A next generation sequencing approach to identify genes that modify the clinical severity of Dravet Syndrome

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Motivation

A parent’s concern:
• What does my child’s mutation type mean for future outcome?
• Why does my child have a different clinical situation than another child with the same mutation?

Modifier genes:
A gene that modifies the expression of a second gene

• Genetic variants that influence the phenotypic outcome of SCN1A haploinsufficiency via the same, a related, or a parallel biological pathway

Scientific value:
• Provide insight into the pathophysiology of epilepsy
• Suggest novel therapeutic strategies for improved treatments
Evidence for Modifiers: Variable Expressivity

SCN1A Missense Variant Segregating
14 affected, 3 unaffected carriers

Goldberg-Stern et al. (2013)

Unaffected carriers
Mildly Affected

Suggests that other factors aside from the primary SCN1A mutation influence clinical manifestation
Experimental Evidence for the Action of Modifier Genes

**Dravet Syndrome model in mice:**
- Mutations in other ion channels may modify spontaneous seizure activity.
- Supports notion that neuronal firing patterns are determined by the net sum of voltage-gated ion channel activity (the “Channelome”)

**Dravet Syndrome in humans:**
- Singh et al. (2009) DS severity correlated with segregation of variants in the closely linked \textbf{SCN9A} gene
- Ohmori et al. (2013) \textbf{CACNA1A} variants may modify the phenotype of DS
- Gaily et al. (2013) \textbf{POLG} variants may increase susceptibility for acute encephalopathy in DS

*No systematic genome-wide searches for modifier genes affecting the severity of SCN1A-related epilepsy*
Next Generation Sequencing (NGS) Approach

- Japanese cohort of 285 individuals
- Focus on affected individuals with **truncating mutations**
- Use **“extreme phenotype”** design
- *First attempt using this approach for any epilepsy syndrome*

Truncating mutations occurring anywhere in *SCN1A* (except last exon) result in **nonsense mediated mRNA decay**

NMD results in loss of 1 copy of *SCN1A* or a single “genetic phenotype”: **haploinsufficiency**
Distribution of $\text{Na}_V 1.1$ variants in Japanese cohort (n=285)

- **55.4% Truncation (n=158)**
- **42.8% Missense (n=122)**

- 91.2% de novo
- Truncation variants appear uniformly throughout $\text{Na}_V 1.1$
- Missense variants appear to cluster
Empirical Cumulative Distribution of Variants

Accumulation of truncation variants follows uniform distribution (through exon 25): do not reject test for evenness of distribution (p=0.562)

Missense variants cluster: reject test for evenness of distribution (p=0.006)
Japanese Cohort: Mild and Severe Phenotypes

Assessment of Intellectual Deficit
Classified according to DQ and IQ tests, which varied by age of child: Tanaka-Binet, WISC-IV, Enjoji Scale of Infant Analytical Development, Kyoto Scale of Psychological Development 2001, Kinder Infant Development Scale, and Tsumori-Inage Infant Mental tests

Assessment of Motor Skills
Considered motor skills, categorized two groups: those who can run and walk versus those that cannot

<table>
<thead>
<tr>
<th></th>
<th>Mild</th>
<th>Severe</th>
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<tbody>
<tr>
<td>DQ/IQ</td>
<td>&gt;50</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Ambulatory</td>
<td>yes</td>
<td>partial/no</td>
</tr>
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</table>
Truncations: more rapid and uniform rate of progression to severe cognitive phenotype

Logistic regression to predict progression to severe phenotype incorporating both age of onset and years since first seizure.

- Missense mutations: Age of onset better predicts rate of progression.
- Truncations: more rapid and uniform rate of progression to severe phenotype.
Efficacy of Add-on AEDs: Truncation versus Missense

**Truncation**

1. Stiripentol
2. Topiramate
3. Bromide
4. Levetiracetam

**Missense**

1. Clonazepam
2. Bromide
3. Topiramate
4. Stiripentol

Stiripentol and Clonazepam significantly more effective for patients with truncations and missense variants, respectively.
Experimental Design: Extreme Phenotype Approach

**Strategy to increase efficiency in identifying modifier genes**

Perform Whole Exome Sequencing (WES) on individuals at both ends of phenotype distribution. “Because the frequency of alleles that contribute to the trait are enriched in one or both phenotypic extremes, a modest sample size can potentially be used to identify novel candidate genes and/or alleles” (Emond et al. Nature Genetics 2012)

