
**Variants:** Includes 33 unique variants collected from patients who participated in the CACNA1A Natural History Study. Specific variants are listed in Table 2.

**Diagnosis/Symptoms:** EA2 (Episodic Ataxia Type 2), FHM1 (Familial Hemiplegic Migraine Type 1), developmental delay, intellectual disability, seizure, PTU (Paroxysmal Tonic Upward Gaze), head injury or loss of consciousness, ASD (Autism Spectrum Disorder), depression, ADHA (Attention-Deficit/Hyperactivity Disorder), OCD (Obsessive Compulsive Disorder).

This study summarizes the results of the CACNA1A Natural History Study headed by Dr. Wendy Chung at Columbia University Irving Medical Center. Data were collected between January 2021 and December 2021 through an online medical history questionnaire completed by a patient or caregiver/parent. The variants reported are those previously classified as pathogenic or likely pathogenic. The authors looked for patterns in genotype-phenotype correlation. In other words, is there a correlation between genetic make-up (specific variants) and symptoms? They used several different software programs to look at homologous conservation (if the variant position is always the same among organisms, it may be of high importance), and help predict whether variants were LOF (loss-of-function) where the calcium ion channel cannot open correctly or GOF (gain-of-function) where the calcium ion channel opens too much. Severity scores were also generated. All data were compiled and any patterns that emerged were noted.

Data was collected from 47 participants with ages ranging from 1-40 years old. The majority of participants were children under 18 years, 85% (40/47). Only 15% (7/47) were adults. There was also a higher percentage of females, 66% (31/47). There were 33 total unique variants reported. Twenty-seven variants were missense (variants that changed the amino acid), 3 were nonsense (variants that created an early STOP codon resulting in a shorter protein), 2 were frameshift (variants that shifted the reading frame for correct protein), and 1 was in-frame deletion (a portion of the gene was missing). Please note that the variants identified in the study may be listed with a different amino acid number than what was shown on a participant’s genetic test report. The authors may have used a different reference RNA sequence than the testing companies, which may shift the variant position within a few places, but the amino acid change remains the same. Twenty-eight variants were *de novo* (not inherited from a parent), 6 were inherited, and 13 were not determined because parents were not tested. The highest reported symptoms were developmental delay, including some intellectual disability (96%), ataxia - episodic or non-episodic (75%), and hypotonia (75%). Hypotonia was the earliest manifestation in 43% of those reporting this symptom. Paroxysmal tonic upward gaze was reported as the first symptom in 36% of participants. Eye movement disorders, including nystagmus, were also seen as early-onset symptoms in over 50% of participants.

For EA2 and FHM1, 36% and 32% were reported as symptoms, respectively. Only four participants reported having both disorders (9%). To investigate whether there was a correlation between LOF or GOF variants and EA2 and FHM1, the authors used a software tool to predict how a specific variant might affect the function or activity of the calcium ion channel (classify them as either LOF or GOF). They did not see any significant correlation between EA2 and LOF, or FHM1 and GOF, as had been reported previously. However, the authors found that those who reported having both EA2 and FHM1 had predicted GOF variants.

The study found that 62% of participants reported having at least one seizure, with 55% reporting epilepsy (two or more unprovoked seizures). Furthermore, there was a correlation of GOF variants with seizures as a phenotype. 38% of participants reported head injuries or loss of
consciousness, including coma, cerebral edema (swelling of the brain), or stroke. The highest reported behavioral diagnosis was ASD (Autism Spectrum Disorder) at 23%. The authors also noted that while depression, anxiety, and ADHD had low incidence rates, it could be due to the fact that adults only made up 15% of the entire study. More studies done in adults with CACNA1A variants are needed to determine long-term behavioral challenges.

To determine a severity ranking of all the variants, the authors used a weighted sum of common symptoms for each variant. They assigned points accordingly to each symptom associated with the variant to calculate a final value. The higher the value, the more severe the variant. Statistical analysis was done using a p-value < 0.01. They found that variants located in the S5 and S6 subunits, which form the pore of the calcium ion channel, had significantly higher severity scores than those in the S3 subunit and linkers between subunits. Surprisingly, there was no significant difference in severity scores of variants in the S4 (voltage sensing) subunit versus S3 and linkers. The authors did not comment further on that observation. The authors also reported that missense GOF variants were significantly more severe than missense LOF variants, and both had significantly higher severity scores than the nonsense (shortened protein) variants. The variant V1392M was the most recurrent (8/47) and had a significantly higher severity score than other variants. This variant also correlated with seizures and status epilepticus (seizures longer than 5 minutes or more than one seizure within 5 minutes). Finally, the authors reported that the study included two families with two CACNA1A-affected individuals each. Their observations were in line with previous data supporting the wide range of variation seen in symptoms within the same families.

Overall, the study showed a higher prevalence of developmental delay, hemiplegic migraine, ASD, and epilepsy than had previously been reported. Higher severity was also predicted in gain-of-function variants and those located in the S5 and S6, the pore-forming subunits of the calcium ion channel. Furthermore, there was a correlation between gain-of-function variants and seizures. The authors note multiple times that there are some differences between the Natural History Study and a similar genotype-phenotype study by Gur-Hartman and colleagues from 2020. The discrepancies could be due to sample size (how many participants) and how the data were collected. The authors also state that their study may not be representative of those with CACNA1A-related disorders and could be biased toward those with more severe conditions that seek clinicians and doctors. Another limitation was that their severity score did not accurately reflect the levels of severity within each individual symptom. However, data for the Natural History Study will continue to be collected, with additional changes to account for variation.

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