all 26 coding exons.[1] All abnormal sequence variants were confirmed by bi-directional sequencing or alternative sequencing chemistry. Studies conducted by Athena Diagnostics, Inc. indicate that mutations in this gene and in similar sequencing tests are detectable at an overall analytic sensitivity approaching 99%. All test results are reviewed, interpreted, and reported by ABMG certified Clinical Molecular Geneticists.

Nucleotides and amino acids are numbered following the internationally accepted Nomenclature set forth by the Ad Hoc Committee on Mutation Nomenclature. The initiator codon, Methionine, is designated as codon number 1 in the cDNA. The "A" of the "ATG" initiator codon is designated as nucleotide +1.

Abbreviations used:
SCN1A (neuronal voltage-gated sodium channel alpha 1 subunit); SMEI (severe myoclonic epilepsy of infancy); SMEB (borderline SMEI); GEFS+ (generalized epilepsy with febrile seizures plus); DNA (deoxyribonucleic acid); PCR (polymerase chain reaction); A(Adenine); G(Guanine); C(Cytosine); T(Thymine).

This testing service is performed pursuant to a PCR license agreement with Roche Molecular Systems, Inc.

References

1. Escayg, A et al (2000) Nat Gen 24: 343-345
2. Wallace, RH et al (2001) Am J Hum Genet 68: 859-865
3. Claes, L et al (2001) Am J Hum Genet 68: 1327-1332
4. Wallace, RH et al (2003) Neurology 61: 765-769
5. Claes, L et al (2003) Hum Mut 21: 615-621
6. Fujiwara, T et al (2003) Brain 126: 531-546

Patient
Christian Millare

Date of Birth
08/23/2005

Sex
M

Social Security Number

Specimen Type
DNA

Test Category
Diagnostic (Symptomatic)

Test Requested
SCN1A DNA Sequencing Test

Requesting Physician

Report to
John Shoffner, MD

Address
One Dunwoody Park

Suite 250

Atlanta, GA 30338

Additional Reports to:

Diagnosis Service Report

Accession Number
07025148

Family Number/Control Number

Patient Number
000000000

Specimen Collection Date
NO Date

Accession Date
05/03/2007

Report Date
06/30/2007

7. Fujiwara, T et al (2004) Epilepsia 45: 140-148
8. Kanai, K et al (2004) Neurology 63: 329-334
9. Ceulemans, BPGM et al (2004) Pediatr Neurol 30: 236-243
10. Mulley, JC et al (pending) Hum Mut (Mutation update, submitted)
11. Guerrini R et al (1998) Epilepsia 39: 508-512

GLOSSARY

DNA sequence variants are deviations from the normal reference sequence of the gene(s) being analyzed. In dominant disorders, mutations must be found in only one allele of a gene to confirm the presence of the disease. However, it is not uncommon to have more than two types of DNA sequence variants detected in a gene. In addition, the clinical significance of individual variant types differs widely. The DNA variant types and additional terminology utilized in the report are explained below. (Consult the Technical Results and Comments section of this report to determine if any of the following apply to this individual.)

Variant Types:

1. **Known disease-associated mutations** (dominant) are documented in the literature to be associated with diseases inherited in a dominant manner. The individual is likely to be affected with, or predisposed to developing, a dominant genetic disease.

2. **Predicted disease-associated mutations** are expected to result in significant alteration of the structure and function of the protein encoded by the dominant gene. Typical examples include frame shift mutations, splicing mutations, nonsense mutations, and deletions or duplications of entire exons. Current literature indicates that DNA sequence variants of this type are associated with disease. However, due to the absence of established genotype-phenotype correlations for this specific DNA sequence variant, this result should be carefully reconciled with this individual's clinical and family history.

3. **Amino acid changes of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. The amino acid change is predicted based on simple interpretations of the genetic code. However, these same types of alterations may sometimes alter normal gene splicing and processing, and thereby cause more significant and unpredictable effects. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

4. **Variants of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. Typical examples include single nucleotide changes in the coding or non-

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Diagnosis Service Report

Patient: Christian Millare	Requesting Physician	Accession Number: 07025148	
Date of Birth: 08/23/2005	Sex: M	Social Security Number	Report to: John Shoffner, MD	Family Number/Kindred Number
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000	
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date	
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007	
	Additional Reports to:	Report Date: 06/30/2007	

coding regions of the gene that are sometimes labeled as "silent mutations" or "intronic polymorphisms." These types of alterations often have no effect, but may sometimes alter normal gene splicing and processing. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

5. Inconclusive test results are those unable to be interpreted as either negative or positive due to a technical problem in the assay, and thus can rule out neither the presence nor absence of abnormalities in these genes. Inconclusive results are typically resolved by analysis of a repeat specimen. There will be no charge for the repeat analysis. Please indicate "REPEAT SPECIMEN" along with the above Athena Accession Number on the requisition. If this test is part of a profile and a positive result was obtained for another gene, the submission of a repeat specimen may not be warranted.

6. Indeterminate test results, while methodologically accurate, are not clinically meaningful due to the lack of published clinical studies correlating test results in this specific category with clinical presentation and/or pathology. Due to the lack of published findings, these test results cannot be interpreted as either normal or abnormal. Indeterminate results are generally caused by test results that fall outside of the established interpretive criteria. Indeterminate test results are not resolved by analysis of a repeat specimen.

7. Benign polymorphisms are DNA sequence variants that have been shown to be present in unaffected control subjects, and are considered "benign" (non-pathogenic) sequence variants. If identified, these are considered normal variations and are not reported in the Technical Results section of this report, but are available upon request.

*** *FINAL REPORT* *** ver 1.0

This test was developed and its performance characteristics determined by Athena Diagnostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes and should not be regarded as investigational or for research only. Athena Diagnostics is licensed under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) to perform high complexity clinical testing. Athena Diagnostics has performed assay validation studies and has developed its laboratory protocols and operating procedures in consultation with experts in the field and in accordance with the standards of the National Committee on Clinical Laboratory Standards (NCCLS).

Laboratory results and submitted clinical information reviewed by,

Sat Dev Batish, PhD, FACMG
Chief Director, Genetics

Narasimhan Nagan, PhD, ABMG
Director, Genetics

Hui Zhu, PhD, ABMG
Director, Genetics

Laboratory oversight provided by Joseph J. Higgins, M.D., F.A.A.N., CLIA license holder, Athena Diagnostics (CLIA # 22D0069726)

Testing performed at:
Athena Diagnostics Four Biotech Park 377 Plantation St Worcester MA 01605

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Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:	Accession Number: 07025148
Date of Birth: 08/23/2005 · Sex: M · Social Security Number:	Report to: John Shoffner, MD	Family Number/Kindred Number:
Specimen Type: DNA	Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)	Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338	Accession Date: 05/03/2007
	Additional Reports to:	Report Date: 06/30/2007

Requisition for Parental Testing - Test Code 536

In order to provide a more comprehensive interpretation of this patient's SCN1A results, Athena Diagnostics is requesting samples from the biological parents of this patient. Athena will perform a target analysis on these samples for variant(s) identified in gene SCN1A only and use the findings to help interpret the patient's SCN1A result(s) at no additional charge. Please use this form as the requisition for parental testing on this patient. Enclose one completed form with each parent's sample and send to Athena. If you have any questions or require shipping kits, please contact Athena customer service at 1-800-394-4493,option 2.

Specimen Requirements: 1 lavendar tube (3.5ml) whole blood **Storage Conditions:** Refrigerate
Shipping Conditions: Room temperature, avoid freezing

Biological Mother Name: ____________	Biological Father Name: ____________
Date of Birth: ____________	Date of Birth: ____________
Address: ____________	Address: ____________
City,State,Zip: ____________	City,State,Zip: ____________
Phone: ____________	Phone: ____________
Specimen Collection Date: ____________	Specimen Collection Date: ____________
Indication for Testing: ________ UPIN: ________	Medical Practitioner Signature: ____________

Testing Authorization and ICD-9 Code: I warrant that this test is either: 1) for the purpose of diagnosing or detecting an existing disease, illness, impairment, symptom or disorder, or 2) that if it is not for such purpose, I have obtained the appropriate prior written consent. This written consent was signed by the person who is the subject of the test (or if that person lacks capacity to consent, signed by the person authorized to consent for that person), and includes: a) a statement of the purpose and description of the test; b) a statement that prior to signing the consent form, the consenting person discussed with the medical practitioner ordering the test the reliability of positive or negative test results and the level of certainty that a positive test result for that disease or condition serves as a predictor of such disease; c) a statement that the consenting person was informed about the availability and importance of further testing, physician consultation and genetic counseling, and provided with written information identifying a genetic counselor or medical geneticist from whom the consenting person might obtain such counseling; d) a general description of each specific disease or condition tested for; and e) the person or persons to whom the test results may be disclosed as indicated above. [illegible]-012 06/2014

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Diagnostic Service Report

Patient: Christian Millare	Requesting Physician:
Date of Birth: 08/23/2005	Sex: M
Social Security Number:	Report To: John Shoffner, MD
Specimen Type: DNA	Address: One Dunwoody Park
Test Category: Diagnostic (Symptomatic)	Suite 250
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338
	Additional Reports To:

Accession Number: 07025148
Family Number/Individual Number:
Patient Number: 000000000
Specimen Collection Date: NO Date
Accession Date: 05/03/2007
Report Date: 06/30/2007

****REVISED REPORT # 1****

CAUTION, Revised Report, disregard previous report.

****See Attached Original Report**

The variant of unknown clinical significance previously reported in the *SCN1A* gene of this patient has been classified as a disease-causing mutation. Please see the Technical Results section below for more information.

Interpretation

POSITIVE

This individual possesses a DNA sequence variant in the *SCN1A* gene that is a previously reported **disease-associated mutation.**[1-10] This test result is consistent with a diagnosis of, or a predisposition to developing, the severe phenotypes associated with *SCN1A* mutations, SMEI or SMEB.[1-10] Please refer to the Technical Results and Comments sections of this report for further information.

Technical Results

DNA Variant 1:Transversion T > A

Nucleotide Position:	1237
Codon:	413
Amino Acid Change:	Tyrosine > Asparagine
Variant Type:	Disease-associated mutation (heterozygous)

No other abnormal DNA sequence variants were identified in the remainder of the coding sequence and intron/exon junction.

Comments

Most Significant result: Analysis of this individual's *SCN1A* gene identified a DNA sequence variant that has been reported in the literature to be associated with SMEI or SMEB, the severe phenotypes associated with *SCN1A* mutations.[1-10] **(disease-associated mutation).** This test result is consistent with a diagnosis of, or a predisposition to developing, SMEI or SMEB.[1-10]

Other variants of less significance: This analysis may also have detected other types of sequence variants as listed in the Technical Results section, a common occurrence for an analysis of this scope. However, in the context of results reported, the presence of additional sequence variants is generally of reduced significance and does not modify the final interpretation of the test results. Benign polymorphisms, if identified, are considered normal and are not reported in the Technical Results section of this report, but are available upon request. Please consult the Glossary for a detailed explanation of "DNA Variant Type" if indicated in the Technical Results section of this report.

Parental testing and other follow up recommendations: Testing of the biological parents (for no additional charge) may help identify whether this sequence variant is *de novo* or inherited. Because *SCN1A* mutations can be inherited, this individual's family members may be at risk for

EXHIBIT 2
ALL-STATE LEGAL

Patient
Christian Millare

Date of Birth: 08/23/2005 | Sex: M | Social Security Number:

Specimen Type
DNA

Test Category
Diagnostic (Symptomatic)

Test Requested
SCN1A DNA Sequencing Test

Requesting Physician:

Report to
John Shoffner, MD

Address
One Dunwoody Park
Suite 250
Atlanta, GA 30338

Additional Reports to:

Diagnostic Service Report

Accession Number
07025148

Family ID# / Medical Record Number:

Patient Number
000000000

Specimen Collection Date
NO Date

Accession Date
05/03/2007

Report Date
06/30/2007

possessing or inheriting these mutations. Careful reconciliation of this molecular data with this individual's clinical and family history is highly recommended. Athena recommends genetic counseling for this individual and his or her family members, and consideration of parental testing. Please contact Athena Client Services at 1-800-394-4493 or visit www.athenadiagnostics.com for further information on parental testing. The attached requisition forms will facilitate the submission and processing of parental specimens for this testing service.

Background Information: SCN1A encodes for the neuronal voltage-gated sodium channel alpha 1 subunit protein. Mutations in the SCN1A gene have been associated with several overlapping epilepsy syndromes ranging from severe to mild phenotypes (SMEI, SMEB, and GEFS+).1-10 The severe phenotypes include SMEI, Severe Myoclonic Epilepsy of Infancy or Dravet syndrome, and SMEB (SMEI borderline) with some, but not all, of the classical features of SMEI. GEFS+, Generalized Epilepsy with Febrile Seizures Plus, encompasses a range of phenotypes from febrile seizures to mild generalized epilepsies, and less commonly, includes severe epileptic encephalopathies. SMEI has a poor prognosis, including developmental delay and refractory seizures. Furthermore, **a confirmed diagnosis of SMEI may significantly guide treatment decisions.**11

SCN1A mutations fall broadly into two groups.1-10 Truncation mutations that severely disrupt the gene are usually associated with severe phenotypes such as SMEI. Missense mutations are associated with a range of phenotypes from mild to severe. Most mutations that cause SMEI are de novo, or sporadic (arise in the affected individual rather than being inherited)3,5,10 an inheritance pattern that can be confirmed by testing of parents. Familial mutations are usually associated with mild phenotypes but can be seen in SMEI. It is noteworthy that non-penetrant mutation carriers and variable expression of affected carriers have been observed in these syndromes in some families. This suggests that modifying factors influence the expression of disease, and indicates that the molecular analysis must be carefully reconciled with the clinical presentation and family history.

Limitations of analysis: Mutations not detected by this analysis may include large deletions and large insertions. Furthermore, this test does not detect potential pathogenic mutations in the promoter, 5' flanking untranslated, 3' untranslated and non-sequenced intronic regions of the gene. Mutations in patients exhibiting mosaicism may not be detectable by automated sequencing technology.

Methods

Analysis of the *SCN1A* gene was performed by PCR amplification of highly purified genomic DNA, followed by automated uni-directional DNA sequencing of the 26 exons of the *SCN1A* gene, including the highly conserved exon-intron splice junctions (e.g. GT....AG) between all 26 coding exons.1 All abnormal sequence variants were confirmed by bi-directional sequencing or alternative sequencing chemistry. Studies conducted by Athena Diagnostics, Inc. indicate that mutations in this gene and in similar sequencing tests are detectable at an overall analytic sensitivity approaching 99%. All test results are reviewed, interpreted,

Diagnosis Service Report

Patient:
Christian Millare

Date of Birth: 08/23/2005
Sex: M
Social security number:

Specimen Type:
DNA

Test Category:
Diagnostic (Symptomatic)

Test Requested:
SCN1A DNA Sequencing Test

Requesting Physician:

Report To:
John Shoffner, MD

Address:
One Dunwoody Park
Suite 250
Atlanta, GA 30338

Additional Reports To:

Accession Number:
07025148

Family Number/Patient Number:
000000000

Specimen Collected On:
NO Date

Accession Date:
05/03/2007

Report Date:
06/30/2007

and reported by ABMG certified Clinical Molecular Geneticists.

Nucleotides and amino acids are numbered following the internationally accepted Nomenclature set forth by the Ad Hoc Committee on Mutation Nomenclature. The initiator codon, Methionine, is designated as codon number 1 in the cDNA. The "A" of the "ATG" initiator codon is designated as nucleotide +1.

Abbreviations used:
SCN1A (neuronal voltage-gated sodium channel alpha 1 subunit); SMEI (severe myoclonic epilepsy of infancy); SMEB (borderline SMEI); GEFS+ (generalized epilepsy with febrile seizures plus); DNA (deoxyribonucleic acid); PCR (polymerase chain reaction); A(Adenine); G(Guanine); C(Cytosine); T(Thymine).

This testing service is performed pursuant to a PCR license agreement with Roche Molecular Systems, Inc.

References

1. Escayg, A et al (2000) Nat Gen 24: 343-345
2. Wallace, RH et al (2001) Am J Hum Genet 68: 859-865
3. Claes, L et al (2001) Am J Hum Genet 68: 1327-1332
4. Wallace, RH et al (2003) Neurology 61: 765-769
5. Claes, L et al (2003) Hum Mut 21: 615-621
6. Fujiwara, T et al (2003) Brain 126: 531-546
7. Fujiwara, T et al (2004) Epilepsia 45: 140-148
8. Kanai, K et al (2004) Neurology 63: 329-334
9. Ceulemans, BPGM et al (2004) Pediatr Neurol 30: 236-243
10. Mulley, JC et al (pending) Hum Mut (Mutation update, submitted)
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GLOSSARY

DNA sequence variants are deviations from the normal reference sequence of the gene(s) being analyzed. In dominant disorders, mutations must be found in only one allele of a gene to confirm the presence of the disease. However, it is not uncommon to have more than two types of DNA sequence variants detected in a gene. In addition, the clinical significance of individual variant types differs widely. The DNA variant types and additional terminology utilized in the report are explained below. (Consult the Technical Results and Comments section of this report to determine if any of the following apply to this individual.)

Variant Types:

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2. **Predicted disease-associated mutations** are expected to result in significant alteration of the strucure and function of the protein encoded

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Diagnosis Service Report

Patient: Christian Millare	Referring Physician:		Accession Number: 07025148
Date of Birth: 08/23/2005	Sex: M	Social Security Number:	Report to: John Shoffner, MD	Family Number/Internal Number:
Specimen Type: DNA			Address: One Dunwoody Park	Patient Number: 000000000
Test Category: Diagnostic (Symptomatic)			Suite 250	Specimen Collection Date: NO Date
Test Requested: SCN1A DNA Sequencing Test			Atlanta, GA 30338	Accession Date: 05/03/2007
			Additional Reports to:	Report Date: 06/30/2007

by the dominant gene. Typical examples include frame shift mutations, splicing mutations, nonsense mutations, and deletions or duplications of entire exons. Current literature indicates that DNA sequence variants of this type are associated with disease. However, due to the absence of established genotype-phenotype correlations for this specific DNA sequence variant, this result should be carefully reconciled with this individual's clinical and family history.

3. **Amino acid changes of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. The amino acid change is predicted based on simple interpretations of the genetic code. However, these same types of alterations may sometimes alter normal gene splicing and processing, and thereby cause more significant and unpredictable effects. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

4. **Variants of unknown significance** are DNA sequence variants that are detected reproducibly, but have not been correlated with clinical presentation and/or pathology in the current literature, nor do they result in a readily predictable effect upon protein structure and function. Typical examples include single nucleotide changes in the coding or non-coding regions of the gene that are sometimes labeled as "silent mutations" or "intronic polymorphisms." These types of alterations often have no effect, but may sometimes alter normal gene splicing and processing. Since these types of sequence variants are similar to those observed in disease-associated mutations and benign polymorphisms, the nature of this variation prohibits definitive interpretation.

5. **Inconclusive** test results are those unable to be interpreted as either negative or positive due to a technical problem in the assay, and thus can rule out neither the presence nor absence of abnormalities in these genes. Inconclusive results are typically resolved by analysis of a repeat specimen. There will be no charge for the repeat analysis. Please indicate "REPEAT SPECIMEN" along with the above Athena Accession Number on the requisition. If this test is part of a profile and a positive result was obtained for another gene, the submission of a repeat specimen may not be warranted.

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7. **Benign polymorphisms** are DNA sequence variants that have been

Patient:
Christian Millare

Date of Birth: 08/23/2005 Sex: M Social Security Number:

Specimen Type:
DNA

Test Category:
Diagnostic (Symptomatic)

Test Requested:
SCN1A DNA Sequencing Test

Referring Physician:

Report to:
John Shoffner, MD

Address:
One Dunwoody Park
Suite 250
Atlanta, GA 30338

Additional Reports to:

Diagnosis Service Report

Accession Number:
07025148

Family Number/Clinical Number:

Patient Number:
000000000

Specimen Collection Date:
NO Date

Accession Date:
05/03/2007

Report Date:
06/30/2007

shown to be present in unaffected control subjects, and are considered "benign" (non-pathogenic) sequence variants. If identified, these are considered normal variations and are not reported in the Technical Results section of this report, but are available upon request.

***** *REVISED REPORT* ***** ver 1.0

This test was developed and its performance characteristics determined by Athena Diagnostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes and should not be regarded as investigational or for research only. Athena Diagnostics is licensed under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) to perform high complexity clinical testing. Athena Diagnostics has performed assay validation studies and has developed its laboratory protocols and operating procedures in consultation with experts in the field and in accordance with the standards of the National Committee on Clinical Laboratory Standards (NCCLS).

Laboratory results and submitted clinical information reviewed by,

Sat Dev Batish, PhD, FACMG
Chief Director, Genetics

Narasimhan Nagan, PhD, ABMG
Director, Genetics

Hui Zhu, PhD, ABMG
Director, Genetics

Laboratory oversight provided by Joseph J. Higgins, M.D., F.A.A.N., CLIA license holder, Athena Diagnostics (CLIA # 22D0069726)

Testing performed at:
Athena Diagnostics Four Biotech Park 377 Plantation St Worcester MA 01605

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(800) 394-4493 : (508) 756-2886

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Diagnosis Service Report

Patient: Christian Millare	Requesting Physician:
Date of Birth: 08/23/2005	Sex: M
Social Security Number:	Report to: John Shoffner, MD
Specimen Type: DNA	Address: One Dunwoody Park
Test Category: Diagnostic (Symptomatic)	Suite 250
Test Requested: SCN1A DNA Sequencing Test	Atlanta, GA 30338
	Additional Reports to:

Accession Number: 07025148
Family Number/Unit Number:
Patient Number: 000000000
Specimen Collection Date: NO Date
Accession Date: 05/03/2007
Report Date: 06/30/2007

Requisition for Parental Testing - Test Code 536

In order to provide a more comprehensive interpretation of this patient's SCN1A results, Athena Diagnostics is requesting samples from the biological parents of this patient. Athena will perform a target analysis on these samples for variant(s) identified in gene SCN1A only and use the findings to help interpret the patient's SCN1A result(s) **at no additional charge.** Please use this form as the requisition for parental testing on this patient. Enclose one completed form with each parent's sample and send to Athena. If you have any questions or require shipping kits, please contact Athena customer service at 1-800-394-4493,option 2.

Specimen Requirements: 1 lavendar tube (8.5ml) whole blood **Storage Conditions:** Refrigerate
Shipping Conditions: Room temperature, avoid freezing

Biological Mother Name: _____ Biological Father Name: _____

Date of Birth: _____ Date of Birth: _____

Address: _____ Address: _____

City,State,Zip: _____ City,State,Zip: _____

Phone: _____ Phone: _____

Specimen Collection Date: _____ Specimen Collection Date: _____

Indication for Testing: _____ UPIN: _____ Medical Practitioner Signature: _____

Testing Authorization and ICD-9 Code: I warrant that this test is either: 1) for the purpose of diagnosing or detecting an existing disease, illness, impairment, symptom or disorder, or 2) that if it is not for such purpose, I have obtained the appropriate prior written consent. This written consent was signed by the person who is the subject of the test (or if that person lacks capacity to consent, signed by the person authorized to consent for that person), and includes: a) a statement of the purpose and description of the test; b) a statement that prior to signing the consent form, the consenting person discussed with the medical practitioner ordering the test the reliability of positive or negative test results and the level of certainty that a positive test result for that disease or condition serves as a predictor of such disease; c) a statement that the consenting person was informed about the availability and importance of further testing, physician consultation and genetic counseling, and provided with written information identifying a genetic counselor or medical geneticist from whom the consenting person might obtain such counseling; d) a general description of each specific disease or condition tested for; and e) the person or persons to whom the test results may be disclosed as indicated above. 012.5x2.0

De-novo mutations of the sodium channel gene *SCN1A* in alleged vaccine encephalopathy: a retrospective study

Samuel F Berkovic, Louise Harkin, Jacinta M McMahon, James T Pelekanos, Sameer M Zuberi, Elaine C Wirrell, Deepak S Gill, Xenia Iona, John C Mulley, Ingrid E Scheffer

Lancet Neurol 2006; 5: 488–92
Published Online April 20, 2006
DOI:10.1016/S1474-4422(06)70446-X

See Reflection and Reaction page 465

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Correspondence to: Prof Samuel F Berkovic, Epilepsy Research Centre, Austin Health, West Heidelberg, Victoria 3081, Australia
s.berkovic@unimelb.edu.au

Summary

Background Vaccination, particularly for pertussis, has been implicated as a direct cause of an encephalopathy with refractory seizures and intellectual impairment. We postulated that cases of so-called vaccine encephalopathy could have mutations in the neuronal sodium channel α1 subunit gene (*SCN1A*) because of a clinical resemblance to severe myoclonic epilepsy of infancy (SMEI) for which such mutations have been identified.