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<tr>
<td>Years since onset</td>
<td>9.9</td>
<td>5.4</td>
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<tr>
<td>Cognitive (IQ/DQ)</td>
<td>&gt;50</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Ambulatory</td>
<td>yes</td>
<td>partial/no</td>
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**Severe Group:** 11 Individuals progressing to severe phenotype most rapidly after seizure onset

**Mild Group:** 11 Individuals remaining mildly affected for longest time after seizure onset
Experimental Design: Mutation “Phenotype”

Include only *de novo* truncation mutations causing haploinsufficiency

Position of truncating mutations in 11 most extreme individuals in mild and severe categories
Experimental Design: Population Structure

Single “ethnic group”:

- minimizes population structure issues
- reduces potential diversity of modifier variants
Experimental Design: Single Variant Approach

Model 1: Common Variant model (low effect size)

- Do we enrich for a variant in one phenotypic category?

Model 2: Rare Variant Model (moderate effect)

- Do we find rare variants limited to mild (protective) or severe (damaging) category?
Candidate Low Impact Common Variants

Test 3 over 1% in Japan pop

Chrom19
KCNN1
KANK2
PLIN3
Common Variants Enriched in Mild and Severe Groups

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mild</th>
<th>Severe</th>
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<tr>
<td>KCNN1</td>
<td>1 2 2 2 2 1 2 2 2</td>
<td>0 1 1 1 1 0 1 1 1 1 0</td>
</tr>
<tr>
<td>BRAT1</td>
<td>0 0 0 1 0 0 0 0 0</td>
<td>1 2 1 0 1 1 1 1 1 2 0</td>
</tr>
<tr>
<td>BRAT1</td>
<td>0 0 0 1 0 0 0 0 0</td>
<td>1 2 1 0 1 1 1 1 1 2 0</td>
</tr>
<tr>
<td>PLIN3</td>
<td>1 1 0 0 1 1 1 1 1</td>
<td>2 2 2 2 2 2 1 2 1 2 2</td>
</tr>
</tbody>
</table>

KCNN1: excess alt allele in mild class

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<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Het</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>Alt</td>
<td>0.20</td>
<td>0.30</td>
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</table>

BRAT1: excess alt allele in severe class

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<tr>
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<td>0.20</td>
</tr>
<tr>
<td>Alt</td>
<td>0.20</td>
<td>0.30</td>
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</table>
Rare Variants Identified in Mild and Severe Groups

<table>
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<tr>
<th>Genotype</th>
<th>Mild</th>
<th>Severe</th>
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<td>CACNA1A</td>
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<td>0 0 0 0 0 1 0 0 0 0</td>
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</tr>
<tr>
<td>SCN9A</td>
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</tr>
<tr>
<td>CACNA1S</td>
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<td>0 0 0 0 0 0 0 0 0 0</td>
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<tr>
<td>CACNA1S</td>
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<td>0 0 0 0 0 0 0 0 0 0</td>
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<tr>
<td>KCNK18</td>
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<tr>
<td>TRPV3</td>
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<td>PCLO</td>
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<td>GABRQ</td>
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<tr>
<td>GABRQ</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

2 Homozygous alt 1 Heterozygote 0 Homozygous ref

4 variants in previously reported “modifiers”

9 variants in newly identified genes

More rare alleles found in severe group (14) than in mild group (2)
Gene Ontology: Prevalence of Channelome Candidates

Candidate Genes

Rare Variants

Common Variants

Mild Genotype Severe

CACNA1A
CACNA1S
SCN9A
SCN9A
CACNA1S
CACNA1S
KCNA18
TRPV3
HLA-DQa1
GABRQ
KCNN1
KANK2
HTRA2
LRRC41
PLIN3

Intracellular signaling
- protein phosphorylases
- ubiquitin ligases
- scaffold proteins
- membrane transporters
- neurotransmitter receptors

Other categories (n=3)
- intermediary metabolism
- cell adhesion/migration
- extracellular matrix
- transcription factors
- immunity