Methods We retrospectively studied 14 patients with alleged vaccine encephalopathy in whom the first seizure occurred within 72 h of vaccination. We reviewed the relation to vaccination from source records and assessed the specific epilepsy phenotype. Mutations in *SCN1A* were identified by PCR amplification and denaturing high performance liquid chromatography analysis, with subsequent sequencing. Parental DNA was examined to ascertain the origin of the mutation.

Findings *SCN1A* mutations were identified in 11 of 14 patients with alleged vaccine encephalopathy; a diagnosis of a specific epilepsy syndrome was made in all 14 cases. Five mutations predicted truncation of the protein and six were missense in conserved regions of the molecule. In all nine cases where parental DNA was available the mutations arose de novo. Clinical-molecular correlation showed mutations in eight of eight cases with phenotypes of SMEI, in three of four cases with borderline SMEI, but not in two cases with Lennox-Gastaut syndrome.

Interpretation Cases of alleged vaccine encephalopathy could in fact be a genetically determined epileptic encephalopathy that arose de novo. These findings have important clinical implications for diagnosis and management of encephalopathy and, if confirmed in other cohorts, major societal implications for the general acceptance of vaccination.

Introduction

The sudden occurrence of seizures and developmental regression after vaccination in previously healthy infants led to the implication of a causal link, especially with pertussis vaccination.[1,2] Extensive debate ensued, but subsequent epidemiological studies did not lend support to the view of a causal association between immunisation and permanent brain damage.[3–5] In individual cases, however, the perception of causality can be difficult to challenge, especially if no alternative cause is identified, and has led to successful litigation. Public interest in this issue is high with a vocal minority urging avoidance of vaccination,[6] with the grave consequence of a potential resurgence of preventable serious childhood illnesses.[10] This issue is difficult to clarify largely because the diagnostic features of vaccine encephalopathy have never been defined. Reported cases have an apparent temporal relation to vaccination (varying from <1 day to 14 days) and typically have multiple seizure types with developmental arrest or regression.[2,3,7,8,11–16]

There are various causes of seizures and developmental regression in infancy, some of which have been previously misdiagnosed as vaccine encephalopathy.[17] A particular epilepsy syndrome, severe myoclonic epilepsy of infancy (SMEI), has become increasingly recognised. SMEI begins in the first year of life in previously healthy children. Hemiclonic seizures, which may be long lasting, are characteristic and can be associated with fever. Myoclonic, absence, tonic–clonic, and partial seizures also occur. The epilepsy is refractory and developmental regression ensues.[18,19] The syndrome is associated with more than 100 different mutations in the neuronal sodium channel α1 subunit gene *SCN1A*. Most cases of SMEI have such mutations, although the exact percentage is still debated. Around half the mutations truncate the protein and about 95% are de novo.[20–27]

We noted a similarity between the clinical pattern of SMEI and alleged cases of vaccine encephalopathy. Thus, we postulated that *SCN1A* mutations might underlie such cases where the physician or family believed that vaccination was causal. This finding would imply that the encephalopathy was not fundamentally caused by vaccination, but was due to a genetically determined, age-specific, epileptic encephalopathy.

Methods

Patients

This retrospective study of post-vaccination cases was nested within a larger study of 96 patients with unexplained encephalopathies and seizures beginning in the first year of life. We recruited participants from child neurologists around Australia and New Zealand during 2002 and 2003 for whom clinical details and DNA were obtainable and other causes of epileptic encephalopathies (perinatal,

post-traumatic, post-infectious, metabolic, and structural, etc) were excluded by appropriate metabolic and imaging studies. A few referrals were also accepted from outside Australasia. The study was approved by the Human Research Ethics Committee of Austin Health. Written informed consent was obtained from parents, guardians, or the appropriate government authority.

Cases were systematically classified on the basis of an exhaustive review of medical records from child neurologists, paediatricians, hospitals, and other treating doctors. Source records from initial medical presentations were sought to determine the precise onset details relative to vaccination. No specific neurological phenotype has been described for vaccine encephalopathy, so all cases were coded as vaccine encephalopathy when a relation to vaccination had been previously claimed and our review showed that the first seizure occurred within 72 h of vaccination. The time interval has no agreed definition, but on the basis of the published work we selected the time frame of documented seizure onset within 72 h of vaccination.[3,5,10,12,14,15]

All patients had epileptic encephalopathy (refractory seizures and developmental slowing); febrile seizures and other benign epilepsies were excluded. Epileptic seizures and epilepsy syndrome were diagnosed according to the International League Against Epilepsy classifications.[19,28] For this study, SMEI was diagnosed if all the following characteristics were present: onset in the first year with hemiclonic or generalised seizures; previous normal development; evolution of myoclonic seizures and generalised spike-wave discharges; and subsequent neurological deterioration. In Lennox-Gastaut syndrome, tonic seizures, atypical absences, and slow spike-wave on EEG were regarded as characteristic. Lennox-Gastaut syndrome can evolve from West syndrome with infantile spasms and hypsarrhythmia. The term borderline SMEI (SMEB), introduced by Japanese authors,[18,26] was used for cases without key features of SMEI (eg, lack of generalised spike-wave discharges, lack of myoclonus, few or atypical seizure types).

Procedures

After clinical classification of the epilepsy syndrome, molecular analysis was done on genomic DNA extracted from patients' venous blood samples. All 26 exons of *SCN1A* were PCR amplified with flanking intronic primers and standard PCR conditions (primers available on request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature before being analysed by denaturing high-performance liquid chromatography (dHPLC) on the WAVE 3500HT instrument (Transgenomic, NE, USA). Amplicons showing altered dHPLC chromatogram patterns compared with normal control DNA were sequenced from independent PCR products in both directions on an ABI 3700 sequencer (Applied Biosystems, CA, USA). Numbering of each mutation was taken from the start codon ATG of the full length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where a mutation was identified, the parents' DNA (if available) was checked for the mutation by direct sequencing.

Sequence changes were identified as mutations rather than as normal polymorphisms if they were not reported as common variants and they resulted in the generation of stop codons or deletions or, for missense mutations, if they resulted in a non-conservative amino-acid change and arose de novo, if parental DNA was available. Specific missense mutations were further validated by excluding them with dHPLC screening from a panel of anonymous Australian blood donors used as the control population.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

14 patients were identified for whom vaccination had been judged as causative of the epileptic encephalopathy and our review confirmed seizure onset within 72 h of vaccination. The patients were aged 2·5–47 years at the time of study (mean 12 years [SD 11]). They were 2–11 months old (5·4 months [2·6]) at the onset of the illness, which followed vaccination by 1–48 h (22 h [15 h]). The vaccines included pertussis in all cases (table).

The first seizure was described as hemiclonic (n=5), generalised clonic or tonic–clonic (n=6), infantile spasms (n=1), tonic (n=1), and unclassified (n=1). The first seizure was definitely associated with fever (>38°C) in five patients, six were afebrile, and in three the temperature was not recorded. Status epilepticus (seizures lasting ≥30 min) occurred at presentation in six cases. All cases had severe epilepsy with multiple seizure types and intellectual disability. Our review of the subsequent clinical course led to diagnosis of SMEI in eight patients, SMEB in four, and Lennox-Gastaut syndrome in two. In the two patients with Lennox-Gastaut syndrome, spasms and hypsarrhythmia occurred early, representing the known evolution from West syndrome. MRI showed no focal lesions and no evidence of destructive or inflammatory processes; scans in all cases were either normal (n=8) or showed varying degrees of diffuse atrophy and delayed myelination (n=6).

Molecular genetic analysis showed heterozygous mutations of *SCN1A* in 11 of 14 cases. These mutations were predicted to lead to truncation of the protein in five cases (three frameshift and two non-sense mutations); the other six were missense mutations (figure).[29,30] A display of evolutionary conservation of the residues where the mutations were found is shown in the webfigure. None of the six missense mutations were identifed in the blood donor control population; a

See Online for webfigure

	Age at study (years)	Age at onset (months)	Seizure onset post vaccination (h)	Vaccine type	First seizure			Later seizures	Epilepsy syndrome	SCN1A mutation	De-novo mutation
					Febrile	Status epilepticus	Seizure type				
1	17·5	8	24	2nd TA	N	N	Hemiclonic	Ab, At, H, T, GTCS, SE	SMEI	Frameshift C1354fsX1359	Y
2	2·5	2·5	24	1st TA	Y	N	Hemiclonic	Ab, At, H, M, T, CPS	SMEI	Missense R946H	Y
3	5	3	5	3rd TA	Y	Y	Hemiclonic	H, F, GTCS, SE	SMEB	Frameshift K1077fsX1079	Y
4	4·5	7	48	3rd TA	N	Y	Hemiclonic	Ab, At, H, M, GTCS,	SMEI	Nonsense R1407X	Y
5	4	6	12	3rd TA	N	Y	GC	Ab, F, M, GTCS, SE	SMEI	Missense R1645Q	Y
6	12	3	24	1st TA	Y	Y	GCS	H, M, GCS, GTCS, SE	SMEI	Missense E1238D	Unknown
7	6·5	2	9	1st TA	N	N	GC	Ab, H, M, T, GTCS, SE,	SMEI	Frameshift N1509fsX1511	Y
8	13·5	6	6	3rd TA	N	Y	GTCS	F, M, SGTCS, SE	SMEB	Missense C1396G	Y
9	4·5	7	24	3rd PV	Y	Y	Hemiclonic	H, M, SGTCS, SE,	SMEI	Missense Y413N	Y
10	47	6	24	1st TA	Unknown	Unknown	Unknown	At, F, M, GTCS, SE,	SMEB	Nonsense W384X	Unknown
11	8	4	36	2nd TA	Unknown	N	GCS	F, M, GTCS, SE,	SMEI	Missense F403L	Y
12	16·5	11	24	3rd TA	Y	N	GTCS	Ab, At, M, Sp, GTCS	SMEB	None detected	NA
13	13·5	7	1	3rd TA	Unknown	N	Spasms	Sp, T, At, M, GTCS, SE,	LGS	None detected	NA
14	14·5	2·5	48	1st TA	N	N	Tonic	Sp, T, F, M, GTCS, SE	LGS	None detected	NA

TA=triple antigen (diptheria, pertussis, tetanus); PV=pentavalent vaccine (diptheria, pertussis, tetanus, inactivated polio, and haemophilus); GTCS=generalised tonic-clonic seizures; GCS=generalised clonic seizures; GC=generalised convulsion (uncertain if tonic-clonic or clonic), M=myoclonic seizures, Ab=absences, At=atonic, SE=status epilepticus, CPS=complex partial seizures, H=hemiclonic; SGTCS=secondarily generalised tonic-clonic seizures; F=focal seizures; Sp=spasms; T=tonic, SMEI=severe myoclonic epilepsy of infancy, SMEB=borderland SMEI; LGS= Lennox-Gastaut syndrome; NA=not applicable.

Table: **Clinical characteristics of 14 patients with alleged vaccine encephalopathy**

minimum of 130 and maximum of 149 control samples were successfully screened for each mutation. In nine of the 11 patients with *SCN1A* mutations for whom samples from both parents were available, the mutations were absent in parental DNA and thus arose de novo. In patient six, parental DNA was not available. In patient ten, the mother was tested and did not have the mutation and the father was deceased. This patient had a deceased brother who was also said to have seizures beginning after vaccination, but medical records were destroyed and this could not be verified. Correlation of the clinically diagnosed phenotype with the molecular analyses showed that the sodium channel mutations were confined to the cases diagnosed as SMEI (eight of eight cases) or SMEB (three of four cases) and were absent in patients who had Lennox-Gastaut syndrome.

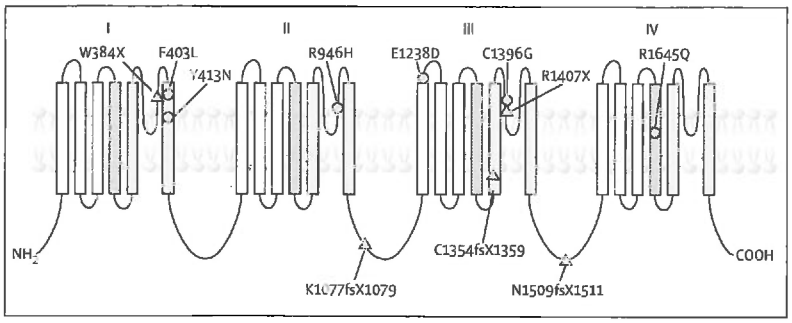

Figure: **Schematic representation of the proposed structure of SCN1A protein**
The protein comprises four homologous domains (I–IV), each with six transmembrane segments. Segments five and six (blue) form the ion channel pore and segment four (grey) is the voltage sensor.[27] The relative location of the six missense mutations (green circles) and five mutations possibly causing protein truncation (pink triangles) in the 11 cases with alleged vaccine encephalopathy are shown. The missense mutations predominantly occurred in the exons coding for the pore forming segments, as previously described in SMEI.[10]

Discussion

In this retrospective cohort of unexplained encephalopathies in the first year of life, 14 patients were judged by clinicians and families to have a vaccine encephalopathy and had documented seizure onset within 72 h of vaccination. With careful electroclinical analysis, we established that the clinical syndrome was SMEI in eight patients and the related syndrome SMEB in four. Eleven patients were shown to have mutations in the sodium channel gene *SCN1A*, which is now a well established finding in SMEI.[20–27] Two of the mutations have been reported before in association with SMEI or SMEB (R946H, R1407X) after nomenclature were standardised to the full length isoform given in Genbank accession number AB093548.[21,26,27] The mutations led to truncation of the protein in five cases, consistent with previous reports of truncation mutations being responsible for about half of SMEI cases with *SCN1A* mutations.[27] The other six patients had missense mutations. All are likely to be pathogenic as they have not been reported in control populations nor were they found in our controls, and the observed missense mutations affect highly conserved amino-acid sites (data not shown), are in regions where SMEI mutations have been previously described,[10] and arose de novo in all cases where both parents were tested.

There is no satisfactory case definition of the specific neurological phenotype in vaccine encephalopathy; indeed, even the temporal relation to immunisation is loose with cases described with onset of symptoms from less than 1 day to 14 days post vaccination.[2,3,5,8,11–16] Although we showed that SMEI or SMEB were important phenotypes in vaccine encephalopathy we were surprised

that no less than 12 of 14 patients were diagnosed as such with careful phenotypic analysis. We do not know if this finding is representative of cases in other centres, but previous reports of seizures in SMEI being associated with vaccination as well as fever lend support to our findings.[5,11,12] The multiple seizure types in SMEI and SMEB can make diagnosis difficult for observers unfamiliar with these disorders; patients can be judged as having an unclassified form of epilepsy and intellectual disability. The discovery of *SCN1A* mutations has led to improved awareness and diagnosis of these severe infantile encephalopathies.[13]

Scientific and medicolegal controversy of vaccine encephalopathy has spanned seven decades.[1,4] We suspect that the nature of cases has changed because of increasingly sophisticated clinical and neurological diagnoses and investigations. Some patients had coma at onset whereas others had seizures with subsequent regression. In the early research, detailed analysis led to the conclusion that some alleged cases were probably due to heterogeneous causes, including viral encephalitis and Reye's syndrome.[17] The molecular delineation of genetic encephalopathies with phenotypes of SMEI and SMEB now seems to be another major piece in the heterogeneous diagnostic puzzle of alleged vaccine encephalopathies.

The genetically determined epilepsy syndromes of SMEI and SMEB typically arise in association with de novo *SCN1A* mutations, presumably due to mutations in the gametes or in the very early post-fertilisation period.[20–24,26,27] In alleged vaccine encephalopathy the assumption of vaccination as a cause has been reinforced by the absence of a family history of severe epilepsy. Now, the molecular findings could explain the nature of the encephalopathy and the usual lack of family history since around 95% of mutations in SMEI occur de novo.[20–22]

SMEI often begins with febrile seizures and fever is frequently associated with seizures early in the clinical course. In the presence of *SCN1A* mutations, vaccination can still be argued to be a trigger for the encephalopathy, perhaps via fever or an immune mechanism. Our experimental design does not address this issue, but the role of vaccination as a significant trigger for the encephalopathy is unlikely for several reasons. First, although vaccination might trigger seizures as shown by the increased risk of febrile seizures on the day of triple antigen or MMR vaccination, there is no evidence of long-term adverse outcomes.[6–8] Second, less than half our patients had documented fever with their first seizure, which indicates that fever is not essential. Third, our neuroimaging data showed no evidence of an inflammatory or destructive process. Finally, truncation and missense mutations reported in conserved parts of *SCN1A* have not been found in many hundreds of healthy patients.[20,22,23,25,26] Thus, individuals with such mutations seem to develop SMEI or SMEB whether or not they are immunised in the first year of life. We do not think that avoiding vaccination, as a potential trigger, would prevent onset of this devastating disorder in patients who already harbour the *SCN1A* mutation.

The mechanism by which *SCN1A* mutations cause SMEI is unknown. Few causative mutations have so far been subjected to functional analysis, and the results are inconsistent; however, these mutations are presumed to cause abnormal neuronal excitability.[14,15] Studies of less severe mutations of *SCN1A* that cause milder phenotypes have also produced conflicting results dependent on the techniques and the model system investigated.[16,17] Definitive data from neuronal systems have yet to emerge. Moreover, because many of the mutations associated with SMEI cause truncation of the protein, these proteins are unlikely to be expressed at the cell surface; thus poorly understood changes to sodium-channel density, stoichiometry, and function might all contribute to the phenotypes observed. Further study in neuronal systems, and ideally whole animal models, is needed to clarify the complex functional effects of *SCN1A* mutations.

We did not find a molecular explanation for three patients with alleged vaccine encephalopathy. These could be chance associations of vaccination with other causes leading to the onset of encephalopathies. Other cases could be due to large deletions or undiscovered mutations in non-translated parts of the *SCN1A* gene or perhaps due to rare mutations in related genes, such as *GABRG2*[38] and *SCN2A*.[39]

Although epidemiological studies have cast doubts on the hypothesis of vaccination as a cause of encephalopathy,[4–7] families, the medical profession, and society remain difficult to reassure of the lack of causality in individual patients in whom vaccination and onset of encephalopathy were coincidental. For individual cases, this problem is particularly significant in the legal setting. For society, fear of adverse consequences of vaccination is a major factor in suboptimum immunisation rates. The identification of a genetic cause of encephalopathy in a particular child should finally put to rest the case for vaccination being the primary cause. Confirmation of our findings by others would be of value in determining their generalisability and the broad societal implications.

Cases of vaccine encephalopathy should be carefully assessed clinically for characterisitcs of SMEI or SMEB, and testing for *SCN1A* mutations should be considered. Correct diagnosis will reassure the family as to the true cause, remove the blame of having vaccinated the child, direct appropriate treatment, and allow realistic planning for prognosis. Specific treatment regimens for seizures in SMEI are emerging with controlled data showing the effectiveness of stiripentol,[40] and uncontrolled open studies suggesting avoidance of lamotrigine[41] and probable benefit of topiramate.[42] Medical and societal energies that have focused on the alleged association with vaccination need to be redirected towards the care of these severely handicapped individuals and towards novel approaches to treat and ultimately prevent these encephalopathies.

Contributors

SFB developed the hypothesis and wrote the first draft. Analysis of clinical data was done principally by IES, JMM, JTP, and SFB, and also by SMZ, ECW, and DSG. Molecular analysis was undertaken by LH, XI, and JCM. All authors critically revised the first draft and approved the final manuscript.

Conflicts of interest

SFB, IES, and JCM have received research support and honoraria from Bionomics Ltd. Bionomics Ltd has licensed a diagnostic test for *SCN1A* mutations.

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The spectrum of *SCN1A*-related infantile epileptic encephalopathies

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The relationship between severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) and the related syndrome SMEI-borderland (SMEB) with mutations in the sodium channel alpha 1 subunit gene *SCN1A* is well established. To explore the phenotypic variability associated with *SCN1A* mutations, 188 patients with a range of epileptic encephalopathies were examined for *SCN1A* sequence variations by denaturing high performance liquid chromatography and sequencing. All patients had seizure onset within the first 2 years of life. A higher proportion of mutations were identified in patients with SMEI (52/66; 79%) compared to patients with SMEB (25/36; 69%). By studying a broader spectrum of infantile epileptic encephalopathies, we identified mutations in other syndromes including cryptogenic generalized epilepsy (24%) and cryptogenic focal epilepsy (22%). Within the latter group, a distinctive subgroup designated as severe infantile multifocal epilepsy had *SCN1A* mutations in three of five cases. This phenotype is characterized by early onset multifocal seizures and later cognitive decline. Knowledge of an expanded spectrum of epileptic encephalopathies associated with *SCN1A* mutations allows earlier diagnostic confirmation for children with these devastating disorders.

Keywords: *SCN1A*; SMEI; SMEB; epileptic encephalopathy; channelopathies

Abbreviations: dHPLC = denaturing high performance liquid chromatography; GEFS+ = generalized epilepsy with febrile seizures plus; ICEGTC = intractable childhood epilepsy with generalized tonic clonic seizures; LGS = LennoxGastaut syndrome; MAE = Myoclonicastatic epilepsy; PCR = polymerase chain reaction; SCN1A = sodium channel alpha 1 subunit gene; SMEB = SMEI-borderland; SMEB-M = SMEI-borderland-myoclonic seizures; SMEB-O = SMEI-borderland more than one feature; SMEB-SW = SMEI-borderland-spike wave; SMEI = severe myoclonic epilepsy of infancy

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Introduction

SCN1A, the gene encoding the sodium channel alpha 1 subunit, has emerged as the most important of the epilepsy genes currently known (Mulley *et al.*, 2005). *SCN1A* mutations underlie more than 70% of patients with the epileptic encephalopathy severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) (Dravet *et al.*, 1982; Claes *et al.*, 2001; Mulley *et al.*, 2005). More than 170 documented mutations are associated with SMEI and the related syndrome of borderland SMEI, known as SMEB. Truncation mutations account for nearly 50% of mutations found in SMEI, with the remainder comprising missense, splice site and deletion mutations (Mulley *et al.*, 2005). These mutations affect many domains of the gene with suggested clustering of missense mutations occurring in the N- and C-termini and the S5–S6 pore-forming regions of the protein (Kanai *et al.*, 2004). Recently intragenic and whole gene deletions have been identified in a few cases of SMEI without truncation, missense or splice-site mutations (Madia *et al.*, 2006; Mulley *et al.*, 2006; Suls *et al.*, 2006).

Approximately 95% of *SCN1A* mutations in SMEI patients arise *de novo*. The remaining cases have familial mutations with milder phenotypes in other family members often consistent with the generalized epilepsy with febrile seizures plus (GEFS+) spectrum (Scheffer and Berkovic, 1997; Escayg *et al.*, 2000; Singh *et al.*, 2001; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Scheffer, 2003; Kimura *et al.*, 2005). Recently, germline and somatic *SCN1A* mutational mosaicism has been reported in unaffected parents (or parents mildly affected with febrile seizures) where their children have SMEI or SMEB (Depienne *et al.*, 2006; Gennaro *et al.*, 2006; Marini *et al.*, 2006; Morimoto *et al.*, 2006).

SMEI or Dravet syndrome is a distinctive syndrome with seizure onset in the first year of life, typically beginning with prolonged febrile hemiclonic seizures or generalized tonic–clonic seizures (Dravet, 1978; Dravet *et al.*, 1982, 2005). The disorder evolves with other seizure types such as myoclonic, focal, absence and atonic seizures developing between 1 and 4 years of age. Development is normal in the first year of life followed by developmental slowing and regression. Pyramidal signs and ataxia may evolve. Cognitive outcome is usually poor and seizures remain refractory for those who survive to adulthood (Jansen *et al.*, 2006).

The phenotypic spectrum of patients with *SCN1A* mutations has been extended beyond SMEI. The related syndrome SMEB (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004) refers to children who lack several of the key features of SMEI such as myoclonic seizures or generalized spike-wave activity (Sugama *et al.*, 1987; Dravet *et al.*, 2005). In two studies, 26% (7/27) and 88% (15/17) of SMEB patients were found to have *SCN1A* mutations respectively (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004). As with SMEI, these mutations are spread throughout the gene with a mixture of types of mutation including truncation, missense and splice-site changes (Mulley *et al.*, 2005). A subgroup of SMEB has been variously described as intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC, originally called high voltage slow waves grand mal by Japanese authors) or Severe idiopathic generalized epilepsy of infancy with generalized tonic–clonic seizures. These infants have generalized tonic–clonic seizures beginning in the first year of life without the evolution of other seizure types and they follow a similarly unfavourable developmental course to children with SMEI (Fujiwara *et al.*, 1992; Kanazawa, 1992, 2001; Sugama *et al.*, 1987; Doose *et al.*, 1998). In one series, 7/10 ICEGTC patients had missense mutations in *SCN1A* (Fujiwara *et al.*, 2003); truncation, missense and splice-site mutations were reported in 3/18 patients described as severe idiopathic generalized epilepsy of infancy (Ebach *et al.*, 2005). We reported the only case so far of West syndrome with an *SCN1A* mutation (Wallace *et al.*, 2003).

Given the overlapping yet heterogeneous clinical features of these epilepsy syndromes, we postulated that *SCN1A* mutations may be associated with other phenotypes. Here we studied unselected patients with severe epileptic encephalopathies (including SMEI) with onset primarily during the first year of life.

Material and methods

Clinical methods

Patients with epileptic encephalopathies of unknown cause were referred by paediatric neurologists and neurologists from Australia and around the world. Epileptic encephalopathies are defined as disorders in which there is a temporal relationship between deterioration in cognitive, sensory and motor function and epileptic activity comprising frequent seizures and/or extremely frequent 'interictal' paroxysmal activity (Nabbout and Dulac, 2003). Cases were only included where magnetic resonance imaging was normal or showed non-specific features without a definite aetiology. A subset of 14 patients, included in this study, with so-called 'vaccine encephalopathy' has been published previously (Berkovic *et al.*, 2006).

Electroclinical data were obtained on all patients with specific emphasis on early seizure history including age of onset, occurrence of status epilepticus, presence of fever sensitivity, clinical photic sensitivity and evolution of other seizure types. A detailed early developmental history was obtained with attention to acquisition of early milestones, timing of plateau or regression of development and current functioning. Other important details included general and neurological examination, family history of seizure disorders and results of EEG, video-EEG monitoring and neuroimaging studies. Results of other available investigations such as chromosomal analysis were also obtained.

SMEI was defined according to the following criteria: onset in the first year of life of convulsive seizures which were hemiclonic or generalized; myoclonic seizures; other seizure types which could include focal seizures, absence seizures, atonic seizures, tonic

seizures; normal development in the first year of life with subsequent slowing including plateauing or regression; generalized spike-wave activity and either normal MRI or non-specific findings.

SMEB was divided into subgroups based on the absence of specific features that are regarded as required for the diagnosis of SMEI. SMEB-M referred to patients who did not have myoclonic seizures but otherwise satisfied SMEI criteria. SMEB-SW defined patients who had all the SMEI criteria but had never had generalized spike-wave activity documented on EEG. SMEB-O referred to patients who had more than one feature that was not in keeping with SMEI; examples include absence of generalized spike-wave activity recorded on EEG, a normal developmental outcome and absence of myoclonic seizures. SMEB included cases with ICEGTC where they followed the same course but only had convulsive seizures.

Cryptogenic generalized epilepsy (CGE) denoted individuals who have multiple seizure types, generalized sharp and slow activity and intellectual disability with no known aetiology. Lennox–Gastaut syndrome (LGS) defined patients with tonic seizures and slow generalized spike-wave activity and abnormal development (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Beaumanoir and Blume, 2005). Myoclonic–astatic epilepsy (MAE) referred to individuals with myoclonic–astatic seizures and other generalized seizure types with generalized spike-wave activity and variable developmental outcome (Doose *et al.*, 1970; Guerrini *et al.*, 2005).

A further subgroup was called cryptogenic focal epilepsy where an individual had focal seizures and uni- or multifocal EEG epileptiform patterns. These individuals showed a variable degree of intellectual disability and usually had normal neuroimaging. Several individuals were included with abnormal neuroimaging that did not account for the clinical presentation such as hydrocephalus, bilateral periventricular leucomalacia, etc.

Other syndromes were defined according to the ILAE classification (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Patients were called 'unclassified' if we had insufficient evidence to make a syndrome diagnosis or the patient did not fit into a recognized syndrome despite detailed evaluation.

The Austin Health Human Research Ethics Committee approved this study. Informed consent was obtained from the parents or guardians of minors and from adult subjects of normal intellect. In the case of adults with intellectual disability, legal consent was obtained from the appropriate government authority or legal guardian.

Molecular analysis

Molecular analysis was carried out on genomic DNA extracted from venous blood. All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR) using flanking intronic primers and standard PCR conditions (primers available upon request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature to form heteroduplex products which were analysed by denaturing high performance liquid chromatography (dHPLC) on the Transgenomic WAVE 3500HT instrument (dHPLC conditions available upon request). Amplicons showing altered dHPLC chromatogram patterns were sequenced in both directions from independent PCR products, on an ABI 3700 sequencer. The final subset of patients (43) was screened by direct sequencing of PCR products (without prior dHPLC screening) by Athena Diagnostics under diagnostic conditions. The numbering for each mutation is taken from the start codon ATG of the full-length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where an *SCN1A* mutation was detected, the appropriate amplicon from parental DNA (where available) was tested by DNA sequencing to distinguish between *de novo* and familial variants. Mutations or rare variants were distinguished from coding single nucleotide polymorphisms which have previously been reported (Escayg *et al.*, 2001).

Results

Clinical diagnoses

One hundred and eighty-eight patients were recruited from Australia (110), Canada (27), United Kingdom (23), New Zealand (20), Israel (4), USA (3) and Denmark (1) with seizure onset in the first 2 years of life. These included 14 cases who were negative for *SCN1A* mutations on single-stranded conformation analysis in our previous study (Wallace *et al.*, 2003); the eight positive cases and two, who were negative on sequencing, are not included in the data presented here.

Our total cohort contained 66 with SMEI, 36 with SMEB including the various subcategories, 25 with cryptogenic generalized epilepsy, 18 with cryptogenic focal epilepsy, 10 with MAE and 12 with LGS. The remaining cases had a range of other syndromes or were unable to be classified (Table 1).

Table 1 *SCN1A* mutations in patients with epileptic encephalopathies

	Total	*SCN1A* mutation
SMEI	66	52
SMEB	36	25
SMEB-O	16	10
SMEB-SW	14	11
SMEB-M	4	3
ICEGTC	2	1
Cryptogenic generalized epilepsy	25	6
Cryptogenic focal epilepsy	18	4
Myoclonic–astatic epilepsy	10	2
Lennox–Gastaut syndrome	12	1
West syndrome	5	
Idiopathic spasms	1	
Early myoclonic encephalopathy	1	
Progressive myoclonic epilepsy	1	
Alternating hemiplegia of childhood	1	
Unclassified	12	
Total	188	90

SMEI, severe myoclonic epilepsy of infancy; SMEB-SW, SMEI borderland without generalized spike wave; SMEB-M, SMEI borderland without myoclonic seizures; SMEB-O, SMEI borderland lacking more than one feature of SMEI; ICEGTC, intractable childhood epilepsy with generalized tonic–clonic seizures.

Molecular analysis

Of the 188 patients examined, 90 (48%) had *SCN1A* mutations. Ninety-four sequence variants were identified in the 90 mutation positive patients as four children each had two changes. In each child, the putative pathogenic variant was distinguished from the likely non-pathogenic variant; the latter was not included in further analyses (see later and Supplementary Table). The majority of mutations are novel (72/90, 80%), reinforcing the mutational heterogeneity characteristic of *SCN1A*. Of the 90 cases, DNA was available from 76 sets of parents and 73/76 (96%) were *de novo* mutations.

Amino acid alignments of the missense mutations show that they affect conserved domains of the protein in other human alpha channels (SCN2A, SCN3A and SCN8A), chimpanzee, rat, mouse, *Fugu* and *Drosophila* consistent with their interpretation as pathogenic mutations (Supplementary Fig. S1). Moreover, the probability that these missense mutations are pathogenic mutations is supported by their *de novo* origin (in 34/37 cases where parents have been examined) and previously published observations in SMEI.

SMEI

Fifty-two of the 66 (79%) of patients with SMEI had *SCN1A* mutations (Table 1). Forty-four percent (23/52) of the SMEI-related mutations were non-sense or frameshift mutations resulting in protein truncation, 39% (20/52) were missense mutations and the remaining 17% (9/52) were intronic splice donor or splice acceptor site changes. These mutations were spread throughout the gene with the majority of missense mutations (14/20, 70%) localized to the transmembrane regions of the protein, in particular the S5–S6 loop of domain II that forms part of the ion channel pore (Supplementary Table, Fig. 1A). In contrast, 57% (13/23) of truncation mutations were positioned in the intracellular loops of the protein (Supplementary Table, Fig. 1A).

Parental DNA was available for testing for 42/52 SMEI patients who were mutation positive. Analysis of the DNA from these parents confirmed that all 42 mutations were *de novo*.

There were four patients with two sequence variants that posed challenges in clinico-molecular interpretation (Supplementary Table). Patient 2 had two *SCN1A* sequence variants: one was a *de novo* missense change (Y84C) affecting a highly conserved amino acid site (Supplementary Fig. S1) and the second was a splice acceptor site change found to have a maternal origin. The mother was unaffected; the maternal grandfather had a history of convulsions until 7 years but was negative for the splice acceptor site change. There was no further seizure history within this family suggesting that the change within the splice site was probably a benign variant. Therefore this variant was not considered in the determination of mutation frequencies.

Patients 6, 32 and 38 also had two sequence variants detected but parental DNA was unavailable in order to ascertain which variant was *de novo* and thus the likely pathogenic mutation (Supplementary Table). Patient 6 had two intronic mutations detected, both potentially pathogenic. In the absence of parental DNA we can only assume that one is likely to be pathogenic. The intron IVS3-13T→A change was chosen as the most likely variant to affect splicing since it is within the consensus C/T run in the splice acceptor site. Patient 32 had both a missense (E1238D) and an intronic donor splice site mutation. Since the missense change affected a highly conserved amino acid site (Supplementary Fig. S1), this was considered to be the true mutation. Patient 38 had a truncation mutation (R1525X) also seen in Patient 39 with SMEI and in a previous study (Supplementary Table) (Kearney *et al.*, 2006). Patient 38 also had a missense change not as highly conserved as most missense mutations (Supplementary Fig. S1) so the truncation mutation was considered the likely pathogenic mutation. The second variant found in each case has not been included in the mutational analyses.

Simultaneous double mutation in the same patient is a theoretical possibility, as is a *de novo* mutation adversely interacting with a pre-existing rare variant. However, in the absence of definitive evidence from other SMEI cases and

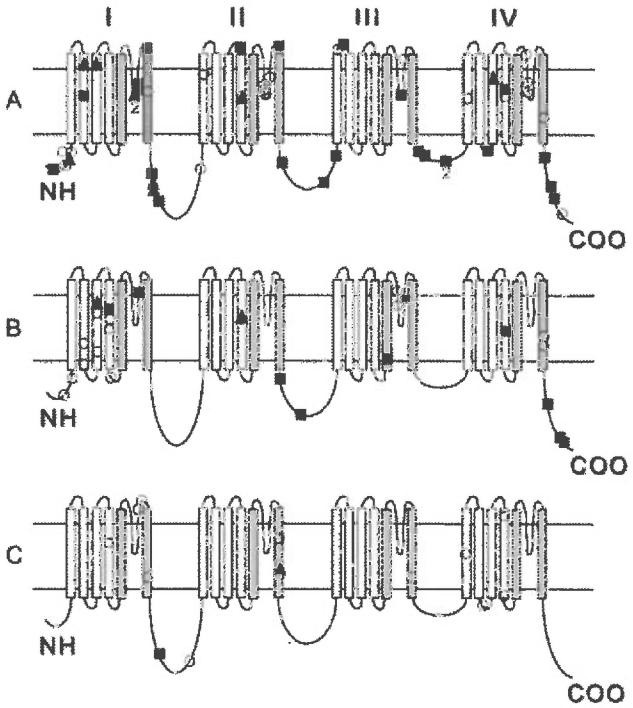

Fig. 1 Schematic representation of mutations in *SCN1A* in patients with **(A)** SMEI, **(B)** SMEB and **(C)** other phenotypes. Refer to Supplementary Table for details. The SCN1A protein consists of four domains designated I–IV, each contains six transmembrane segments designated S1–S6. ■ = truncation, ○ = missense, ▲ = splice-site mutations.

absence of parental DNA to establish *de novo* origin, the most parsimonious explanation is that of a single mutational event unless proven otherwise.

SMEI—borderland

SCN1A mutations were identified in 25/36 (69%) patients with SMEB including all subcategories (Table 1). Over half of these changes were missense mutations (13/25) with 40% (10/25) being truncation mutations; the remaining two were splice-site mutations. The mutations were spread throughout the gene with the majority (18/25, 72%) localized to the transmembrane domain regions. Missense mutations were clustered in the S2–S4 transmembrane segments of domain I (Fig. 1B).

Analysis of parental DNA from 22/25 patients with mutations confirmed 95% (21/22) were *de novo*. Patient 63 had a paternally inherited mutation (A239T). The proband's father had febrile seizures plus (Scheffer and Berkovic, 1997) and the paternal grandmother had unclassified seizures. Both individuals were found to carry the A239T change, which when taken together with the amino acid conservation of this residue (Supplementary Fig. S1), reinforces the status of this variant as a true pathogenic mutation of *SCN1A*. The family had a bilineal family history of seizures as the proband's mother had febrile seizures and did not carry the *SCN1A* mutation (Supplementary Fig. S2).

Cryptogenic generalized epilepsy

Of the 25 patients with cryptogenic generalized epilepsy, six (24%) had mutations. None of the mutations have been previously reported, however the T226M in Patient 78 was also seen in Patient 61 within this cohort with SMEB-O (Supplementary Table). Four mutations arose *de novo*; parental DNA was unavailable for one patient and for Patient 82 the mutation (M973V) was found in her unaffected father. There was no family history of seizures but the amino acid conservation at this site is reasonably strong (Supplementary Fig. S1) providing circumstantial evidence that it is a true mutation. If so, then it must be non-penetrant in the father or else function as a susceptibility allele acting in tandem with other unidentified susceptibility genes responsible for the phenotype in the proband.

The six cases with mutations had heterogeneous phenotypes with onset between 1.5 and 12 months (Table 2). Two had a phenotype with onset in the first 2 months of life and abnormal early development but other features were similar to SMEI. Patient 80 fixed and followed and smiled by 6 weeks when seizures began. Development slowed from 6 weeks: he sat late, walked at 18 months and development stagnated from 2 years. He died at 13 years. Patient 78 had seizure onset at 8 weeks, smiled at 3 months, never sat or acquired words.

The other four cases presented a mixed picture, but generalized spike wave and focal discharges were usually

Table 2 Clinical features of *SCN1A* mutation positive patients with diagnosis other than SMEI and SMEB

Patient	Age at study (years)	Seizure onset (months)	Seizure types	Intellect	Neurological signs	Epilepsy classification	*SCN1A* mutation	Inheritance
78	5	2	GTCS, H, MJ, F, NCS	Severe ID	Increased tone, later generalized hypotonia	CGE	T226M	De novo
79	23	5.5	FS, GTCS	Mild-moderate ID	None	CGE	A395P	De novo
80	14	1.5	GTCS, H, At, MJ, F, SE	ID	None	CGE	V422E	De novo
81	14	12	FS, GTCS, aAb, MJ, SE	ID	Ataxia, intermittent movement disorder	CGE	S626G	ND
82	35	9	FS, GTCS, MJ, F-SG	Low average	None	CGE	M973V	Paternal
83	3	6	FS, GTCS, MJ	Normal	None	CGE	IVSI5+IG→T	*De novo*
84	16	7	FS, GTCS, MJ, F, NCS	Mild ID	Mild generalized spasticity	CFE (SIMFE)	F575fsX622	*De novo*
85	5	4.5	F, H, SE	ID	None	CFE (SIMFE)	FI543S	Maternal
86	20	5	GTCS, MJ, F, T	Moderate ID	Ataxia, mild left hemiparesis	CFE (SIMFE)	RI596C	*De novo*
87	5	18	FS, F-SG, SE	Normal	None	CFE	RI657H	*De novo*
88	21	0.75	IS, At, aAb, T, SE, NCS	ID	Mild right hemiparesis	LGS	RI636Q	*De novo*
89	11	4	FS, GTCS, MJ, At, T, H	ID	None	MAE	R393C	*De novo*
90	12	13	FS, F, MA, MJ	Moderate ID	None	MAE	GI480V	*De novo*

FS, febrile seizures; aAb, atypical absence seizures; At, atonic seizures; F, focal seizures (not hemiclonic/unilateral); GTCS, generalized tonic–clonic seizures; H, hemiclonic; IS, infantile spasms; MA, myoclonic–astatic; MJ, myoclonic jerks; NCS, non-convulsive status epilepticus; SE, status epilepticus; SG, secondary generalization; T, tonic seizures; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; LGS, Lennox–Gastaut syndrome; MAE, myoclonic–astatic epilepsy; SIMFE, severe infantile multifocal epilepsy; ID, intellectual disability; ND, not done.

seen. The severity of the seizures varied with some only having generalized tonic–clonic seizures, which settled by adult life.

Cryptogenic focal epilepsy

Of 18 patients with cryptogenic focal epilepsy within this cohort of infantile epileptic encephalopathies, four (22%) had mutations (Table 1). Five cases presented with severe infantile multifocal epilepsy with developmental delay and are described later. Three had mutations: two (Patients 84 and 86) arose *de novo* (F575fsX622, R1596C) and one was maternally inherited. The latter (Patient 85) had a putative mutation (F1543S) that was highly conserved (Supplementary Fig. S1) and was carried by her unaffected mother, and may represent a susceptibility allele.

One (Patient 87) had recurrent febrile status epilepticus with onset at 18 months (Table 2). Twenty-four episodes of status epilepticus occurred, some with focal features with variable lateralization. MRI was normal. The patient died at 5 years due to complications of status epilepticus. He had a *de novo* missense *SCN1A* mutation (R1657H).

Severe infantile multifocal epilepsy

Five cases had this phenotype with seizure onset at a mean of 4 months. Of those with *SCN1A* mutations (Patients 84, 85 and 86, Table 2), onset occurred at mean of 5.5 months (4.5, 5 and 7 months) compared with 6- and 8-week onsets in the other two cases. Each child had multiple types of focal seizures with varying semiology. EEG studies showed abundant multifocal epileptiform activity typically with no (or exceptional) generalized or bilaterally synchronous discharges. The three patients with mutations had MRI brain studies; two showed mild atrophy. The remaining two had CT brain scans; one showed mild right sided atrophy.

Developmental delay became evident in all cases. In the two cases that were mutation negative, seizures began at 6 and 8 weeks concurrent with the recognition that developmental delay was present. In the three cases with *SCN1A* mutations, early development was normal with developmental slowing noted at the ages of 16 months, 3–4 years and 6 years even though seizure onset occurred at 4.5, 5 and 7 months, respectively (Table 2). Developmental outcome was poor with intellectual disability ranging from mild (one case, mutation positive: Patient 85), moderate (three cases, two had mutations: Patients 84 and 86) to severe (one case).

Other phenotypes

De novo SCN1A mutations were identified in 2/10 patients with MAE (Patients 89 and 90) and 1/12 patients with LGS (Patient 88) (Tables 1 and 2). No mutations were identified in patients with West syndrome, idiopathic spasms, early myoclonic encephalopathy, progressive myoclonic epilepsy, alternating hemiplegia of childhood or the 12 cases that could not be classified.

Discussion

The sodium channel alpha 1 subunit gene, *SCN1A*, is currently the most clinically relevant epilepsy gene. Mutations in *SCN1A* are an important cause of SMEI and SMEB and its subset ICEGTC (Claes *et al.*, 2001; Mulley *et al.*, 2005). Recently we showed that so-called 'vaccine encephalopathy' should be regarded as SMEI/SMEB on clinical and molecular grounds (Berkovic *et al.*, 2006). Whilst *SCN1A* was originally associated with a small proportion of patients with the mild phenotypes characteristically seen in the GEFS+ syndrome (Escayg *et al.*, 2000; Mulley *et al.*, 2005), mutations within this gene have been identified far more often in patients with more severe forms of epilepsy. This study examines epileptic encephalopathies beginning early in life and expands the phenotypic spectrum of *SCN1A* defects beyond that previously recognized, to now include patients with cryptogenic generalized epilepsy and cryptogenic focal epilepsy.

The majority of mutations identified in the 90 children in this study were novel (72/90, 80%), whereas 18 (20%) had been previously published (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Wallace et al., 2003; Fukuma *et al.*, 2004; Mulley *et al.*, 2005; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006). This expanded list of mutations, taken together with those reviewed by Mulley *et al.* (2005), provides an essential mutational database for use as an interpretative aid for diagnostic laboratories offering *SCN1A* mutation testing. Unlike some disorders where mutations are largely concentrated in 'hot spots', the mutations within *SCN1A* are widely distributed throughout the gene.

Parental DNA was available in 84% (76/90) of cases of which 96% (73/76) arose *de novo* and 4% (3/76) were familial. Familial *SCN1A* mutations have been previously reported in around 5% of SMEI where family members have mild GEFS+ phenotypes, as we observed here (Supplementary Fig. S2) (Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Mulley *et al.*, 2005). In these probands, it is likely that their disorder has a multifactorial basis where *SCN1A* is a major but not the sole contributing gene. This would explain the marked disparity in phenotypic severity between the proband and their relatives. This model could explain probands 63, 82 and 85 where the parent was unaffected or had a mild phenotype. It is worth noting that these probands had a range of phenotypes including cryptogenic generalized and cryptogenic focal epilepsies.

Given the current state of knowledge, the majority of *SCN1A* mutations remain novel. This creates a challenge in determining whether new variants are pathogenic or not. Where the variant is *de novo* or results in truncation of the protein, then the likelihood of it being pathogenic is

extremely high; 79 (88%) of our 90 positive cases fitted these criteria. In cases with missense changes, where DNA from parents is unavailable, or where an unaffected transmitting parent is identified, the case for pathogenicity rests on circumstantial evidence provided by evolutionary conservation of protein structure. Definitive functional studies are rarely available for this particular ion channel. In the four cases with two rare *SCN1A* variants, one of the two was assessed as more likely to be relevant to the observed phenotype. In cases where an unaffected transmitting parent is identified, these changes may be incidental benign variants, incompletely penetrant pathogenic variants or represent a susceptibility allele that contributes to the phenotype in a polygenic manner. Another alternative is that the variant has a major effect on the proband, whereas the transmitting parent has unrecognized protective molecular mechanisms.

SMEI

This study reinforces the high frequency of *SCN1A* mutations in patients with SMEI. The initial report described mutations in 7/7 cases (Claes *et al.*, 2001). Subsequently, large series from a number of centres have reported mutations in 61–87% cases consistent with our finding of 79% reported here (Ohmori *et al.*, 2002, 2003; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Fukuma *et al.*, 2004). Lower mutation rates of 35% (33/93) and 33% (55/169) have been reported (Nabbout *et al.*, 2003*a*; Suls *et al.*, 2006) and of 33% (8/24) by our laboratory (Wallace *et al.*, 2003). The latter study used single-strand conformation analysis for mutation detection, a rapid screening technology less sensitive than dHPLC used here. DHPLC has >96% sensitivity and specificity (Xiao and Oefner, 2001). Additional direct sequencing was performed in five cases (two negative). Fourteen of the remaining negative SMEI cases from our study were tested by dHPLC (3 cases) or direct sequencing (11 cases) here. Eight mutations were identified (two by dHPLC and six by sequencing), bringing the mutation rate to 16/24 (66%) for those cases reported in our original study (Wallace *et al.*, 2003). Of our *SCN1A* mutation negative SMEI cases on dHPLC, 2 of 13 (15%) were subsequently found to have whole exon deletions detected by multiple ligase-dependent probe amplification (Mulley *et al.*, 2006). Other SMEI cases lacking point mutations have been shown to have microdeletions including the *SCN1A* gene (Madia *et al.*, 2006; Suls *et al.*, 2006).

SMEB

We found 69% of our SMEB cases had *SCN1A* mutations. This figure is higher than the 26% reported by Fukuma *et al.* (2004) and more in keeping with the 88% mutation rate of Ohmori *et al.* (2003). The majority of SMEB mutations detected in this study were novel changes (17/25, 68%), with eight mutations being previously reported in patients with SMEI (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Wallace *et al.*, 2003; Fukuma *et al.*, 2004; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006).