Channelome
- potassium
- sodium
- calcium

2 Homozygous alt 1 Heterozygote 0 Homozygous ref
KCNQ2 Variant Found in 3 Individuals with Severe Outcome

Benign Familial Neonatal Convulsions: Novel Mutation in a Newborn

Benign familial neonatal convulsions are a rare, autosomal-dominant form of neonatal epileptic syndrome. It can occur 1 week after birth, and usually involves frequent episodes, but with a benign course. The diagnosis depends on family history and clinical features. The mutant gene locates at 20q13, a voltage-gated potassium-channel gene (KCNQ2). Our patient exhibited an uneventful delivery course and onset of seizures at age 2 days. The general tonic seizures were unique and asymmetric, with frequencies of >20 per day. Results of examinations were within normal limits, including biochemistry and brain magnetic resonance imaging. Abnormalities included a small ventricular septum defect on cardiac sonography unrelated to the seizures, and nonspecific, multiple, high-voltage sharp waves and spike waves occurring infrequently in the central region on electroencephalogram. After phenobarbital and phenytoin use, the seizures persisted. On day 12, another antiepileptic drug, vigabatrin (unavailable in the United States), was used, and seizures decreased. A novel mutation of KCNQ2 was identified from a blood sample. The baby had occasional seizures with drug treatment at age 3 months. Benign familial neonatal convulsion should be considered in a baby with a unique seizure pattern and positive family history. Genetic counseling and diagnosis are mandatory. © 2009 by Elsevier Inc. All rights reserved.

- Same variant reported in proband with Benign Neonatal Convulsions
- Inherited from heterozygous affected mother
- Clustered, general asymmetrical tonic-clonic seizure

KCNQ2 encodes K\(_{\text{QT}}\), a voltage-gated potassium channel important for determining the excitability of neurons

More than 80 mutations in KCNQ2, most in the pore region and the intracellular C-terminal domain
Ashkenazi Kindred: SCN1A p.Lys1372Glu

14 affected, 3 unaffected carriers

Dravet Syndrome

Unaffected carriers

Mildly Affected

Are there modifier genes protecting against the epilepsy phenotype?
Genomic Position of p.Lys1372Glu mutation: DIIIIS5

- Singleton in SCN1A databases
- Not present in ExAC Browser
- Evolutionarily highly conserved site
- Predicted to be damaging by several *in silico* methods
- Of 19 other known missense mutations in DIIIIS5, 18 are associated with DS
- Predicted to stabilize the closed state

![Diagram of SCN1A protein domain structure](image)
Ashkenazi Kindred: WES

Performed WES on 9 members of family expressing all 3 phenotypes

E+: SCN1A p.K1372E

Models Tested:

• Fully protective dominant
• Fully protective recessive
• Mildly protective dominant
• Mildly protective recessive
• Additive
• Test candidate variants for mildly protective phenotype on remainder of kindred
2nd Ashkenazi SCN1A mutation: p.Leu375Ser in DI(S5-S6)

- Singleton in SCN1A databases
- Not present in ExAC Browser
- Evolutionarily highly conserved site
- Predicted to be damaging by several in silico methods
- Three unpublished cases of substitution at 375 all with DS (phenylalanine instead of serine)
- Of 81 other known missense mutations in DIS5S6, 75 are associated with DS
- Predicted to destabilize channel regardless of conformation
Ashkenazi Kindred: p.Leu375Ser (S+)

S+ found in all three unaffected carriers, inherited from paternal grandmother

- No history of epilepsy in grandmother's family
- Could this mutation protect carriers of the E+ mutation (benign GOF)?
- Variants with same segregation pattern as S+ identified in candidate modifier genes
- Candidates fall in similar functional pathways as in Japanese analysis
- Cellular model for both S+ and E+ mutations under construction

E+: SCN1A p.K1372E
S+: SCN1A p.L375S

Unaffected carriers

E+: SCN1A p.K1372E
S+: SCN1A p.L375S
Test hypothesis that SCN1A-S\(^+\) variant modulates the pathogenic effect of SCN1A-E\(^+\) (i.e., intra-locus complementation).

- Functional effects of E\(^+\) and S\(^+\) individually on Na\(_V\)1.1 channel in a cellular model
- Infer S\(^+\) balances loss of function caused by E\(^+\) using *in silico* approach (i.e., benign GOF)
Summary

Japanese Cohort

• Excess of rare variants in severe class (channelome)
• Single statistical ‘hit’ at locus that affects stress response
• Must validate single modifier locus in larger cohort

Ashkenazi Kindred

• 2\textsuperscript{nd} SCN1A variant (benign GOF) may protect pathogenic effects of segregating variant (LOF), or…
• Fully protective variant at a distinct locus (protective modifier) may co-segregate with pathogenic SCN1A variant (several candidates identified by WES)
Gene Network Interactions

Hypothetical gene network showing interactions between genes. Some gene pairs have positive interactions, whereas other gene pairs have negative interactions. Together, these interactions result in a final output phenotype.

Implications For Modifier Studies

• Different roles for common variants with low effect size, and rare variants of moderate effect size

• Rare variants distinguish individuals

• Additional factors that modify the ‘neural environment’ (e.g., stress and gender) may be important

• All of above complicate strategies to design comprehensive therapeutic approach (tension between common factor and individualized genetic background)
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