SMEB is distinguished from SMEI by the absence of specific features. The question of whether myoclonic seizures are an essential component of a SMEI phenotype remains controversial (Ogino *et al.*, 1988; Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Ohmori *et al.*, 2003; Fukuma *et al.*, 2004; Dravet *et al.*, 2005). Dravet and colleagues observed that myoclonic seizures may be segmental or occur immediately prior to convulsive seizures and they postulate that subtle myoclonus may be missed (Dravet *et al.*, 2005). Our data suggest that myoclonic seizures are not obligatory as three of four patients with an SMEI phenotype lacking only obvious myoclonic seizures (SMEB-M) carried a *SCN1A* mutation. Similarly, generalized spike-wave activity is considered the EEG hallmark of SMEI, but we found that 11/14 (79%) of our patients with a SMEI picture without demonstrated generalized spike-wave activity (SMEB-SW) had mutations.

Our findings in SMEB have important implications for the 'lumpers and splitters' debate. Whilst Ohmori and co-workers (2003) found a higher mutation rate in SMEB (88%) than SMEI (72%), our larger study shows the reverse. Moreover, three mutations are associated with both SMEI and SMEB (Patients 4 and 54, 8 and 59, 48 and 74) in this study. Similarly, eight cases have a mutation previously associated with the alternate phenotype (Supplementary Table). The recent ILAE classification proposal suggests the new name of Dravet syndrome for SMEI (Engel, 2001). In terms of clinical utility, we suggest that it may be more helpful to conceptualize SMEI and SMEB as a spectrum and incorporate both under the eponym of Dravet syndrome. This would also resolve the inaccuracy in terminology arising from the absence of myoclonic seizures in some cases of SMEI despite 'myoclonic' being part of the syndrome's name.

SCN1A mutations in SMEI and SMEB

Our data show similar results to those previously summarized in our review of *SCN1A* mutations (Mulley *et al.*, 2005), with mutations comprising 43% (33/77) truncation and 43% (33/77) missense changes. The proportion of missense (39% versus 52%) and truncation (44% versus 40%) mutations is similar in SMEI and SMEB. Our new data fail to fully confirm previous observations of a predilection for missense mutations to occur in the ion channel pore region (Kanai *et al.*, 2004), with only 15/33 (46%) in this region. Previous studies suggested clustering of missense mutations in SMEI in the S5–S5 loops of domain I and II (Mulley *et al.*, 2005) but here, clustering in domain I was not seen (Fig. 1A).

No consistent pattern of clustering has emerged in SMEB although 18/25 mutations were located in the transmembrane domains (Fig. 1B). Here, clustering of mutations was noted in the S2–S4 transmembrane segments of domain I, in contrast to patterns seen previously where clustering in domain II was observed (Mulley *et al.*, 2005). More data are required in order to establish if a true pattern of clustering exists.

Broader phenotypes of *SCN1A* mutations (Table 3)

The specific generalized epilepsy syndromes of MAE and LGS had a low yield of mutations with 2/10 and 1/12 positive cases respectively confirming that *SCN1A* is rarely associated with these syndromes (Wallace *et al.*, 2001; Nabbout *et al.*, 2003*b*; Ebach *et al.*, 2005). The nosological boundaries between these disorders, SMEI, SMEB and other cryptogenic generalized epilepsies are blurred. Indeed, in the large group of patients with cryptogenic generalized epilepsy of early onset where a more specific syndromal diagnosis could not be reached, 6/25 had *SCN1A* mutations. Two patients had a phenotype with features similar to SMEI but had onset in early infancy with abnormal early development and a more severe course. Others had heterogeneous phenotypes of generalized epilepsy with intellectual disability including those previously recognized in GEFS+ families (Scheffer and Berkovic, 1997; Singh *et al.*, 1999).

In patients classified as cryptogenic focal epilepsy, we identified a clinical subgroup who presented with a devastating multifocal epileptic encephalopathy. Of the five cases, three had *SCN1A* mutations. We designated this group severe infantile multifocal epilepsy (SIMFE) as onset is in the first year of life and multiple seizure types occur, with the most prominent being focal seizures. Multiple types of focal seizures occur including complex partial seizures of temporal lobe origin and hemiclonic seizures. Video-EEG telemetry showed that the variation in seizure semiology was not due to seizure spread patterns. Focal myoclonus may occur or even be brought out by specific anti-epileptic drugs known to exacerbate myoclonic seizures, such as vigabatrin. Patients may also have convulsive or non-convulsive status epilepticus, tonic seizures with focal features and tonic–clonic seizures. Interictal EEGs show abundant multifocal epileptiform discharges. These individuals do not have generalized spike-wave activity on EEG. Their MRI brain scans are normal or show non-specific features. They usually have normal early development followed by cognitive decline, with the refractory seizure disorder culminating in intellectual disability. Abnormal neurological signs such as ataxia and spasticity may evolve. The factor that distinguishes these children from SMEI is the absence of generalized absence and myoclonic seizures, generalized spike-wave activity on EEG, and that their cognitive decline may be later than the second year of life. These children had a severe, progressive and hitherto puzzling phenotype, where extensive investigations had been performed searching for an aetiology such as muscle biopsy, lumbar puncture and liver biopsy.

Similar cases are described in the literature by many authors (Noriega-Sanchez and Markand, 1976; Markand, 1977; Blume, 1978; Malik *et al.*, 1989; Ohtsuka *et al.*, 1990, 2000; Burnstine *et al.*, 1991; Ohtahara *et al.*, 1995; Nabbout and Dulac, 2003; Yamatogi and Ohtahara, 2003). Some clinicians regard this phenotype as being the later evolution of a 'burnt out' symptomatic generalized epilepsy, but these patients never have the EEG signature of generalized spike-wave activity. The phenotype could also be regarded as part of 'severe epilepsy with multiple independent spike foci' described by Ohtahara and colleagues where generalized minor seizures are also emphasized (Ohtsuka *et al.*, 1990; Ohtahara *et al.*, 1995; Yamatogi and Ohtahara, 2003, 2006). This group incorporates a heterogeneous array of causes

Table 3 Epileptic encephalopathies with *SCN1A* mutations

	SMEI ($n = 66$)	SMEB ($n = 36$)	CGE ($n = 25$)	CFE ($n = 13$)	SIMFE ($n = 5$)
Average age seizure onset (months)	5.5	6	9.5	8	4
Clinical features					
Hemiclonic and/or generalized convulsions	Always	Always	Often	Often	Often
Myoclonic seizures	Always	Often	Often	Occasional	Often
Other focal seizures	Often	Often	Occasional	Always	Always
Other generalized seizures	Often	Often	Often	Occasional	Rare
EEG					
Generalized spike wave	Always	Occasional	Often	Rare	No
Multifocal epileptiform activity	Occasional	Occasional	Occasional	Occasional	Always
SCN1A mutations	52 (79%)	25 (69%)	6 (24%)	1 (8%)	3 (60%)
Truncation	23	10	–	–	1
Missense	20	13	5	1	2
Splice site	9	2	1	–	–

SMEI, severe myoclonic epilepsy of infancy; SMEB, SMEI borderland; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; SIMFE, severe infantile multifocal epilepsy.

such as tuberous sclerosis and birth asphyxia. In contrast, SIMFE encompasses those patients hitherto without a known cause, with 3/5 found to have mutations of *SCN1A*. SIMFE is an important group of patients with a devastating epileptic encephalopathy who are presently difficult to classify. The discovery of *SCN1A* mutations as the basis of their disorder avoids further potentially invasive investigations for alternative causes and assists in targeting therapy. For example, anti-epileptic drugs that exacerbate myoclonic seizures, such as vigabatrin and tiagabine, should be avoided.

This extensive study of the role of *SCN1A* mutations in epileptic encephalopathies beginning in the first year of life has, not surprisingly, expanded the phenotypic spectrum. Disorders are initially identified in a 'pure cohort' with a specific group of essential features. As the molecular basis is determined, phenotype–genotype correlation results in broadening of the phenotypic spectrum to include milder cases or seemingly unrelated disorders. This is just beginning to be possible in epileptology, as *SCN1A* is the first gene shown to have a role in epilepsies previously regarded as cryptogenic. An important finding is that children with an epileptic encephalopathy with multifocal features in the setting of normal MRI may have *SCN1A* mutations as may children with cryptogenic generalized epilepsy. A strong indicator for *SCN1A* analysis is an epileptic encephalopathy with seizure onset before 1 year of age, even if cognitive decline does not occur for several years thereafter. The social and economic benefit in making a definitive diagnosis in children with epileptic encephalopathies cannot be underestimated. Neurologists continue to perform investigations looking for an aetiology in children with cryptogenic encephalopathies such that establishing a definitive molecular diagnosis is cost-effective. More importantly, families are very grateful for a specific diagnosis especially with the treatment and genetic counselling implications that a *SCN1A* mutation carries.

Supplementary material

Supplementary material is available at *Brain* Online.

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TABLE 2: *SCN1A* mutations in patients with epileptic encephalopathies

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
1	SMEI	M	4.5	Exon 1	c.41delT	F14fsX91	N-terminal	*De novo*	Novel
2	SMEI	F	4	Exon 1	c.251A>G	Y84C	N-terminal	*De novo*	Novel
				Intron 9	*c.1378-1G>A*	*IVS9-1G>A*	*DI-DII linker*	*Maternal*	*Novel - non pathogenic*
3	SMEI	F	7	Intron 1	c.265-1G>A	IVS1-1G>A	N-terminal	*De novo*	Novel
4	SMEI	F	6	Exon 2	c.301C>T	R101W	N-terminal	*De novo*	Novel - this study [SMEB-SW]
5	SMEI	M	5	Exon 2	c.302G>A	R101Q	N-terminal	*De novo*	Fukuma et al., 2004 [SMEB]
6	SMEI	F	4	Intron 3	c.474-13T>A	IVS3-13T>A	DIS2	ND	Novel
				Intron 4	*c.602+5G>A*	*IVS4+5G>A*	*DIS3*	*ND*	*Novel - non pathogenic*
7	SMEI	F	5.5	Exon 4	c.495_496insGTGAATC	T166fsX170	DIS2	*De novo*	Novel
8*	SMEI	F	5	Intron 4	c.602+1G>A	IVS4+1G>A	DIS3	*De novo*	Fujiwara et al., 2003 [SMEI] This study [SMEB]
9	SMEI	F	3	Intron 7	c.1028+1G>T	IVS7+1G>T	DIS5-S6 loop	*De novo*	Novel - this study [SMEI]
10	SMEI	M	3	Intron 7	c.1028+1G>T	IVS7+1G>T	DIS5-S6 loop	*De novo*	Novel - this study [SMEI]
11	SMEI	F	2.25	Exon 8	c.1048_1049delAT	M350fsX355	DIS5-S6 loop	*De novo*	Novel
12*	SMEI		6	Exon 8	c.1055_1056delTG	V352fsX355	DIS5-S6 loop	*De novo*	Novel
13*	SMEI	M	4	Exon 9	c.1197C>A	Y399X	DIS5-S6 loop	*De novo*	Novel
14	SMEI	F	4	Exon 9	c.1207T>C	F403L	DIS6	*De novo*	Novel
15	SMEI	F	7	Exon 9	c.1237T>A	Y413N	DIS6	*De novo*	Novel
16	SMEI	F	3.5	Exon 10	c.1639_1640delAA	K547fsX569	DI-DII linker	ND	Novel
17*	SMEI	F	3	Intron 10	c.1662+2T>C	IVS10+2T>C	DI-DII linker	*De novo*	Novel
18*	SMEI	M	9.5	Intron 10	c.1663-1G>C	IVS10-1G>C	DI-DII linker	*De novo*	Novel
19	SMEI	F	5	Exon 11	c.1687delC	L563fsX622	DI-DII linker	*De novo*	Novel
20	SMEI	M	4	Exon 11	c.2021A>G	D674G (long isoform)	DI-DII linker	*De novo*	Novel
21	SMEI	M	6	Exon 13	c.2348T>C	L783P	DIISI	*De novo*	Novel
22*	SMEI	F	8	Exon 14	c.2562delA	G854fsX876	DIIS3-S4 loop	*De novo*	Novel
23*	SMEI	F	4	Intron 14	c.2589+3A>T	IVS14+3A>T	DIIS4	*De novo*	Novel
24	SMEI	M	7	Exon 15	c.2831T>A	V944E	DIIS5-S6 loop	*De novo*	Novel
25*	SMEI	F	6	Exon 15	c.2833T>C	F945L	DIIS5-S6 loop	ND	Novel

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
26	SMEI	M	2.5	Exon 15	c.2837G>A	R946H	DIIS5-S6 loop	*De novo*	Fukuma et al., 2004 [SMEB]
27*	SMEI	F	4	Exon 15	c.2849G>A	G950E	DIIS5-S6 loop	*De novo*	Novel
28	SMEI	F	6	Exon 15	c.2893C>T	Q965X	DIIS5-S6 loop	ND	Novel
29	SMEI	M	3.5	Exon 16	c.3096delA	E1032fsX1045	DII-DIII linker	*De novo*	Novel
30	SMEI	M	7	Exon 17	c.3462delT	G1154fsX1163	DII-DIII linker	*De novo*	Novel
31	SMEI	F	5	Exon 18	c.3561_3562delAA	Q1187fsX1215	DII-DIII linker	*De novo*	Novel
32	SMEI	M	3	Exon 19	c.3714A>C	E1238D	DIIIS1-S2 loop	ND	Novel
				Intron 14	*c.2589+1G>C*	*IVS14+1G>C*	*DIIS4*	*ND*	*Novel - non pathogenic*
33	SMEI	F	7	Exon 19	c.3733C>T	R1245X	DIIIS1-S2 loop	ND	Nabbout et al., 2003 [2x SMEI]
34	SMEI	F	7	Exon 21	c.4219C>T	R1407X	DIIIS5-S6 loop	*De novo*	Sugawara et al., 2002 [SMEI]
									Fukuma et al., 2004 [SMEI]
35	SMEI		6	Exon 22	c.4321G>C	A1441P	DIIIS5-S6 loop	*De novo*	Novel
36	SMEI	M	2	Exon 24	c.4526delA	N1509fsX1511	DIII-DIV linker	*De novo*	Novel
37*	SMEI	F	4.5	Exon 24	c.4547C>A	S1516X	DIII-DIV linker	*De novo*	Sugawara et al., 2002 [2x SMEI]
38*	SMEI	F	8.5	Exon 24	c.4573C>T	R1525X	DIII-DIV linker	ND	Kearney et al., 2006 [SMEI]
									This study [SMEI]
				Exon 11	*c.1811G>A*	*R604H*	*DI-DII linker*	*ND*	*Novel - non pathogenic*
39	SMEI	F	5	Exon 24	c.4573C>T	R1525X	DIII-DIV linker	ND	Kearney et al., 2006 [SMEI]
									This study [SMEI]
40	SMEI	F	8	Exon 25	c.4633A>G	I1545V	DIVS1	*De novo*	Novel
41	SMEI	M	4	Exon 25	c.4794T>A	Y1598X	DIVS2-S3 loop	*De novo*	Novel
42	SMEI	M	9	Intron 25	c.4853-14T>G	IVS25-14T>G	DIVS3	*De novo*	Novel
43	SMEI	M	4	Exon 26	c.4933C>T	R1645X	DIVS4	*De novo*	Fukuma et al., 2004 [SMEI]
44	SMEI	F	6	Exon 26	c.4934G>A	R1645Q	DIVS4	*De novo*	Novel
45	SMEI	M	3	Exon 26	c.5119T>G	F1707V	DIVS5-S6 loop	ND	Novel
46	SMEI	M	4	Exon 26	c.5162C>G	T1721R	DIVS5-S6 loop	*De novo*	Novel
47*	SMEI	M	9	Exon 26	c.5176T>C	W1726R	DIVS5-S6 loop	*De novo*	Novel
48	SMEI	F	4	Exon 26	c.5347G>A	A1783T	DIVS6	*De novo*	Novel - this study [SMEB-SW]
49	SMEI	F	6	Exon 26	c.5436G>A	W1812X	C-terminal	*De novo*	Novel
50*	SMEI	F	4	Exon 26	c.5656C>T	R1886X	C-terminal	*De novo*	Mancardi et al., 2006 [SMEI]

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
51*	SMEI	M	6	Exon 26	c.5710C>T	Q1904X	C-terminal	*De novo*	Novel
52	SMEI	M	5	Exon 26	c.5765T>C	I1922T	C-terminal	ND	Novel
53	SMEB-O	M	3	Exon 1	c.235G>C	D79H	N-terminal	*De novo*	Novel
54	SMEB-SW	F	8	Exon 2	c.301C>T	R101W	N-terminal	*De novo*	Novel - this study [SMEI]
55	SMEB-SW	M	7	Exon 4	c.512T>A	I171K	DIS2	*De novo*	Novel
56	SMEB-O	F	6	Exon 4	c.523G>A	A175T	DIS2	*De novo*	Novel
57	SMEB-O	F	3	Exon 4	c.580G>A	D194N	DIS3	ND	Mancardi et al., 2006 [SMEI]
58	SMEB-SW	F	7	Exon 4	c.596C>G	T199R	DIS3	*De novo*	Novel
59*	SMEB-O	M	4	Intron 4	c.602+1G>A	IVS4+1G>A	DIS3	*De novo*	Fujiwara et al., 2003 [SMEI] Marini et al. 2006 [SMEI] This study [SMEI]
60	SMEB-O	F	6	Exon 5	c.664C>T	R222X	DIS4	*De novo*	Claes et al., 2001 [SMEI] Nabbout et al., 2003 [2x SMEI]
61	SMEB-O	F	6.5	Exon 5	c.677C>T	T226M	DIS4	*De novo*	Novel - this study [CGE]
62	SMEB-SW	M	6	Exon 5	c.680T>G	I227S	DIS4	ND	Nabbout et al., 2003 [3x SMEI]
63	SMEB-SW	M	6	Exon 6	c.715G>A	A239T	DIS4-S5 loop	Paternal	Novel
64	SMEB-O	F	6	Exon 8	c.1152G>A	W384X	DIS5-S6 loop	ND	Novel
65	SMEB-SW	M	7	Intron 14	c.2589+2T>A	IVS14+2T>A	DIIS4	*De novo*	Novel
66	SMEB-SW	M	5.5	Exon 16	c.3022G>T	E1008X	DII-DIII linker	*De novo*	Novel
67	SMEB-O	F	3	Exon 16	c.3231delA	K1077fsX1079	DII-DIII linker	*De novo*	Novel
68	SMEB-M	F	5	Exon 21	c.4062delT	C1354fsX1359	DIIIS5	*De novo*	Novel
69*	SMEB-M	M	9	Exon 21	c.4168G>A	V1390M	DIIIS5-S6 loop	*De novo*	Ohmori et al., 2002 [SMEI]
70	SMEB-SW	F	6	Exon 21	c.4186T>G	C1396G	DIIIS5-S6 loop	*De novo*	Novel
71	SMEB-SW	F	6.5	Exon 21	c.4279C>T	Q1427X	DIIIS5-S6 loop	*De novo*	Novel
72	SMEB-M	F	4.5	Exon 26	c.4949_4950insT	I1650fsX1672	DIVS4	*De novo*	Novel
73	SMEB-O	F	4	Exon 26	c.5339T>C	M1780T	DIVS6	*De novo*	Nabbout et al., 2003 [SMEI]
74	SMEB-SW	F	10	Exon 26	c.5347G>A	A1783T	DIVS6	*De novo*	Novel - this study [SMEI]
75*	SMEB-SW	F	4	Exon 26	c.5536_5539delAAAC	K1846fsX1856	C-terminal	*De novo*	Claes et al., 2001 [SMEI] Wallace et al., 2003 [SMEI] Kearney et al., 2006 [SMEI]

Patient	Phenotype	Sex	Onset (mths)	Location	Nucleotide change	Protein change	Position	Inheritance	Published [phenotype] / Novel #
76	SMEB-O	F	3	Exon 26	c.5674C>T	R1892X	C-terminal	*De novo*	Sugawara et al., 2002 [SMEI] Fukuma et al., 2004 [2x SMEI]
77	ICEGTC	F	7	Exon 26	c.5741_5742delAA	Q1914fsX1943	C-terminal	*De novo*	Novel
78	CGE	M	2	Exon 5	c.677C>T	T226M	DIS4	*De novo*	Novel - this study [SMEB]
79	CGE	M	5.5	Exon 9	c.1183G>C	A395P	DIS5-S6 loop	*De novo*	Novel
80	CGE	M	1.5	Exon 9	c.1265T>A	V422E	DIS6	*De novo*	Novel
81	CGE	M	12	Exon 11	c.1876A>G	S626G	DI-DII linker	ND	Novel
82	CGE	F	9	Exon 15	c.2917A>G	M973V	DIIS6	Paternal	Novel
83	CGE	F	6	Intron 15	c.2946+1G>T	IVS15+1G>T	DIIS6	*De novo*	Novel
84	CFE (SIMFE)	F	7	Exon 11	c.1724delT	F575fsX622	DI-DII linker	*De novo*	Novel
85	CFE (SIMFE)	F	4.5	Exon 25	c.4628T>C	F1543S	DIVS1	Maternal	Novel
86*	CFE (SIMFE)	F	5	Exon 25	c.4786C>T	R1596C	DIVS2-S3 loop	*De novo*	Novel
87*	CFE	M	18	Exon 26	c.4970G>A	R1657H	DIVS4	*De novo*	Novel
88	LGS	M	0.75	Exon 26	c.4907G>A	R1636Q	DIVS4	*De novo*	Novel
89*	MAE	M	4	Exon 9	c.1177C>T	R393C	DIS5-S6 loop	*De novo*	Novel
90	MAE	M	13	Exon 24	c.4439G>T	G1480V	DIIIS6	*De novo*	Novel

Novel mutations refer to those not previously reported, note that some novel mutations are recurrent in this cohort

* Patients sequenced by Athena Diagnostics

Mutations in italics were not considered pathogenic, see text for explanation, and are not included in figure 1.

SMEI = Severe Myoclonic Epilepsy of Infancy, SMEB-SW = SMEI Borderland without generalised spike wave, SMEB-M = SMEI Borderland without myoclonic seizures, SMEB-O = SMEI Borderland lacking more than 1 feature of SMEI, ICEGTC = Intractable Childhood Epilepsy with Generalised Tonic Clonic seizures, CGE = Cryptogenic Generalised Epilepsy,

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Lamotrigine and Seizure Aggravation in Severe Myoclonic Epilepsy

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Summary: *Purpose:* In severe myoclonic epilepsy of infancy (SME), multiple drug-resistant focal and generalized seizure types occur. Lamotrigine (LTG), found effective in many generalized and partial seizures, has been little used in severe childhood epilepsy syndromes with multiple seizure types. We studied the effects of LTG in SME.

Methods: Twenty-one patients with SME, aged 2–18 years, were treated with LTG, 20 in add-on and one in monotherapy. LTG was started at 0.2–2.5 mg/kg/day and increased to 2.5–12.5 mg/kg/day. For each seizure type, excluding atypical absences, >50% variations compared with the 2 months preceding LTG were considered indicators of response, also taking into account the degree of disability each seizure type produced.

Results: LTG induced worsening in 17 (80%) patients, no change in three, and improvement in one. There was >50% increase in convulsive seizures in eight (40%) of 20 patients. Myoclonic seizures worsened in six (33%) of 18 patients. Of five patients improving in at least one seizure type, four had concomitant worsening of more invalidating seizures. Clear-cut worsening appeared within 3 months in most patients but was insidious in some. LTG was suspended in 19 patients after 15 days–5 years (mean, 14 months) with consequent improvement in 18.

Conclusions: The pronounced seizure deterioration during LTG treatment was not attributable to the natural course of the disease and could be a direct effect of therapeutic LTG doses. LTG treatment seems inappropriate in SME. **Key Words:** Lamotrigine—Severe myoclonic epilepsy—Seizure worsening.

Severe myoclonic epilepsy (SME) in infants (1) is one of the most disabling epileptic syndromes. Seizures begin during the first year of life in previously normal children as generalized or unilateral attacks, facilitated by fever, and often occurring in the form of status epilepticus. Such seizures are followed later, between ages 1 and 4 years, by myoclonus, atypical absences, and complex partial seizures, accompanied in some children by clinical photosensitivity. Often coinciding with the onset of myoclonus, there is a slowing in psychomotor development, patients being variably mentally retarded from school age on. All seizure types are extremely resistant to drug treatment. Although SME is diagnosed only in ~1% of patients with epilepsy (2), the management of these patients is particularly time demanding and costly, as they undergo multiple periods of hospitalization and antiepileptic drug (AED) trials in which almost all combinations of available drugs are tried.

Lamotrigine (LTG) has proven to be effective in the treatment of many generalized and partial seizure types in both adults and children (3–5). Although LTG is currently used in various childhood epilepsy syndromes (4, 6,7), conclusive data on its efficacy in severe epilepsies with multiple seizure types are scanty. Add-on LTG management of Lennox–Gastaut syndrome and nonspecific forms of symptomatic generalized epilepsies has been evaluated, with several open studies (7–10) and one controlled study (11). Results appear to be favorable, as reflected in reduction of atonic, tonic, and atypical absence seizures. No data on the efficacy of LTG in SME are available to date.

After preliminary observations of seizure aggravation in four patients (12), we examined the results of LTG treatment in 21 patients with SME, the majority of whom had been treated with add-on in the framework of prospective studies including children affected by different types of severe epilepsies.

PATIENTS AND METHODS

Data were collected in three centers. Twenty-one patients with SME, aged 2–18 years (mean, 9 years 1

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month) were treated with LTG, 20 in add-on and one in monotherapy. All patients had poorly controlled epilepsy. Nineteen had two or more types of seizures, and two had one seizure type only (Table 1). All patients had been treated with various antiepileptic drugs (AEDs), singly or in various combinations, which had failed to achieve seizure control. LTG was added to the preexisting drug regimen, starting with a daily dosage of 0.2–2.5 mg/kg (the smaller starting dosages were used to minimize the risk of skin rashes, especially in VPA-treated patients) and increased to a maximum dose of 2.5–10 mg/kg/day according to type of comedication and clinical response. A twice-daily dosing regimen was used in all patients. AEDs used at the start of LTG treatment are shown in Table 1. Concurrent AEDs were adjusted depending on patients' response, but on account of the lack of positive clinical response, in most patients previous medication could not be tapered. One patient (patient 12) was enrolled in a prospective trial of LTG monotherapy that was continued for 1 year. Sixteen patients were included in prospective studies of add-on LTG treatment. Data for the remaining four patients were collected retrospectively. All patients underwent EEG during wakefulness and sleep while receiving LTG treatment. However, only in 12 of the 21 patients was one EEG performed within the 2 months preceding LTG treatment, and another, designed to be comparable, during treatment. Throughout the period of LTG treatment, patients were seen regularly every 1–3 months in the clinic for evaluation of clinical efficacy of the drugs and any adverse effects, through general questioning of parents and clinical examination. Seizures were recorded daily in calendars by the patients' relatives. Treatment was continued for as long as it was thought either to improve seizures or to have beneficial effects on patient well-being. LTG was discontinued by reducing the maintenance dose

TABLE 1. *Main clinical findings of 21 patients with severe myoclonic epilepsy and effects of LTG treatment*

Pat No. sex	Age at beginning of LTG	Age at follow-up	Seizure types before LTG	Associated AEDs	Max LTG dose (mg/kg/day	Improved seizures	Worsened seizures	Appearance of new seizures	Duration of LTG treatment	Follow-up after LTG discontinuation	Overall LTG effect
1/F	6 yr 5 mo.	7 yr 6 mo	CP, AA, UCl, Myocl	VPA, CZP	5	No	UCl	No	7 mo	6 mo	Aggravation
2/M	2 yr 8 mo	5 yr 7 m	GCl, SP, CP, SG	VPA, CLB	4.2	No	GCl	No	4 mo	2 yr 6 mo	Aggravation
3/F	2 yr	5 yr	GCl, Myocl, T	VPA, CZP, PGB	5	No	Myocl	No	4 mo	2 yr 8 mo	Aggravation
4/M	4 yr 2 mo	6 yr 3 mo	UCl, Cl, AA SP, Myocl	VPA	4.1	SP, Myocl	No	GTC	5 mo	1 yr 8 mo	Aggravation
5/M	5 yr 10 mo	7 yr 3 mo	GTC, AA, Myocl	VPA, CZP	10	No	Myocl	No	5 mo	1 yr	Aggravation
6/F	3 yr 10 mo	5 yr	SP, Myocl, AA, GTC	VPA, CZP	2.5	No	GTC	No	1 yr 2 mo	3 mo	Aggravation
7/F	10 yr 10 mo	12 yr	SP, SG, AA, GCl, GTC, T, Myocl	VPA, ESM, CZP	3.3	No	GCl	No	2 mo	1 yr	Aggravation
8/F	5 yr	7 yr	UCl, SG, AA, Myocl	GVG, CZP	10	No	GTC	No	7 mo	1 yr 7 mo	Aggravation
9/M	12 yr	15 yr	GTC, Myocl, AA	VPA, CZP	5	GTC, Myocl	No	Myocl-at.	1 yr 1 mo	2 yr	Tremor-Aggravation
10/F	3 yr 7 mo	5 yr 4 mo	GTC, AA, CP, Myocl	PB, CZP	7.5	No	Myocl	No	7 mo	1 yr 2 mo	Aggravation
11/M	9 yr 6 mo	11 yr	AA, Myocl, GCl, CP	VPA, CLB, PB, GVG	3	No	CP	No	0.5 mo	1 yr 6 mo	Aggravation
12/M	18 yr	22 yr	Myocl	No	5.5	No	Myocl	No	1 yr	3 yr	Aggravation
13/M	3 yr	4 yr 4 mo	Myocl, GTC	VPA, ESM	5	No	GTC	No	5 mo	1 yr	Aggravation
14/F	4 yr 9 mo	13 yr	GCl	PHT, CLB	5	No	No	No	1 yr 3 mo	7 yr	No change
15/M	10 yr 2 mo	12 yr	GCl, Myocl	VPA, GVG	4	No	No	No	1 yr	10 mo	No change
16/F	4 yr	6 yr 6 mo	GCl, GTC, Myocl	VPA, CLB, PGB	5.3	No	GCl	No	8 mo	1 yr 10 mo	Aggravation
17/M	4 yr 7 mo	7 yr 6 mo	GTC, Myocl, Myocl Ab	VPA, CZP	2.5	GTC	Myocl	Myocl status	2 yr 2 mo	9 mo	Aggravation
18/F	9 yr 10 mo	17 yr	GTC, CP, GCl, Myocl, SP	VPA, CLB	7.4	Cl	Myocl	No	5 yr	—	Aggravation
19/F	2 yr 3 mo	4 yr 6 mo	GTC, Myocl, GCl, AA, Unil	VPA, CZP, PGB	4	No	Unil	No	3 mo	2 yr	Aggravation
20/F	12 yr 3 mo	14 yr 6 mo	UCl, GTC	GVG, CZP, CLB	4.4	UCl, GTC	No	No	2 yr 3 mo	Still taking LTG	Improvement
21/F	5 yr	10 yr 5 mo	AA, Myocl, GTC, Myocl status	PB, CZP	12.5	No	No	No	5 yr 4 mo	Still taking LTG	No change

F, female, M, male, AA, atypical absences; at, atonic, Cl, clonic; CP, complex partial; GCl, generalized clonic; GTC, generalized tonic–clonic; Myocl, myoclonic; Myocl Ab, myoclonic absences; SG, secondarily generalized; SP, simple partial; T, tonic; UCl, unilateral clonic; Unil, unilateral; CLB, clobazam; CZP, clonazepam, ESM, ethosuximide; GVG, vigabatrin; LTG, lamotrigine; PGB, progabide; PHT, phenytoin; PB, phenobarbial; VPA, valproate.

by 25 mg/week or to 50% for 2 weeks and then to 25% for a further 2 weeks.

To assess the effects of LGT, seizure frequency was determined for the 2 months preceding LTG introduction and was compared with the frequency during LTG treatment. Variations >50% were taken as indicators of improvement or worsening. Because epilepsy syndromes with multiple seizure types may show different degrees of disability and varying response to treatment, we assessed the efficacy of LTG relative to each seizure type, excluding atypical absences, and expressed a global impression of seizure-related disability. In general, convulsive seizures were considered more severe than myoclonic seizures, which in turn were more severe than complex partial seizures. To estimate seizure-related disability, we also took the specific seizure characteristics of each patient into account, because extensive intra- and interindividual variation was observed in any given category. For example, prolonged clonic seizures followed by protracted postseizure sedation were found to be far more disabling than brief tonic–clonic seizures occurring during sleep, although both are convulsive seizures. Because clinical detection of subtle seizures such as atypical absences is arbitrary, especially in mentally retarded children, for the latter, we merely requested parents to provide us a global impression of the modification in total quantity of absences.

RESULTS

Results are summarizd in Table 1. LTG treatment produced worsening of the epileptic syndrome in 17 (80%) patients, resulted in no substantial change in three and in improvement in one. Table 2 shows the cumulative effect on the main seizure types. There was appearance of new seizure types in three patients. Analysis of the distribution of the various drugs administered as comedication in patients who showed no seizure exacerbation compared with those with worsening suggested that the aggravation was not attributable to a particular association with other AEDs.

TABLE 2. *Cumulative effects of LTG on the main seizure types (21 patients)*

Main seizure types		Worsening >50%	Improvement >50%	No change
Myoclonic	18	6	2	10
Absence	12	0	0	12
Gen Clonic	10	3	1	6
Gen TC	12	3	3	6
CP	5	1	0	4
SP	5	0	1	4
UCI	5	2	1	2

Gen, generalized; TC, tonic–clonic; CP, complex partial; SP, simple partial, UCI, unilateral clonic.

The most important finding was the >50% increase in the frequency of convulsive seizures (unilateral, generalized clonic, and generalized tonic–clonic) in eight (40%) of 20 patients. Myoclonic seizures also worsened in six (33%) of 18 patients, in one of whom a first episode of myoclonic status occurred after LTG administration. Although an improvement in at least one seizure type was found in five patients (patients 4, 9, 17, 18, and 20), four of them were considered to have had no improvement because there was a concomitant worsening of more disabling types of seizures (patients 4, 9, 17, and 18).

In most patients, seizure worsening appeared between 15 days and 3 months after the start of LTG treatment, occurring in three of them during titration. In some patients, however, worsening was slow and insidious, becoming evident over several months.

LTG was suspended in 19 patients after 15 days to 5 years (mean, 14 months) from its introduction. In 18 patients, seizures improved with reversal to levels preceding LTG treatment. In 14 of them, including the one receiving LTG monotherapy (patient 12), improvement occurred on LTG discontinuation, without introducing further medication, whereas in the remaining five, LTG was replaced by another drug. After LTG discontinuation, patient 12 was maintained drug free for 1 year because the parents were skeptical about the usefulness of any treatment. Mean duration of follow-up after cessation of LTG treatment was 21 months (range, 1 month–7 years). The parents of four children who had improved or unchanged seizures claimed to have noticed an improvement in their child's behavior and alertness (better contact, less irritable). For this reason, treatment was continued in some of the unchanged patients (14, 15, and 21) at their parents' request, even after a lack of efficacy on seizures had been observed.

Of the 12 patients who underwent EEG in the 2 months before and during LTG therapy, three (patients 6, 7, and 17) showed increase in interictal paroxysmal EEG abnormalities, two (patients 1 and 16), a moderate slowing in background EEG activity; six (patients 8–13) showed no change, and one, an improvement, with marked reduction of interictal paroxysmal activity (patient 20).

DISCUSSION

Although pediatric experience with LTG is still limited, this drug has been reported to be effective in controlling generalized and partial seizures in childhood and to be of particular value in the management of absence seizures (7).

The poor results we observed in SME, with seizure worsening in 80% of patients, have no easy explanation. Severe childhood epileptic syndromes are particularly

prone to AED-induced seizure aggravation (13,14), through mechanisms that are as yet poorly understood. Although the majority of reports referred to West and Lennox–Gastaut syndrome, aggravation of generalized convulsive seizures in SME also has been reported with CBZ treatment (15,16).

Exacerbation of seizure frequency has been reported in some studies involving LTG in children (4,7), although no mention was made as to whether this adverse effect involved a particular seizure type or a specific syndrome. However, in these studies, the number of patients who worsened did not exceed 11%, a rate of worsening no higher than that observed in patients with drug-resistant seizures after addition of placebo (17).

Although periods of spontaneous improvement or worsening are quite commonly observed in severe epilepsies (18), especially in children, the rate of deterioration we recorded during LTG treatment was too pronounced to be attributed to the natural course of the disease in most patients. On the other hand, deterioration could not be ascribed to concurrent tapering of previous medication or to a particular combination of LTG with other drugs. It appears, therefore, that deterioration of convulsive and myoclonic seizures in SME should be related to a direct effect of LTG administered at therapeutic doses.

Although in three patients, severe worsening occurred during LTG titration, increase in seizures was insidious rather than showing the sudden onset that generally occurs after inappropriate drug choice or paradoxic reaction (13,14). This may have been due to the slow increase in LTG dose we adopted at the beginning of treatment.

The few available data on the effect of LTG in epilepsies with predominant myoclonic seizures have suggested that lack of efficacy or worsening are found in a substantial proportion of both adult and pediatric patients (7,19,20). However, in these studies, the type of myoclonic epilepsy is infrequently specified. On the other hand, LTG can be effective in myoclonus associated with typical 3-Hz spike-and-wave discharges of myoclonic astatic epilepsy, juvenile myoclonic epilepsy, and myoclonic absences (21,22).

A characteristic of SME is the multiplicity of seizure types observed, with multiple brain areas appearing able to generate seizures. In this syndrome, it has been suggested that myoclonic seizures would seem to be more an expression of local than of generalized epileptogenesis (23). Yet the known efficacy of LTG in convulsive seizures (24) makes the deterioration we observed in this type of seizure particularly surprising. SME is a neurobiologically unitary syndrome, as testified by its cryptogenicity, low convulsive threshold, homogeneous clinical presentation, and strong family history of epilepsy (25–54%; 25–29) with several affected siblings including monozygotic twins (1,30,31). Multifocal microdysgenesis of cerebral and cerebellar cortex, observed by Renier and Renkawek (23) in one autopsied case, could be the neuropathologic substrate for this syndrome. Although such structural changes need further confirmation, they could account for the high epileptogenicity.

LTG is reported to be effective in the treatment of both typical and atypical absence seizures (9,32,33). Even though these reports have not been supported by quantification of seizures with EEG monitoring, the amount of clinical evidence gathered so far seems to indicate that LTG is a powerful drug against absence seizures of epilepsies with abundant spike–wave activity. In SME, generalized spike and wave activity, although present in almost all patients, is scanty. Atypical absences were described by the parents of our patients as showing no substantial change in frequency with LTG. However, because assessment of atypical absences in our study was performed without prolonged EEG monitoring, we do not believe our experience can provide any reliable information concerning the effects of LTG on this type of seizure in SME.

Although results of medical treatment are in general disappointing in SME (34), VPA and BZDs are preferable to other drugs. In these patients, PHT offers no obvious advantage and may produce more severe side effects than PB (1,2). CBZ may worsen myoclonus and atypical absence seizures (35,36). ESM may be helpful in reducing myoclonus. Among the new AEDs, VGB may lead to interesting results, reducing convulsive seizures, but only in older patients in whom myoclonus is no longer a prominent symptom (37).

The poor results obtained, with a marked tendency toward seizure aggravation, suggest that use of LTG is inappropriate in SME.

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Carbamazepine-exacerbated Epilepsy in Children and Adolescents

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Forty-nine children and adolescents whose seizures reportedly worsened while receiving carbamazepine (CBZ) were studied retrospectively. Twenty-six patients met criteria for excellent documentation of carbamazepine-exacerbated seizures. Four epileptic syndromes were particularly affected: childhood absence epilepsy; focal symptomatic, frontal lobe epilepsy; Lennox-Gastaut syndrome; and severe myoclonic epilepsy of infancy. Eight of the 26 patients developed new-onset absence seizures and three patients with established absence epilepsy experienced absence status. Other seizure types, including atonic, tonic-clonic, and myoclonic, developed in eight patients treated with CBZ, and new generalized spike-and-wave discharges were observed in electroencephalograms of nine patients. CBZ is a widely used, effective antiepileptic drug, particularly for partial or partial complex seizures; however, if uncontrolled, generalized seizures occur after CBZ is prescribed for children or adolescents with absence or mixed seizures, a trial of CBZ discontinuation is warranted. The data reported here do not permit calculation of the incidence of this phenomenon.

Horn CS, Ater SB, Hurst DL. Carbamazepine-exacerbated epilepsy in children and adolescents. Pediatr Neurol 1986;2:340-5.

Introduction

Several authors recently expressed concern about carbamazepine-induced seizures [1-5]. In 1983, Shields and Saslow [1] initially described five children treated with carbamazepine (CBZ) who had explosive onset of atonic, absence, and/or myoclonic seizures. Four of the five patients improved with the discontinuation of CBZ. Johnson et al. [2] reported three pediatric patients with similar difficulties which confirmed the original observations of Shields and Saslow, and added an important finding; the adverse response to CBZ is associated with diffuse spike-and-wave abnormalities of the electroencephalogram (EEG). Sachdeo and Chokroverty [3] reported four adults with childhood absence epilepsy who had increases in absence spells with the use of CBZ which had been prescribed for the control of generalized tonic-clonic seizures. This adverse effect was found to be dose-dependent. Finally, in an elegant study using video-EEG telemetry, Snead and Hosey reported 15 children with CBZ-exacerbated seizures [4]. Of these 15 patients 11 had "atypical" absence, eight had "generalized convulsive," and four had simple partial seizures as components of their epilepsy.

Methods

Records were obtained on 49 children and adolescents followed at Fitzsimons Army Medical Center (FAMC) from January 1983 to December 1985 whose seizures were reported by their parents to have worsened during treatment with CBZ. The following information was obtained from medical records: demographic data, seizure characteristics, associated neurologic abnormalities, antiepileptic drugs, and EEG results. The following factors relative to carbamazepine therapy were reviewed for their status before, during, and after the patients had received the drug:

(1) CBZ dosing;
(2) Other antiepileptic drug treatment;
(3) Seizure quality, type, and frequency;
(4) EEG results; and
(5) Blood levels of antiepileptic drugs.

The data were evaluated to determine if excellent documentation existed to demonstrate that CBZ exacerbated the patient's epilepsy. For classification to be excellent, documentation of factors 1-4 was required before, during, and after CBZ therapy; factor 5 (a therapeutic blood level) was required during CBZ therapy. All descriptions of seizure activity and all interpretations of EEGs were based on the International Classification of Seizures [6] and on the International Classification of Epilepsy [7].

The classifications of the patients' seizures were analyzed to determine which seizure types and syndromes were exacerbated by CBZ, and which were caused or uncovered by the drug. A worsening of seizure activity was defined as:

(1) An estimate of increased seizure frequency of >50% of that reported for a baseline period of one month or more (usually from less than one seizure per day to multiple seizures per day);

(2) An increase of individual seizure duration from seconds to minutes or from minutes to status epilepticus; or,

(3) The description of a different new type of seizure.

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Table 1. Patients with CBZ-exacerbated seizures (before CBZ use)

Patient	Age (Years)	Seizure Types	EEG Findings	Epilepsy Type
1	10	F, Ab	FS, 3 Hz SW	FL
2	15	F, PC, T, TC	FS	FL
3	11	F, PC, TC	FS, PSW	FL
4	6	F, PC	FD	FL
5	16	PC	FD	FL
6	12	M, TC, Un	FS	FL
7	8	PC, TC	FS	TL
8	11	V	FS	OL
9	10	PC	Nl	FS-nfc
10	9	PC, TC	FD	FS-nfc
11	7	Ab	2.5 Hz SW	CA(a)
12	17	Ab, TC	PSW	CA(a)
13	17	Ab	3.5 Hz SW	CA
14	11	Ab	Nl	CA
15	15	Ab, TC	3 Hz SW	CA
16	10	Ab	SW	CA
17	18	Ab, TC	Nl	JA
18	4	TC	Nl	TC
19	5	M, TC	SW	IMC
20	8	M, TC, Un	MFS, SW	EME
21	12	Ab, TC, At, M	3 Hz SW, PSW	CLF-J
22	10	M, TC, Un	SW	SMC
23	6	Ab, PC, TC, At	MFS, PSW	LGS
24	7	Ab, TC, At, Un	2 Hz SW	LGS
25	2	Fb, M, Ab, TC	PSW	SMEI
26	16	Fb, F, T, TC	PSW	SMEI

Abbreviations in Tables 1-3

Seizure Types:

Ab	=	Absence
At	=	Atonic
Fb	=	Febrile
LOC	=	Loss of consciousness
M	=	Myoclonic
C	=	Partial complex
T	=	Tonic
TC	=	Tonic-clonic
Un	=	Unclassified
V	=	Visual
F	=	Focal, all types

EEG Findings:

FD	=	Focal delta
FS	=	Focal spikes
FSW	=	Focal spike-and-wave
MFS	=	Multifocal spikes
Nl	=	Normal
PSW	=	Polyspike-and-wave
SW	=	Spike-and-wave
SSW	=	Slow spike-and-wave
#/Hz-SW	=	#/Hz spike-and-wave

Epilepsy Types:

CA	=	Childhood absence
CA(a)	=	Childhood absence (atypical)
CLF-J	=	Ceroid lipofucinosis-juvenile type
EME	=	Early myoclonic encephalopathy
FL	=	Frontal lobe (focal symptomatic)
FS-nfc	=	Focal symptomatic — not further classified
IMC	=	Idiopathic myoclonic
JA	=	Juvenile absence
LGS	=	Lennox-Gastaut syndrome
M-A	=	Myoclonic-astatic
OL	=	Occipital lobe (focal symptomatic)
SMEI	=	Severe myoclonic epilepsy of infancy
SMC	=	Symptomatic myoclonic
TC	=	Tonic-clonic, generalized
TL	=	Temporal lobe (focal symptomatic)

Other:

N/D	=	Not determined
D	=	Decreased

Table 2. Patients with CBZ-exacerbated seizures (during CBZ use)

Patient	Seizure Types	EEG Findings	Epilepsy Type	Dose-Related Worsening With CBZ
1	Same	FD, 3 Hz SW	FL	Yes
2	Same + Ab, At	FS	LGS	N/D
3	Same + At, Un	SSW	LGS	Yes
4	Same + TC	FS, FSW	FL	N/D
5	Same	FS	FL	N/D
6	Same + Ab	Abnormal	FL	N/D
7	Ab	3 Hz SW	CA	N/D
8	Same	FS	OL	N/D
9	Ab	PSW	CA(a)	N/D
10	Ab	MFS	FS-nfc	Yes (x3)
11	Same + M, At, TC	2.5 Hz SW	LGS	N/D
12	Ab (status)	3 Hz SW, PSW	CA(a)	Yes
13	AB (status)	PSW	CA	N/D
14	Same	3 Hz SW	CA	Yes
15	Same	3 Hz SW	CA	Yes
16	Ab (status)	2.5 Hz SW	CA(a)	N/D
17	Same	3 Hz SW	JA	N/D
18	Same + Ab	2.5 Hz SW	CA	Yes
19	Same + Ab	3 Hz SW	IMC	N/D
20	Same	1-2 Hz SW	EME	N/D
21	Same	2 Hz SW	CLF-J	N/D
22	Same	SW	SMC	N/D
23	Same	MFS, PSW	LGS	Yes
24	Same	2 Hz SW	LGS	N/D
25	Same	2 Hz SW	SMEI	Yes
26	Same + Ab, M	PSW	SMEI	Yes

Results

Twenty-six patients met the criteria for excellent documentation of CBZ-exacerbated epilepsy. Their seizure types, EEG findings, and epilepsy types are listed (Tables 1-3). Twenty-two of these patients had increases in seizure frequency, eight had increases in duration of individual seizures, and eleven had onset of new seizure types (Table 4). Twelve patients had two changes and one patient had all three of these changes in seizure activity.

The most commonly affected seizure type was absence. Eleven patients had increased frequency of their absence seizures while receiving CBZ. Other seizure types that occurred more often were tonic-clonic in seven patients, myoclonic in three, atonic in two, and unclassifiable in two. Eleven other patients developed new-onset absence seizures (including three patients who developed absence status) while receiving CBZ. A variety of other seizure types also developed: atonic in three patients, tonic-clonic in two, myoclonic in two, and unclassifiable in one.

New generalized spike-and-wave discharges were seen on 11 patients' EEGs: 3 Hz spike-and-wave in five, 2.5 Hz spike-and-wave in two, 2 Hz spike-and-wave in two, and polyspike-and-wave in two. Other abnormalities consisted of focal spikes in one tracing and multifocal spike-and-wave in another.

Several epileptic syndromes worsened with CBZ therapy. Four syndromes were affected: childhood absence in six patients; frontal-lobe in six; severe myoclonic epilepsy of infancy in two; and Lennox-Gastaut syndrome in two. Additionally, three patients developed Lennox-Gastaut syndrome and three patients had the "new onset" of childhood absence epilepsy while receiving CBZ. An illustrative case report appears below.

Case Report

This patient was a 17-year-old female who at age 6 years experienced staring spells lasting less than 30 seconds. These episodes occurred multiple times per day, but had been dismissed as inattentiveness. In June 1984, she had a generalized tonic-clonic seizure lasting several minutes. Just prior to that seizure she had been in her normal state of health, although frequent staring spells had been noticed that morning. She was examined in an emergency room, but the etiology of the seizure was not determined, and phenobarbital (3 mg/kg/day) therapy was initiated by her pediatrician. Her EEG

Table 3. Results of CBZ therapy withdrawal

Patient	Improvement Without CBZ	Other New Drugs Without CBZ
1	No Sz	Valproate
2	D Sz	Multiple
3	No Sz	None
4	D Sz	Ethosuximide
5	D Sz	None
6	D Sz	None
7	D Sz	Phenytoin
8	D Sz	None
9	No Sz	None
10	No Sz	Valproate
11	No Sz	None
12	No Sz	Ethosuximide
13	No Sz	None
14	D Sz	None
15	D Sz	Off meds
16	D Sz	None
17	No Sz	Valproate
18	No Sz	Ethosuximide
19	No Sz	None
20	D Sz	Valproate
21	No Sz	Methsuximide
22	D Sz	None
23	D Sz	Multiple
24	D Sz	Methsuximide
25	D Sz	Multiple
26	D Sz	None

revealed 3 Hz polyspike-and-wave discharges. She had no further tonic-clonic seizures, but complained of sedation; CBZ (8 mg/kg/day) was prescribed with a plan to gradually discontinue the phenobarbital. One week later she was hospitalized because of eye-rolling and tonic posturing of her arms. Blood levels of both antiepileptic drugs were considered therapeutic and she was discharged with no change in medications. Enhanced and unenhanced cranial computed tomography was normal. Hemogram and liver function tests were normal.

Eye-rolling recurred daily with increasing frequency by mid-July. CBZ dosage was increased to 14 mg/kg/day. This increase worsened the spells which persisted up to 90 minutes at a time. CBZ level was 6.1 μg/ml (therapeutic range: 4-12 μg/ml) and the phenobarbital level was 31 μg/ml (therapeutic range: 15-30 μg/ml). A child neurology consultation was obtained and CBZ dosage was further increased to 26 mg/kg/day, in three divided doses with a subsequent blood level of 9.6 μg/ml. Her seizures again increased in frequency. An EEG in August demonstrated frequent spike and polyspike discharges, as well as generalized 3 Hz spike-and-wave discharges during hyperventilation. She was re-examined and the addition of CBZ was suspected of having worsened this patient's seizures; therefore, CBZ was discontinued. Four days later after a marked decrease in seizure frequency and duration, ethosuximide was administered at 22 mg/kg/day. At an examination ten days later, no further seizure activity was reported or observed. She has remained seizure-free for two years.

Discussion

CBZ is an effective antiepileptic drug for partial or partial complex seizures, but generalized seizures may be exacerbated by it [1-4]. CBZ exacerbation of generalized seizures in humans is supported by animal research. In the mouse subcutaneous pentylenetetrazol (scPTZ) test, valproate, ethosuximide, clonazepam, and phenobarbital provide ptotection against threshold seizures [8]. These antiepileptic drugs are clinically useful in treating generalized seizures. CBZ and phenytoin, however, do not produce significant protection in the mouse scPTZ test. In this test, seizures are potentiated by CBZ and become more frequent and more prolonged [8].

The current study has identified several primary generalized seizure types to be worsened by CBZ: (1) absence, (2) atonic, (3) myoclonic, and (4) tonic-clonic. These finding are in agreement with previous human studies [1-4]. Tonic-clonic seizures may respond well to CBZ or may be exacerbated by it [4]. This discrepancy occurs because not all tonic-clonic seizures are primary generalized; some are secondary generalized. The seizure problems associated with CBZ also have been reported to be dose-related [3], a phenomenon confirmed in several of our patients (Table 2). As opposed to phenytoin, toxic levels of CBZ are not required for seizure exacerbation [9]. Therapeutic blood levels were documented in our patients.

In the patient population of the present review, four epileptic syndromes were identified as being affected by CBZ, including (1) childhood absence; (2) focal symptomatic, frontal lobe; (3) Lennox-Gastaut; and (4) severe myoclonic epilepsy of childhood. The largest patient group adversely affected by CBZ had absence epilepsy, three of whom experienced absence status. This response also occurred in patients already receiving a succinimide who were treated with CBZ for control of tonic-clonic seizures. EEGs demonstrated increased generalized 3 Hz spike-and-wave activity.

Surprisingly, the second largest group of patients adversely affected by CBZ had frontal lobe epilepsy. Blume and Pillay, in their review of "secondary bilateral synchrony," found that 51% of their patients had a frontal lobe focus [10]. Frontal lobe epilepsy also has been documented to evolve into a mixed seizure syndrome with drop attacks [11]. Furthermore, CBZ increases generalized epileptic discharges in the EEGs of patients with frontal lobe epilepsy [12]. Thus, the adverse effect of CBZ on this epilepsy type may be due to the tendency of CBZ to potentiate secondary generalization.

CBZ worsened seizures in two patients with Lennox-Gastaut syndrome. This result is not surprising because Lennox-Gastaut syndrome is characterized by the two seizure types, absence and atonic, both of which are worsened by CBZ. Four patients exhibited new onset of EEG abnormalities of 2 Hz or slower generalized spike-

Table 4. Patients with worsening of seizure activity during CBZ use

Patient	Increased Frequency of Seizures	Increased Duration of Seizures	New Types of Seizures
1	Yes	Yes	No
2	No	No	Yes
3	No	No	Yes
4	Yes	No	Yes
5	Yes	No	No
6	No	No	Yes
7	Yes	No	Yes
8	Yes	No	No
9	Yes	No	Yes
10	Yes	Yes	Yes
11	Yes	No	Yes
12	Yes	Yes	No
13	Yes	Yes	No
14	Yes	No	No
15	Yes	Yes	No
16	Yes	Yes	No
17	Yes	No	No
18	Yes	No	Yes
19	No	No	Yes
20	Yes	No	No
21	Yes	Yes	No
22	Yes	No	No
23	Yes	Yes	No
24	Yes	No	No
25	Yes	No	No
26	Yes	No	Yes
Total Yes:	22 (85%)	8 (31%)	11(42%)

and-wave discharges while receiving CBZ. This finding suggests that CBZ may exacerbate the Lennox-Gastaut syndrome.

Severe myoclonic epilepsy of infancy is a recently described epileptic syndrome. Prior to this study only two patients at FAMC had this diagnosis. During the study an additional four patients were diagnosed. Five of these patients who were treated with CBZ were reported to be adversely affected, and two had excellent supporting documentation. More patients will need to be studied to relate this effect causally.

Problems with CBZ-exacerbated epilepsy have been established by previous studies [1-4] and further reported in this one. Snead and Hosey [4] demonstrated with prolonged video-EEG telemetry that CBZ can exacerbate generalized, atypical absence seizures.

We chose to evaluate retrospectively our patients whose histories suggested adverse effects of CBZ. This study was performed by careful analysis of clinical courses and standard EEG data.

Because a retrospective evaluation was performed, it is noteworthy that the incidence of CBZ-exacerbated epilepsy could not be calculated in our patient population. Referral bias for the observed CBZ problem also might falsely increase the incidence in this study.

Accumulated evidence supports a primary adverse effect of CBZ on generalized seizure types, as opposed to a coincidental worsening with CBZ as part of the natural evolution of a patient's particular seizure disorder. This effect has been documented in several ways. First, an abrupt exacerbation of generalized/mixed seizures with the introduction of CBZ in therapeutic doses has been observed [1-4]. This exacerbation is illustrated by our case report. Second, a stepwise increase in seizure frequency can occur as CBZ doses and levels are increased [3]. This phenomenon was observed in at least ten of our patients (Table 2). In particular, Patient 10 had this dose-related phenomenon documented with three independent uses of CBZ. Third, withdrawal of CBZ can result in improved seizure control [13]. Five patients had stable seizure rates for months to years on CBZ; these patients became seizure-free after withdrawal of CBZ without the addition of other antiepileptic drugs (Table 3).

Classification of patients by seizure types and epileptic syndromes gives accurate identification of patients at risk for CBZ-exacerbated epilepsy. Caution, therefore, is needed when prescribing CBZ to a child or adolescent with absence or mixed seizures. Patients taking this drug who develop uncontrolled, generalized seizures should be examined for CBZ exacerbated epilepsy. Discontinuation of CBZ may result in a marked improvement in epilepsy control in these patients.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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W135 and Y) meningococcal vaccine. Meningococcal strains can be separated into twelve serogroups, based on their capsular structure. Serogroup B is the most frequent isolated strain. Platonov has vaccinated 18 LCCD patients of whom 2 experienced a meningococci disease, 9 and 12 months after vaccination respectively. Unfortunately, the meningococcal strains of the 2 patients were not serogrouped and characterized further. Because it is very likely that the meningococcal involved had another serogroup than one of the four serogroups included in the vaccine (A, C, W135 or Y), we can not support the conclusion that these cases represent vaccine failures. In the same study, Platonov found a lower frequency of meningococcal disease in a period of 3 years after vaccination than before the vaccination, suggesting a beneficial effect. We have immunized 21 LCCD individuals with the tetravalent meningococcal vaccine and found that 2 patients developed meningococcal disease after vaccination [1]. One C8β-deficient 19-year-old female patient twice developed meningococcal disease with serogroup B (not included in the vaccine), 1 and 3 years after vaccination respectively. The first episode was due to meningococcus B:4:P1.4, and in the second episode a meningococcus B:4:P1.6 was isolated. The second 21-year-old C8β-deficient male patient developed meningococcal disease with serogroup Y (included in the vaccine), but the onset of the disease was more than 3.5 years after vaccination. This patient had developed a significant antibody response to serogroup Y (measured by ELISA) determined 6 months after vaccination. Meningococcal capsular polysaccharides do not activate T-helper cells and induce relatively poor memory. After vaccination, a protective period of 4 years is assumed. Therefore, LCCD patients at risk for meningococcal disease require revaccination each 3–3.5 years with the tetravalent vaccine. The use of vaccine that also confers immunity to serogroup B should be investigated further.

We agree entirely with Cremer and Wahn that LCCD patients should be informed about their risk to contract meningococcal disease in order to lower the threshold for early antibiotic treatment. However, for screening of complement deficiencies we strongly recommend to use also the more sensitive haemolysis-in-gel-assay and not the traditional CH50 and AP50 test only [2].

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Accepted: 22 February 1996

Abbreviations *CBZ* carbamazepine · *SME* severe myoclonic epilepsy in infancy

Sir: Severe myoclonic epilepsy in infancy (SME) is a severe epileptic disorder proposed as a separate entity by Dravet et al. in 1978 [2] which has been regarded as one of the most intractable epilepsies in infancy and childhood. Because this disorder features both generalized and focal seizures [1], it has seemed reasonable to use the antiepileptic drugs which are appropriate for partial seizures including carbamazepine (CBZ).

Within the last 5 years, we have treated seven children with SME. Mean onset of the illness was around 5 months of age. Perinatal events were unremarkable. Precipitating factors of the seizures were mild fever, taking a bath and watching television or other flashing light sources. All patients had frequent episodes of prolonged convulsive seizures more than 30 min and also exhibited partial seizures in addition to other types of seizure. The seizures of these patients were extremely difficult to control. In six of seven patients, we administered CBZ. It was not effective. On the contrary, it aggravated generalized seizures in at least four of the six patients. Since it is difficult to make a correct diagnosis of SME early in the phase of illness, the attending physician may prescribe CBZ for partial seizures. If these seizures are aggravated by CBZ, SME may be suspected. Once a diagnosis of SME has been established, CBZ should not be administered. Our experience is in accord with that of others [3].

References

1. Commission on classification and terminology of the International League against epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:389–399
2. Dravet C (1978) Les epilepsies graves de l'enfant. Vie Med 8:543–548
3. Hurst DL (1987) Severe myoclonic epilepsy of infancy. Pediatr Neurol 3:269–272

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2008-000025

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

* Amended *

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

DATE OF AUTOPSY: 1/6/2008 09:30

REQUESTOR: GARY M WATTS

PROSECTOR: CLAY A. NICHOLS MD

FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

CAUSE OF DEATH: **SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).**

CAN:krh 3-13-08

Electronically Signed Out By Clay A Nichols MD

TOXICOLOGY (SLED): No blood ethanol is identified. Carbamazepine is identified in blood at a level of 1.6 mg/L.

CAN:krh 3-13-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

Amendments

Amended: 3/13/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 1/8/2008

Amended: 4/10/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 3/14/2008

2008-000025

PALMETTO HEALTH RICHLAND

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DEPARTMENT OF PATHOLOGY
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COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

* Amended *

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

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REQUESTOR: GARY M WATTS

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FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

CAUSE OF DEATH SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).

CAN:krh 3-13-08

Electronically Signed Out By Clay A Nichols MD

TOXICOLOGY (SLED): No blood ethanol is identified. Carbamazepine is identified in blood at a level of 1.6 mg/L.

CAN:krh 3-13-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

Amendments

Amended: 3/13/2008 by Karen Hilyer
Reason: TOXICOLOGY RESULTS
Previous Signout Date: 1/8/2008

2008-00025

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

NAME: MILLARE, CHRISTIAN
DOB: 8/23/2005 (Age: 2)
SEX: M
HOSP #: 999A08010
DATE OF DEATH: 1/5/2008
PROVISIONAL REPORTED: 1/7/2008

AUTOPSY #: A08-10
RACE: Caucasian

DATE OF AUTOPSY: 1/6/2008

REQUESTOR: GARY M WATTS
PROSECTOR: CLAY A. NICHOLS MD

PROVISIONAL ANATOMICAL DIAGNOSIS

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

IV. TOXICOLOGY PENDING.

CAUSE OF DEATH SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).

CAN:jh01/07/07

Electronically Signed Out By CLAY A. NICHOLS MD

2008-UCCO25

PALMETTO HEALTH RICHLAND

PALMETTO HEALTH RICHLAND
DEPARTMENT OF PATHOLOGY
5 RICHLAND MEDICAL PARK
COLUMBIA, SOUTH CAROLINA 29203

REPORT OF POSTMORTEM EXAMINATION

NAME: MILLARE, CHRISTIAN

AUTOPSY #: A08-10

DOB: 8/23/2005 (Age: 2)

RACE: Caucasian

SEX: M

CHART #:

DATE OF DEATH: 1/5/2008 09:49

DATE OF AUTOPSY: 1/6/2008 09:30

REQUESTOR: GARY M WATTS

PROSECTOR: CLAY A. NICHOLS MD

FINAL ANATOMICAL DIAGNOSES

I. HISTORY OF DEATH FOLLOWING SUSPECTED SEIZURE. PAST MEDICAL HISTORY REMARKABLE FOR SEIZURES SECONDARY TO MITOCHONDRIAL DISORDER, TYPE NOT SPECIFIED.

II. BITE MARK ON RIGHT LATERAL TONGUE.

III. NO EVIDENCE OF TRAUMATIC INJURY. X-RAYS NEGATIVE.

IV. TOXICOLOGY PENDING.

CAUSE OF DEATH **SEIZURE DUE TO COMPLICATIONS OF MITOCHONDRIAL DISORDER (TYPE NOT SPECIFIED).**

CAN:krh 1-8-08

Electronically Signed Out By Clay A Nichols MD

CLINICAL SUMMARY:

This 2-year-old white male was found unresponsive at his residence on the morning of January 5, 2008. The deceased was found half on and half off of his bed and without evidence of trauma. He was transported to a local emergency room where resuscitative efforts were unsuccessful and death was declared at 9:49 a.m. The deceased has a past history of a seizure disorder caused by a mitochondrial disorder (type not specified). There is no suspicion of foul play from the scene investigation. The history is provided by David Burns of the Richland County Coroner's Office. The Richland County Coroner's Office authorized the autopsy.

CAN:jh01/07/08

GROSS ANATOMICAL DESCRIPTION:

EXTERNAL DESCRIPTION: This is the body of a well-developed, well-nourished white male child appearing compatible with the stated age. The height is 37 inches and the weight is estimated at ~ 40 pounds. The rigor is full and generalized. Purple lividity is posterior and fixed. The body is identified by the coroner's office. The head is covered by light brown hair. The head and face are normally formed and atraumatic. The pupils are equal at 0.2 inch each. The irides are blue. No petechial hemorrhage is identified. There is no evidence of trauma to the nose, mouth or ears. The neck is free of trauma or masses. The chest, abdomen and posterior aspects are appropriately developed and free of trauma. The genitalia are those of a normal male child. There is no evidence of trauma or discharge to the genitals or anus. The upper and lower extremities are appropriately developed and free of trauma.

TOTAL BODY X-RAYS do not identify any evidence of fracture.

EVIDENCE OF MEDICAL INTERVENTION: An endotracheal tube is in place. EKG monitor pads and pacing pads are on the anterior chest. Multiple needle puncture marks are on the arms and ankle. An interosseous catheter is in the right shin.

INTERNAL DESCRIPTION: The body is opened with a standard Y-shaped incision revealing all organs in their normal anatomic relationship. Dissecting through the soft tissues of the chest and neck reveals no evidence of trauma or contusion. Removal of the chest plate reveals unremarkable bilateral chest cavities. Opening the abdominal cavity reveals all organs in their normal anatomic relationship and no evidence of excess fluid accumulation.

CARDIOVASCULAR: The heart weighs 60 grams. The pericardial surface is smooth without adhesions. There is a normal amount of epicardial fat within a normal distribution. The coronary arteries are of normal distribution and demonstrate no atherosclerotic vascular disease. Opening the heart reveals normally formed heart valves without evidence of vegetations, incompetence or stenosis. The papillary muscles are well formed. The myocardium is red brown without evidence of hemorrhage or fibrosis. The vessels arise from the heart in a normal fashion.

RESPIRATORY: There is no obstruction of the upper airway. The larynx, epiglottis and trachea are within normal limits. The trachea divides into the major bronchi in a normal fashion. The right lung weighs 70 grams and the left lung weighs 50 grams. The bronchi divide in a normal fashion through the lung. Examination of the pulmonary vasculature reveals no evidence of embolus or fibrosis of the perivascular bed. There is no evidence of an infectious process of the lungs.

LIVER AND BILIARY: The liver weighs 660 grams. The capsular surface is smooth and glistening. Sectioning the liver reveals a uniform red brown parenchyma without evidence of fibrosis, cirrhosis or neoplasia. The cut surfaces of the liver are smooth. The hepatic vasculature is within normal limits and the biliary tree is grossly unremarkable. The gallbladder demonstrates mild autolysis. Cholelithiasis is not present.

RENAL AND URINARY: The right kidney weighs 50 grams, the left kidney weighs 30 grams. Both

have a normal amount of perirenal fat. The capsules of the kidneys strip easily away revealing smooth cortical surfaces. The cortices and medullae are within normal limits and the corticomedullary junctions are distinct. The renal papillae are well-developed without blunting or necrosis. The renal pelves drain freely to the ureters which are unobstructed to the bladder.

GASTROINTESTINAL: The esophagus is covered with a steel gray mucosa without evidence of ulceration or varices. The esophagogastric junction is distinct without evidence of tearing, ulceration or dysplasia. The stomach has normal rugal folds and autolyzed mucosa without evidence of ulceration or thickening. The stomach contains approximately two ounces of partially digested food. The lower gastrointestinal tract is intact and otherwise unremarkable. An appendix is present.

SPLEEN: The spleen is covered with steel gray slightly wrinkled capsule. The spleen weighs 90 grams. Sectioning the spleen reveals a mulberry red interior with a normal distribution of white and red pulp.

PANCREAS: The pancreas is unremarkable in size, weight or configuration. There is no evidence of peripancreatic cyst, pseudocyst, hemorrhage, calcification or fibrosis.

LYMPHATICS: Unremarkable.

ENDOCRINE: The bilobed thyroid is deep, red brown and within normal limits. There is no evidence of abnormality in the pituitary. The adrenals demonstrate normal cortices and medullae without evidence of neoplasm.

BRAIN: The scalp is reflected revealing no evidence of trauma or contusion. There is no evidence of a skull fracture. Removal of the calvarium reveals no evidence of a subarachnoid, subdural or epidural hemorrhage. The sulci and gyri are normally formed. The cerebral vessels are of normal distribution and without evidence of berry aneurysm formation. Dissecting of the brain reveals a normal distribution of white and gray matter without evidence of hemorrhage or infarction. There is no evidence of a disease process in the mid brain, cerebellum or the cerebrum. The brain weighs 1300 grams.

NECK ORGANS: A bite mark is identified on the right lateral tongue. The hyoid bone is intact. There is no evidence of laryngeal trauma.

TOXICOLOGY: Blood is obtained. Portions of liver, brain, kidney and pulled head hair are retained. A DNA card is obtained. All tissues not retained for diagnostic purposes are returned to the body.

CASSETTE SUMMARY:

1. Heart.
2. Left lung, kidney and adrenal.
3. Right lung, kidney and adrenal.
4. Liver, spleen, pancreas and thyroid.
5. Brain, thymus, skeletal muscle and bit mark right lateral tongue.

CAN:jh01/07/08

MICROSCOPIC DESCRIPTION:

Examination of sample tissues demonstrates varying degrees of congestion and autolysis and are otherwise noncontributory.

TOXICOLOGY: This report is being issued without benefit of a final toxicology report. When a report becomes available, an addendum will be issued.

SUMMARY & COMMENT: This unfortunate child as a result of a seizure due to complications of mitochondrial disorder. The pertinent anatomic findings are summarized under "Final Anatomic Diagnoses".

CAN:krh 1-8-08

***Electronically Signed Out By CLAY A. NICHOLS MD ***

11.11.2004

Chairman's Address – Bionomics Limited 2004 AGM

Ladies & Gentleman

Once again, on behalf of the Board, a warm welcome to our fellow shareholders and invited guests – including in particular Chairman elect Peter Jonson.

<u>Observations about the last 5 years</u>

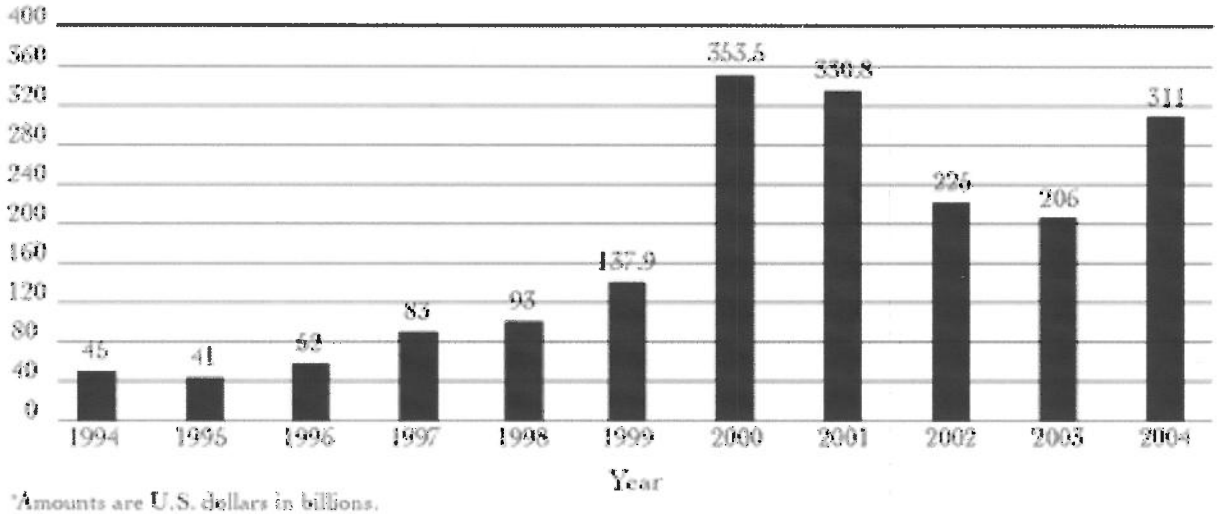
Next month will mark 5 years since Bionomics' ASX listing in 1999. In view of this (together with the Board's decision to extend the term of CEO Deborah Rathjen's contract, and my retirement as Chairman), it is timely to begin this address with some observations on the performance of our Company over that period.

These observations about Bionomics should be viewed against the following industry background:-

- The biotechnology industry continues to produce remarkable results – results which have much enhanced the understanding of the systems and processes involved in serious human disease. For example, in the USA alone, there are over 370 biotechnology derived drugs and vaccines currently in clinical trials, and medical diagnostic tests are increasingly detecting conditions early enough to enable successful treatment of these conditions, (we will hear more about this in the Bionomics context at this meeting).
- Notwithstanding the substantial risks involved in biotechnology research, development and commercialisation (about which we have continually reminded shareholders!) the rewards to the winners for success have continued to be very high, and industry growth prospects are generally strong. Our observation at the 2001 AGM that "this is not a transient, short-lived industry", has indeed proved correct, though of course we acknowledge that this is a high risk/high reward industry with the number of companies that don't make it well exceeding the number of high reward winners!

Market Cap. US Biotechnology Stocks

Market Capitalization, 1994–2004*



*Amounts are U.S. dollars in billions.

Sources: Ernst & Young LLP and BioWorld

Bionomics Limited

- This is an interesting slide - as you can see, over the last five years the market value of the USA Biotechnology industry has (apart from the brief but substantial upward spike in 2000/2001) been trending steadily upwards (from about US$140 billion in 1999 to over US$300 billion now) – all the signs of an industry that is doing thoroughly worthwhile, sustainable things. And there is little doubt that in part these increases in shareholder value were due to the value USA markets place on intellectual property portfolios rather than simply traditional revenues and earnings.

Turning now to Bionomics in particular:-

At the first AGM of Bionomics after ASX listing, we stated that

> "Bionomics is committed to building shareholder value, initially at least by increasing the value of our intellectual property portfolio"

In making this commitment to building our intellectual property base, we recognised that it would likely take sometime for this

IP value to be acknowledged by the Australian sharemarket – a risk that I believe we have communicated to our shareholders. Our view is that this likelihood has indeed come to pass, and that our intellectual property and R & D is materially undervalued by the sharemarket, as evidenced by the following specific and substantial R & D achievements over the last 5 years:-

These achievements have included the discovery of genes associated with different forms of epilepsy and blood vessel growth, validation of genes as drug targets and development of a diagnostic test for severe childhood epilepsy. Bionomics achievements can be measured by our partnerships for therapeutic product discovery and development and worldwide diagnostic marketing, a substantial intellectual property base including granted patents, the award of more R & D Start Grants and two Biotechnology Innovation Fund Grants and, numerous publications in highly regarded international scientific journals.

2003 / 2004 Performance

Further evidence of the tangible value of our R & D is the recent signing of two agreements relating to epilepsy diagnostic tests. Deborah Rathjen will more fully describe the significance of these agreements shortly. Suffice it to say that for a range of reasons, achieving these two agreements is both very pleasing and a great credit to the Bionomics team. These deals have a significance exceeding the material upfront cash inflows to Bionomics that will result, largely by way of recognition that the R & D efforts of the last few years have indeed achieved real, realisable value. The deals will clearly establish the scientific credentials of Bionomics.

On other aspects of last year's performance, Deborah Rathjen will also describe in specific terms corporate and R & D performance over the past year, so I will not pre-empt that here.....In summary, further significant progress was made in both the R & D and corporate areas. Much was achieved, and the Board is well pleased with the results.

Finance

Turning now to finance.....

The significant matters include:-

- **Income for the year to 30 June 2004 was $2.0 million, compared with $1.6 million the previous year. The main component of this income was the second year of our R & D Start Grant payments together with some milestone payments by Nanogen Inc.**
- **In February / March this year, an institutional placement and a shareholders' entitlements issue were successfully completed, raising approximately $5.8 million after expenses. The Board chose this combined structure to ensure the certainty of a capital raising of about $6 million, whilst also respecting the rights of existing shareholders to participate in the capital raising. We were very pleased with the outcome.**
- **Cash on hand at 30 June this year was $8.7 million, which at current cash burn rates will be more than sufficient to meet our self imposed 2 year funding benchmark,**

excluding recently announced additional Grant funding of approximately $270,000 and upfront, milestone and royalty payments resulting from the 2 agreements referred to earlier. In other words, the Company remains in a relatively sound financial position.

- Tight cost control continues, and the Board remains satisfied that the ratio between corporate overheads and administration costs on one hand and real, value adding R & D work on the other hand, is appropriate. (Currently, 68 cents in each dollar spent is on R & D, up from 58 cents four years ago.)
- Finally, the Board's view is that we must, in shareholders interests, take all reasonable steps to maintain this financial underpinning of our programs, particularly of our exciting and growing drug discovery programs. Therefore, as I noted last year, we are continually investigating various sources of additional financing, and will access these sources when and if appropriate – with the interests of existing shareholders first and foremost in mind. These sources include equity raisings; collaborative research and commercialisation arrangements and, provided they fit strategically, corporate merger opportunities.

Corporate Governance

As advised at last year's AGM, Bionomics is advancing our ASX corporate governance compliance and review program over a three year timetable, commencing June 2003. We are committed to achieving and applying a high standard of corporate governance taking into consideration the company's size and the industry in which the company operates.

Specific actions that have been taken are set out in the Annual Report, and details of the timetable are on the Company's website. We are confident, based on the solid progress made to date, that we will meet this timetable, and I gratefully acknowledge the major contribution that Peter Maddern (as Chairman of the Audit and Compliance Committee) has made to this major task.

People

Now some comments about our people:-

- **Last month we announced the extension of Deborah Rathjen's CEO contract for 3 years from its existing expiry date of June next year. In view of Deborah's passionate commitment to Bionomics' success, and her demonstrated capabilities, her decision to extend her contract is in the Board's view very positive for the Company's prospects of converting the excellent R & D work over the last few years into real and substantial increases in shareholder value over the next few years. (In this context the recent diagnostic test agreements are a significant step in making this transition!)**
- **In May 2004 the appointment of Dr Tim Harris to the Bionomics Scientific Advisory Board ("SAB") was announced. Dr Harris is the CEO of leading US biotech company, Structural GenomiX Inc., a company he co-founded in 1999. Tim Harris will add substantial international experience and value in advising on the Company's drug discovery programs.**
- **Also in May, the Company announced the retirements of Professors Mathew Vadas, Erkki Ruoslahti and Ullrich from the SAB, each having served four years, with Professor Vadas having served over that period as co-chair of the**

SAB. The Board thanks them for their valuable roles in the formative years of the Company's scientific development. In particular, we are deeply appreciative to Professor Vadas as scientific co-founder of Bionomics (with Professor Grant Sutherland, who is with us today) for his foresight in recognising the potential of the genomics revolution to lead to better health outcomes for patients and his creativity in guiding the formation of the Company.

- **Without denigrating the significance of it, we will deal with the change of Chairman towards the close of this meeting.**

<u>Concluding Observations</u>

Much has been achieved since Bionomics' formation in 1998 and its ASX listing in 1999. Bionomics is now a recognised leader in understanding the genetics of epilepsy and the processes that drive cancer growth. The Company has matured from a 'virtual' entity with limited internal resources to a Company characterised by substantial scientific substance and achievements, a talented management team and, notwithstanding the risks, excellent growth prospects. The Bionomics team remain deeply committed to turning these

growth prospects into tangible increases in shareholder value – the team is indeed determined to achieve the high reward / winner status that I referred to at the beginning of this address!

Thankyou for being with us, and I now invite Deborah Rathjen to present to us, and in doing so, mention that on Tuesday this week Deborah was awarded the prestigious Inaugural AusBiotech President's Gold Medal for contributions to the Australian biotechnology industry.....over to you Deborah.....

CEO ADDRESS – AGM 2004

INTRODUCTION

Ladies and Gentlemen welcome and thank you for your attendance here today.

2004 has been a year of solid performance both in science and commercialization. We have made significant progress in executing our strategy having put in place licensing arrangements for the epilepsy diagnostic product now available the major market – North America.

SLIDE 2 – KEYS TO SUCCESS FOR BNO

The keys to our success are to target those therapeutic areas where Bionomics has a global competitive advantage – CNS disorders and angiogenesis. Our focus is balanced in favour of maximizing returns to the Company from its R&D. Our strategy is to exploit near term revenue opportunities in diagnostics to fuel our drug development pipeline. Our goal is now to develop a strong intellectual property position on drug compounds in addition to our existing IP in diagnostics, animal models, drug targets and genes. An additional key to our continued success is maintenance of our sound financial position which enables us to compete effectively in our chosen areas in the global biotech business arena.

SLIDE 3 – 2004: A YEAR OF STRONG PROGRESS BY BNO

I'd like to list some of the achievements of 2004 before I begin my operational review.

- We completed a large clinical study in patients with severe myoclonic epilepsy in the first 12 months of life – this study is likely to be regarded as a landmark study in the field.
- Completion of the study enabled the Company to conclude a licensing agreement with US company Athena Diagnostics. This agreement has the potential to secure a solid revenue stream for the Company based on royalty payments and milestone payments linked to sales targets achieved by Athena. We also announced a second epilepsy diagnostic licensing agreement this week, with Genetic Technologies (GTG) to whom Bionomics' granted worldwide rights in return for an upfront payment, royalties and milestone payments linked to sales targets achieved by GTG. Together with Nanogen Inc our current licensees cover each of the key market segments for the delivery of molecular diagnostic tests for epilepsy.
- We now have key drug discovery collaborations in place with WEHI, Southern Cross University and PerkinElmer Inc to implement our drug discovery strategy.
- We have achieved the Company's first Angene™ platform revenues and thus we now have revenues accruing from 2 areas of the company's activities in addition to the grant funding the Company receives for its R&D programs.
- This year has seen the Company create a solid foundation for future licensing of its cancer assets, including the proprietary drug target BNO69.

Bionomics established a US subsidiary Bionomics Inc this year to facilitate both IP ownership from our network of US collaborations and commercialization of IP.

Bionomics now has a part-time business development representative based in Boston to assist the Company in its commercialization activities.

SLIDE 4 – SMEI: SEVERE MYOCLONIC EPILEPSY OF INFANCY

Our CNS business strategy is to generate near term revenues through licensing of epilepsy diagnostic IP, these revenues will then be used to help fund our CNS drug discovery and development pipeline. The first diagnostic application to be licensed is for the diagnosis of SMEI which is a severe form of epilepsy with symptoms of febrile seizures beginning in the first months of life. The condition is associated with a high mortality rate, with up to 18% of children dying from their seizures and up to 50% showing evidence of developmental delay and brain damage in their second year. Early diagnosis is difficult and certain drugs aggravate seizures in these children. Bionomics diagnostic test makes possible earlier and more precise diagnosis which enables clinicians to make appropriate treatment decisions for children.

The development of this diagnostic test by Bionomics' represents a significant commercial advance for the Company following on from the world-renowned research of its collaborators.

SLIDE 5 – SMEI DIAGNOSTIC AVAILABLE IN NORTH AMERICA

Athena Diagnostics is now offering the SMEI test in North America. This slide shows the brochure which is being used by Athena to promote the test to neurologists. The licensing of IP, which underpins the test, to Athena and our other licensees was made possible by the extensive genetic analysis conducted by our collaborators the Women's and Children's Hospital in Adelaide and the University of Melbourne and the large clinical study conducted under the leadership of neurologists Professor Sam Berkovic and Associate Professor Ingrid Scheffer.

The Company is now looking at other epilepsy diagnostic opportunities from within its IP portfolio which are ripe for commercialization.

We know a great deal about the potential market for molecular tests for different forms of epilepsy, including SMEI, based on our knowledge of the number of neurologists, the number of potential patients, and the likely test pricing. For example, up to 230,000 children in the US may be candidates for our test. The fact that we are developing truly innovative products to meet clinical needs means however that it is difficult to prospectively estimate revenues at this early stage.

Under the terms of our licensing agreements with Athena and GTG, Bionomics has received upfront payments and will receive royalties on sales and milestone payments linked to the achievement of revenue targets. The latter may form the basis of a substantial revenue stream for Bionomics from each of its licensees.

SLIDE 6 – DRUG DISCOVERY PROGRAM IS TARGETING LARGE MARKETS WITH UNMET NEEDS

Bionomics drug discovery program is focused on the discovery of drugs which can be used to treat both epilepsy and anxiety. Epilepsy and anxiety are amongst the largest worldwide markets for pharmaceutical products with revenues of the order of US$6

billion and US$14 billion respectively. There is still significant unmet clinical need in the treatment of both epilepsy and anxiety, with a need for fast acting medications which don't cause sedation, addiction or loss of memory. A staggering statistic is that 30% of people with epilepsy don't gain control of seizures with current medications.

SLIDE 7 – PROPRIETARY IONX® DRUG DISCOVERY TECHNOLOGIES UNDERPIN BIONOMICS' DRUG DISCOVERY

The drug discovery program, which receives funding under the federal government's R&D Start grant scheme, utilizes many components of Bionomics' proprietary platform ionX® including our GABA receptor genetics IP together with our patented mouse models and sophisticated brain recording capabilities. It's worth noting that we have developed the world's first animal model of inherited human epilepsy – a mouse model that exhibits human-like seizures.

Our longstanding and highly productive collaboration with the Howard Florey Institute continues to help Bionomics' build the value of the platform.

SLIDE 8 – BIONOMICS INTERNAL CAPABILITIES IN DRUG DISCOVERY ARE NOW UNDERPINNED BY STRONG COLLABORATIONS IN CHEMISTRY

The process of drug discovery and development requires an understanding of genetics and biology. It is a process which involves the screening of chemical libraries against a validated target. Medicinal chemistry is a discipline which then turns hits discovered in a screening program into optimized leads and drug candidates. One of our objectives this year was to fully implement our drug discovery plan. I am pleased to say that we have done this – gaining access to chemical libraries held by the Walter and Eliza Hall Institute (WEHI) in Melbourne and Southern Cross University in Lismore, to the medicinal chemistry expertise of WEHI and the drug candidate optimisation skills of the Centre for Drug Candidate Optimisation at the Victorian College of Pharmacy. Investments have been made in the latest drug discovery technologies helped by Bionomics' strategic alliance with the global company PerkinElmer. The program is now moving forward as planned under the capable direction of the Thebarton-based team at Bionomics with the scene set for significant progress in 2005.

SLIDE 9 – CNS 2004 MILESTONES ACHIEVED

In summary, Bionomics has made some important steps forward this year in our CNS program having:

- completed a large clinical study to validate the SMEI diagnostic test
- secured licensing agreements with Athena Diagnostics and Genetic Technologies
- positioned ourselves for significant progress in drug discovery
- continued to progress our IP, particularly in the areas of diagnostics and drug discovery

SLIDE 10 – ANGIOGENESIS

Angiogenesis is the process by which new blood vessels are formed. Angiogenesis is required for cancers to grow as this video illustrates. Angiogenesis inhibitors are set to become an increasingly important component of anti-cancer treatment. Angiogenesis is

also important in other settings such as eye diseases and inflammation and therapeutic angiogenesis inhibitors are also under development for these indications. These products have been greeted by clinicians and the investment community in the US with enthusiasm. An angiogenesis inhibiting antibody approved by the US FDA in February of this year – Avastin – is anticipated to be a blockbuster drug.

Bionomics angiogenesis program is identifying and validating novel targets for interrupting angiogenesis. We have an active collaboration with the Danish company Genmab A/S for the generation of therapeutic antibodies and we were pleased to have recently achieved further funding from the Biotechnology Innovation Fund and BioInnovation SA to progress our antibody targets. We have also made considerable progress in generating interest in our proprietary platform Angene™ for angiogenesis drug discovery. This interest has recently converted to revenues for Bionomics.

SLIDE 11 – BNO69 PLAYS A MAJOR ROLE IN BREAST CANCER AND ANGIOGENESIS

One of the many exciting targets under investigation by Bionomics is BNO69. Our scientists have demonstrated that molecules which silence the expression of the BNO69 gene inhibit both angiogenesis and breast cancer cell growth. Part of the attractiveness of BNO69 as an angiogenesis target is the fact that silencing disrupts 2 major angiogenic pathways, including the pathway targeted by Avastin.

SLIDE 12 - BNO69 GENE SILENCING INHIBITS BREAST CANCER GROWTH IN ANIMALS

Recent data in animal models of breast cancer have indicated that BNO69 gene silencing is able to reduce the growth of breast cancer. This is a very exciting new development which has boosted significantly our interest in this target as well as the prospects for licensing Bionomics' IP on the target and the gene silencing molecules.

SLIDE 13 – ANGIOGENESIS MILESTONES ACHIEVED

All up our angiogenesis program has had a great year commencing with completion of the Angene™ platform rollout in March. We are now generating revenues from contracts with a biotech company and we will focus on growing these revenues. Importantly for maintaining future upside for the company we have significantly progressed target validation.

SLIDE 14 – THE COMPANY IS IN A SOUND FINANCIAL POSITION

In 2004 Bionomics increased its cash in flows and managed down its losses. With growing revenues from epilepsy diagnostic product licenses and the Angene™ platform the Company continues to be on a sound financial footing.

SLIDE 15 – POSITIVE OUTLOOK FOR 2005

In conclusion Ladies and Gentlemen I am pleased to deliver this report to you as the prospects for Bionomics' and the opportunities before us in 2005 are indeed positive. Our CNS and angiogenesis programs are now generating revenues – our focus is on

growing those revenues. We have a well defined strategy for both near term and longer term revenues with significant upside potential from our drug discovery program.

We have already seen the US launch of our SMEI diagnostic by Athena, only a month or so after signing the licensing agreement, and the signing of a second licensee in GTG, with GTG's recently reported intention of making the test available this month.

Bionomics is without peer in the world in its epilepsy research and ionX™ platform for drug discovery and development. The Company is a clear leader in the niche it occupies and has established a competitive position in angiogenesis.

In the next 12 months we will continue to leverage our world-class science to secure additional licensing deals, with the objective of building sustainable shareholder value.

Thank you once again for your time here today. We appreciate your support and we look forward to reporting our progress to you during the coming year.

Turning gene science into diagnostics and drugs

Annual General Meeting November 2004

Bionomics Limited

Keys to Success for Bionomics

- CNS disorders and Angiogenesis in line with BNO competitive advantage
- Focus balanced in favour of maximizing returns
  - Exploiting near term opportunities in diagnostics to fund drug development pipeline
- IP position on molecules in addition to existing IP in diagnostics, animal models and targets
- Sound financial position

2004: A Year of Strong Progress by BNO

- SMEI clinical study completed
- SMEI diagnostic licensing deals with Athena Diagnostics and Genetic Technologies
- Key drug discovery partnerships in place in epilepsy and anxiety
- First Angene™ platform revenues
- Solid foundation for licensing cancer assets and future prosecution of cancer drug discovery and development
  - Validation of BNO69 in angiogenesis and breast cancer
- Bionomics Inc.

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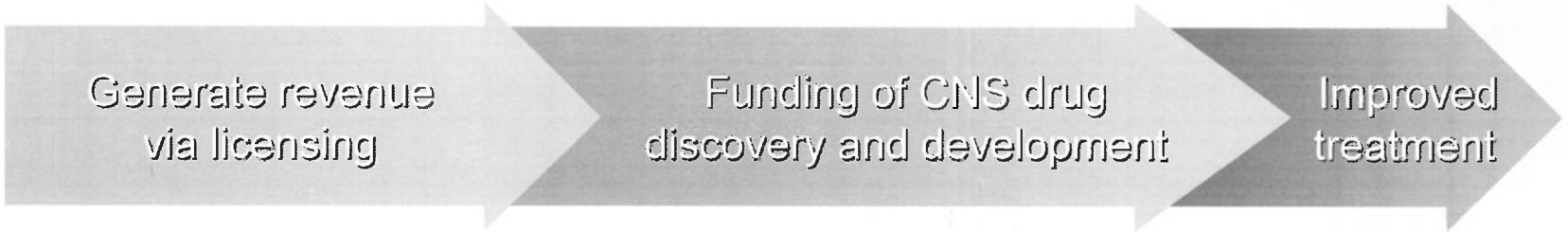
SMEI: Severe Myoclonic Epilepsy of Infancy



- SMEI is a severe form of childhood epilepsy
  - Up to 18% mortality and up to 50% show developmental delay and brain damage
- Early diagnosis difficult and treatment is disappointing
  - Certain drugs can make seizures worse
- Bionomics' SMEI Diagnostic Test meets clinical needs
  - Leads to early and precise diagnosis
  - Enables early choice of appropriate treatment strategies

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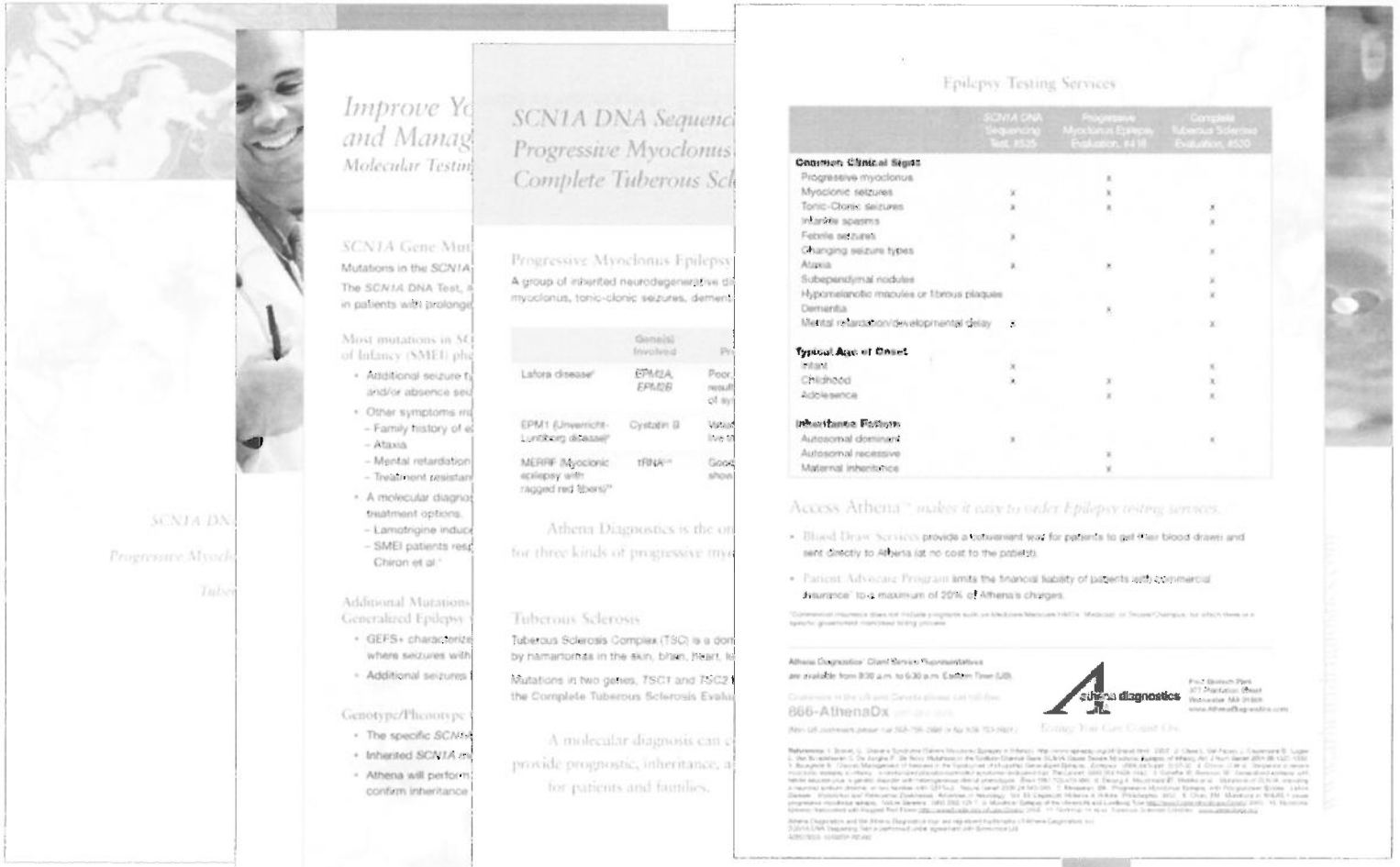
SMEI Diagnostic now available in US



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Bionomics' drug discovery program targeting large markets with unmet needs

Epilepsy

- US$6 billion and growing at 17% pa
- Up to 3% of the population have epilepsy
- Many drugs are poorly tolerated and have many side-effects
- 30% of patients don't gain control of seizures with current drugs

Anxiety

- US$14.5 billion in 2003
- Approximately 2 million Australians suffer from an anxiety disorder
- Patients need medications which are non-sedating, non-addictive and don't affect memory

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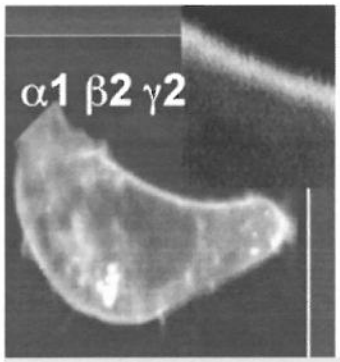
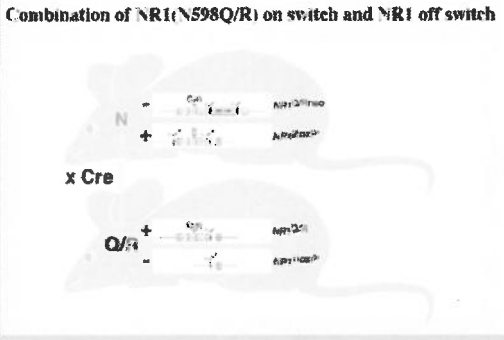
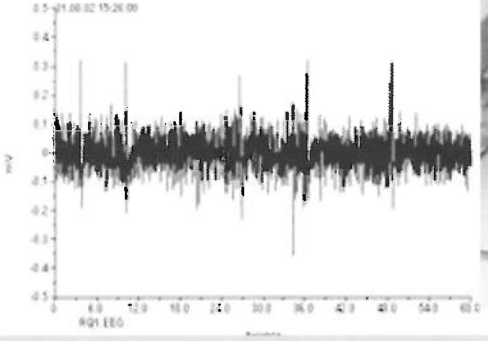
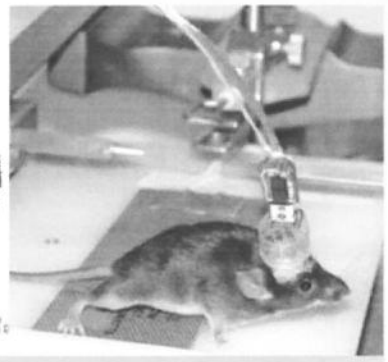
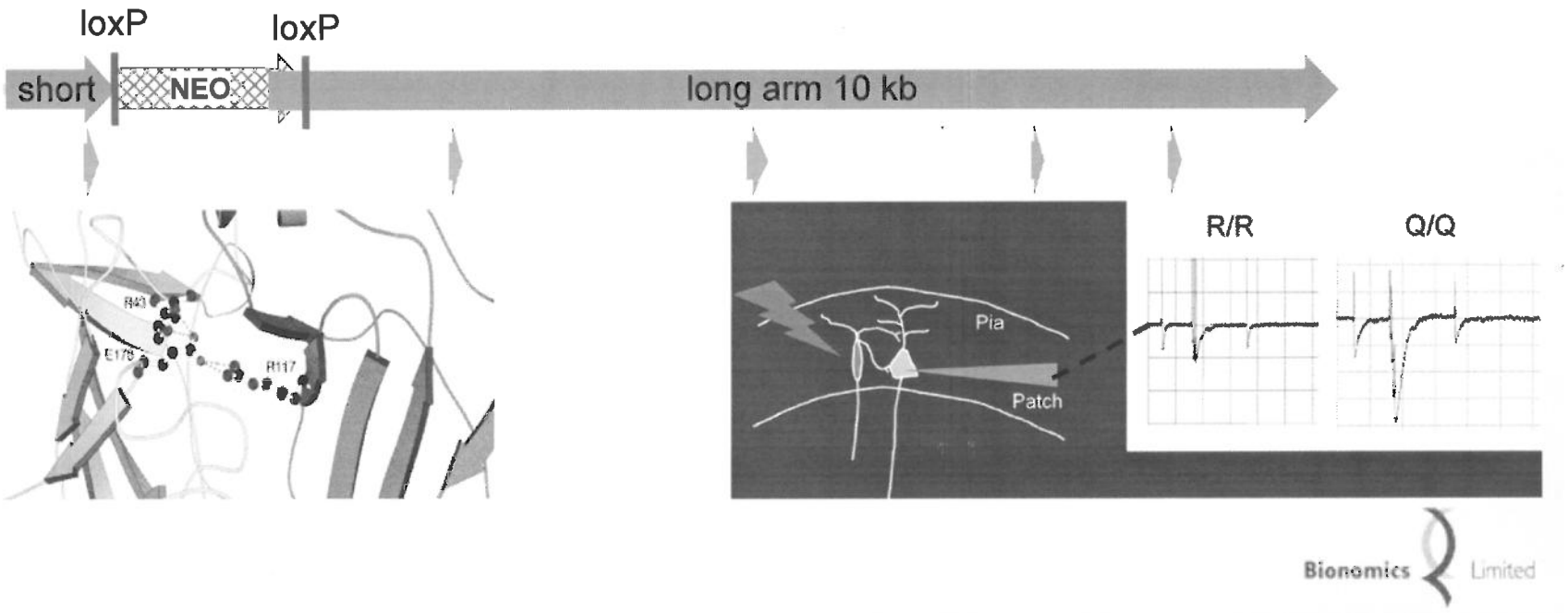
ionX® Drug Discovery Technologies











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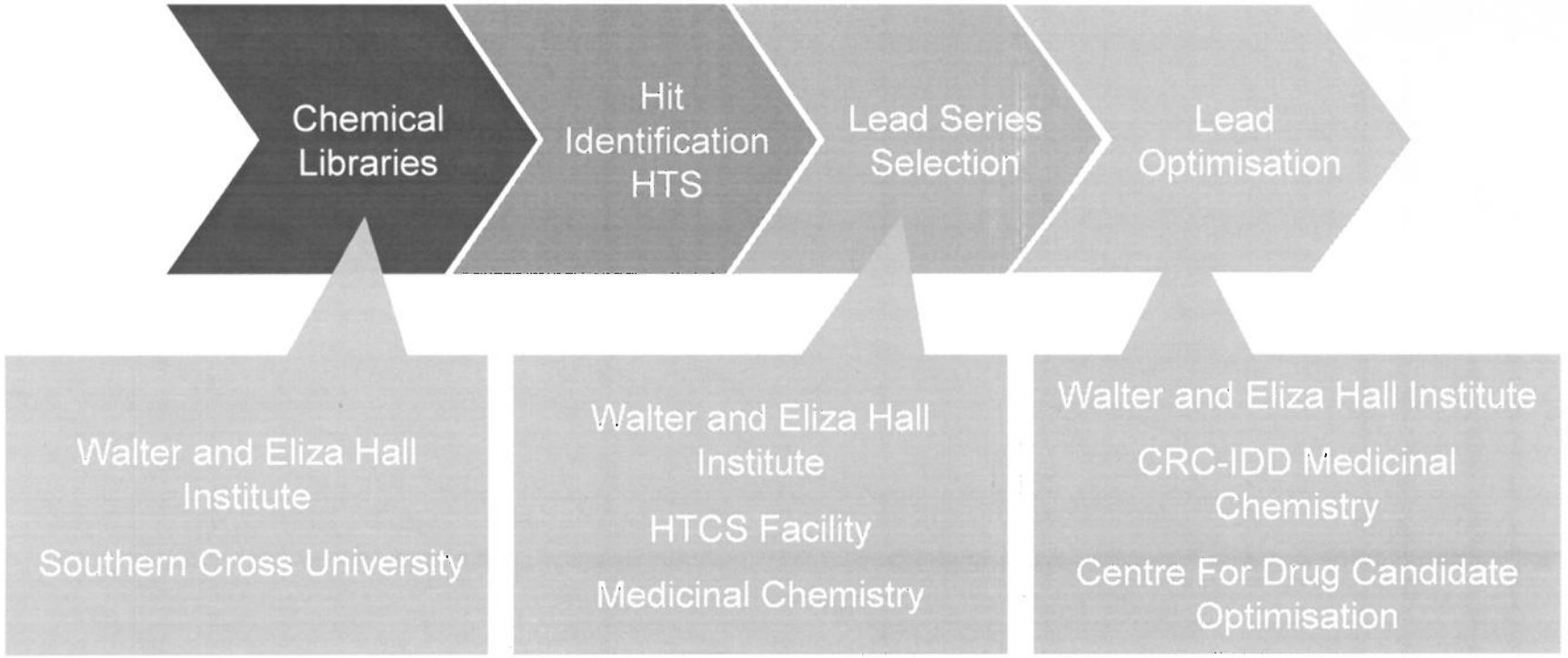
Bionomics – internal capability underpinned by strong collaborations

BIONOMICS



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CNS 2004 Milestones Achieved

- Patent progress to support commercialization of epilepsy diagnostics and drug discovery
- Key drug discovery partnerships
- Athena Diagnostics and Genetic Technologies epilepsy diagnostic product deals
- Completed SMEI clinical study to support initial epilepsy diagnostic product

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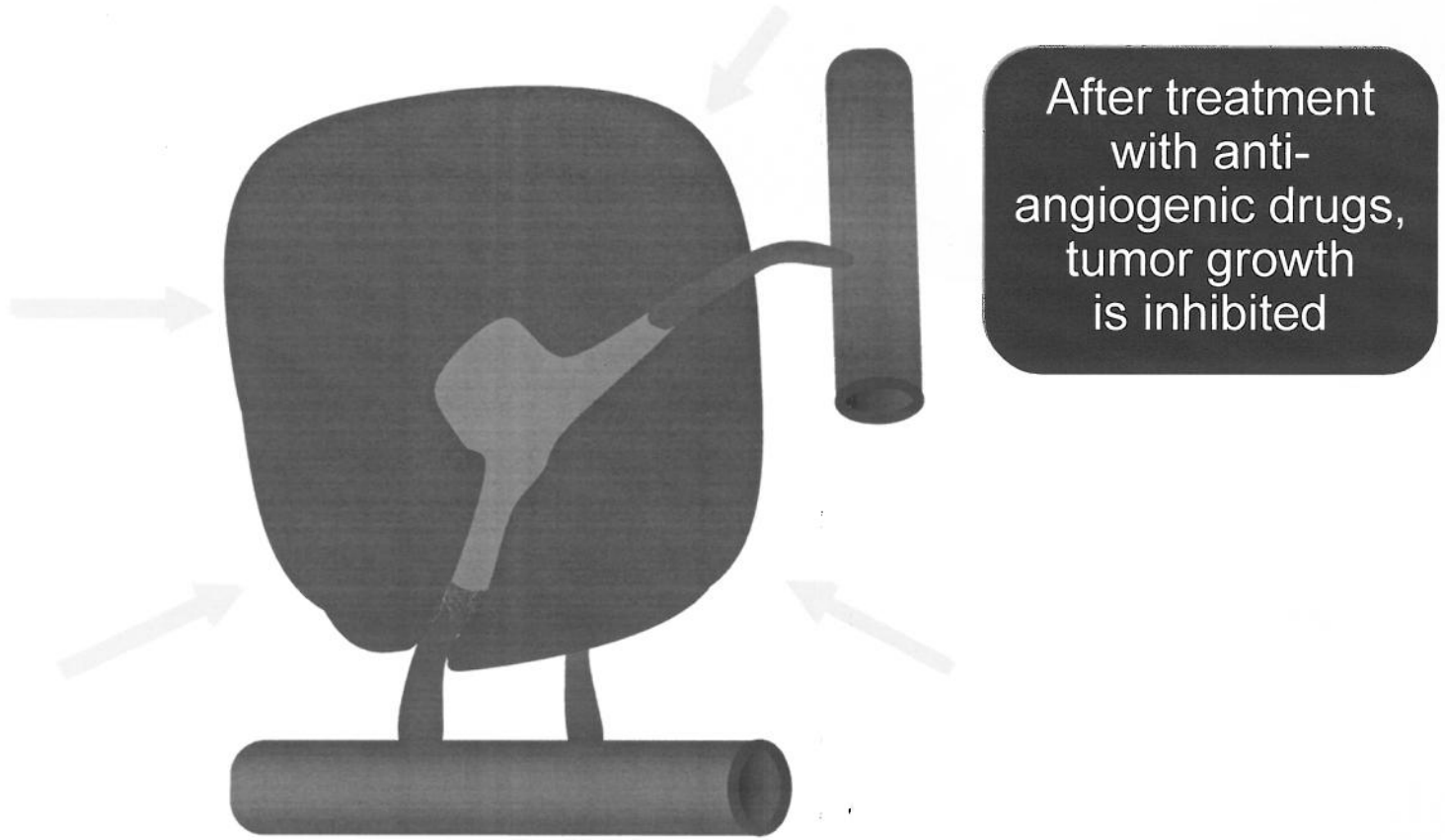
Angiogenesis



After treatment with anti-angiogenic drugs, tumor growth is inhibited

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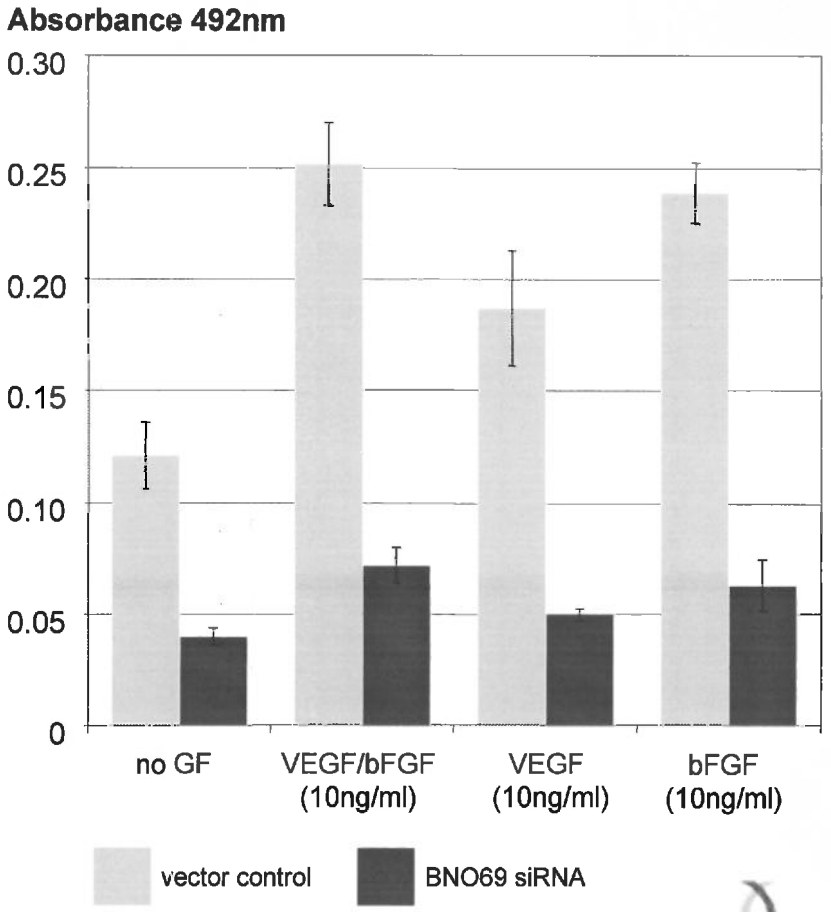
BNO69 plays a major role in Breast Cancer and Angiogenesis

- BNO69 silencing:
  - inhibits angiogenesis and breast cancer cell growth
  - disrupts two major angiogenic pathways, including the pathway targeted by Avastin
  - inhibits breast cancer growth in animals



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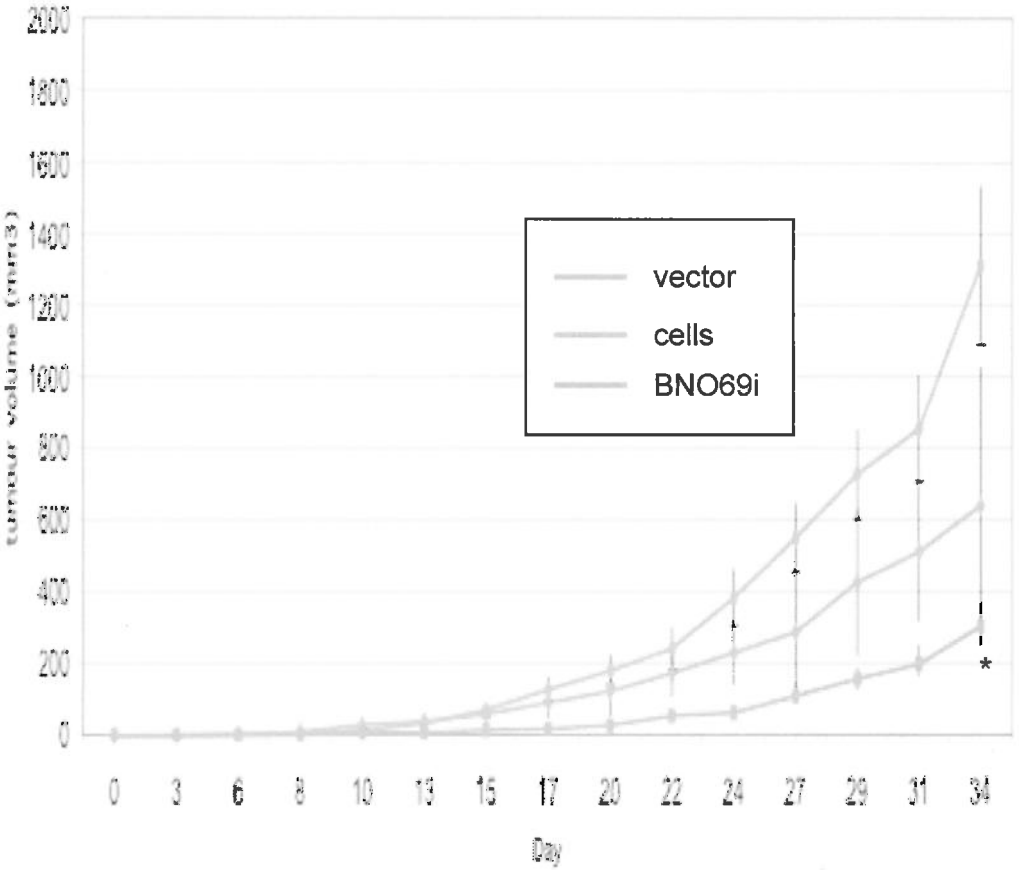
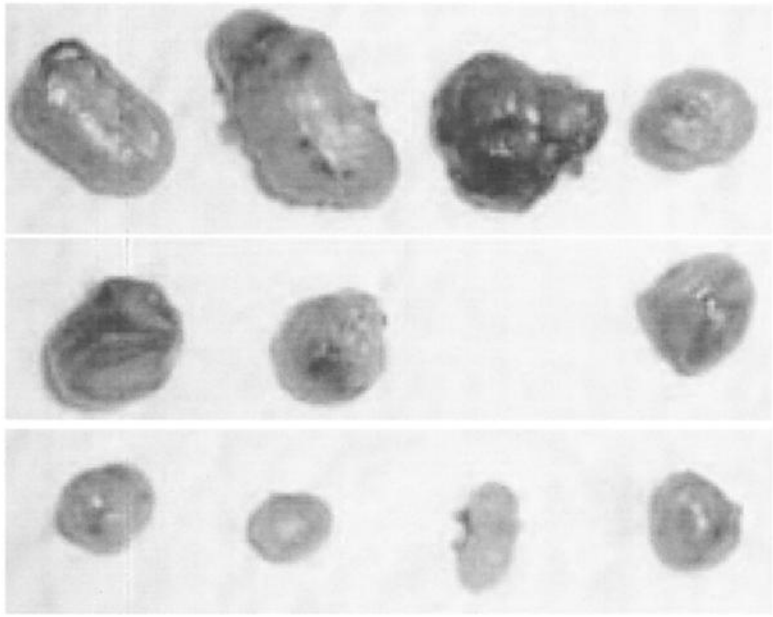
BNO69 Silencing inhibits the Tumorigenic Ability of Breast Cancer cells in vivo





*P<0.05 (One Way ANOVA - post-hoc test - Tukey's)

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Angiogenesis - 2004 Milestones

- Completed Angene™ platform rollout in March
- Generated revenue from screening compounds for fee for service using Angene™ platform
- Progressed target validation
  - BNO69 collaboration with Louisiana Gene Therapy Consortium
  - BNO69 published in *Proceedings National Academy of Sciences (USA)*

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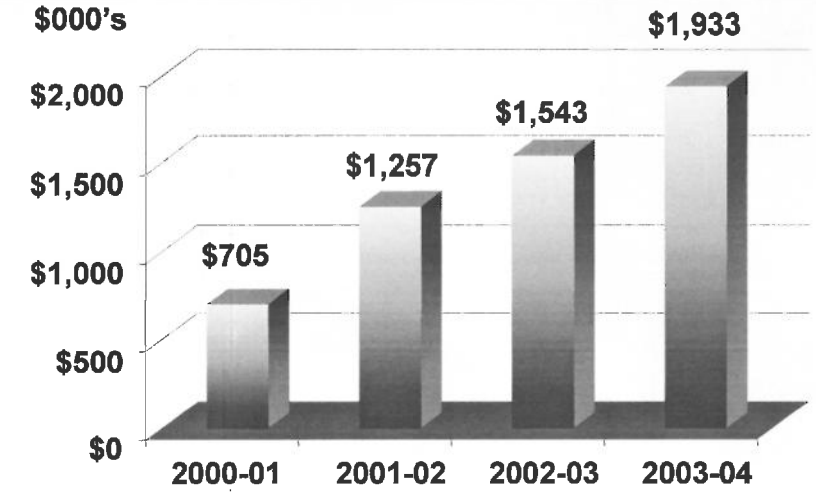
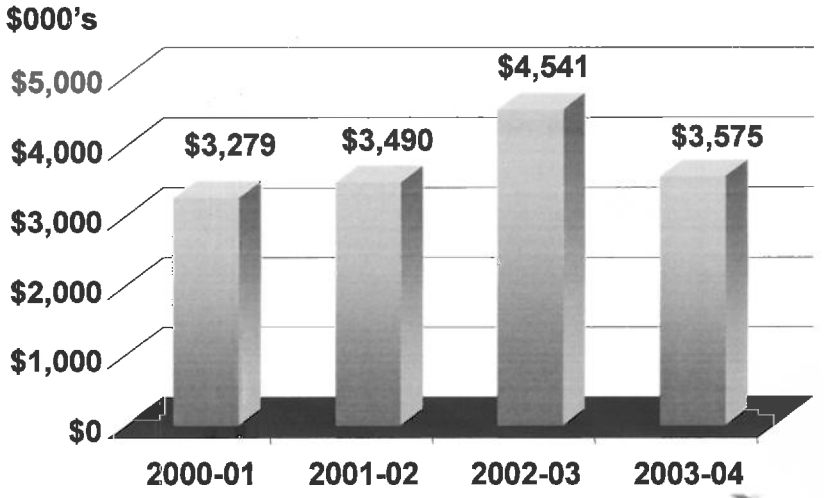
Sound Financial Position

- Increased cash inflows
- Managed down losses
- Growing revenues from epilepsy diagnostic licensing and Angene™ platform





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Positive Outlook for 2005

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Turning gene science into diagnostics and drugs

Annual General Meeting November 2004

Bionomics Limited

Bionomics Limited – Chairman-elect's comments – AGM- 11 Nov-04

Ladies and gentlemen

Thank you for your warm welcome today. It is an honor for me to be appointed Chairman of Bionomics Limited. I shall do my best to build on the excellent foundations established by our retiring Chairman Fraser Ainsworth.

Fraser of course needs no particular commendation from me. He was a founder of this Company, and has guided its fortunes wisely since then. He leaves it in excellent shape, with an exceptional CEO (recently reappointed, to my great relief) and management team.

I strongly believe that Bionomics Limited has a chance to make its mark in one of Australia's most promising, but also most difficult, fields of endeavor. Australia has scientists of world status, and Bionomics works with more than its natural share of such scientists. Its fields of endeavor – epilepsy, anxiety and some cancers - are of immense importance, and will become more important as the world's population ages and becomes wealthier. Its scientists have established sound positions in these fields, and I say "sound' as a deliberately conservative judgment.

The company's financial strategy is also conservative. We aim to have at least two year's cash on hand at any time, and this is the current situation. More importantly, we aim to create early revenue flows by licencing diagnostic tests, with evident success. And when (I do not say "if") we find promising drug candidates we shall seek to licence these to create cash flow to support our leading edge science while minimizing resort to the well of additional financing.

The board of Bionomics Limited has people of great ability and knowledge. It shall be a pleasure, as well as an honor, for me to serve as its Chairman. We shall need a touch of luck, as well as a lot of hard work, to deliver the exceptional returns that shareholders deserve.

Of course, we all know the comment made by the famous golfer about his luck – "The harder I work, the luckier I get."

All I can promise is that we shall work hard. We shall be working hard on important matters with highly talented people. I shall hope for good luck, and look forward to your support in this exciting endeavor.