RATIONALE

- Most seizures in the neonatal period are related to acute brain injury including structural, infectious, metabolic, and immune causes¹
- However, for some patients, neonatal seizures are the first symptom of a genetic epilepsy syndrome
- Variants in the KCNQ2 gene underlie a spectrum of neonatal-onset epilepsies with varying severity, from severe developmental and epileptic encephalopathy (KCNQ2-DEE), affecting ~100–150 new infants per year in the US, to a milder self-limited form²
- KCNQ2 encodes the voltage-gated potassium channel K_v7.2, and partial loss of function causes KCNQ2-related epilepsy which typically presents during the first week of life, with multiple, daily seizures³
- Heterozygous pathogenic variants are typically de novo mutations; however, they can also be inherited (autosomal dominant), and mosaicism has also been reported in some patients³
- No treatments are specifically approved for KCNQ2-DEE
- The K_v7 channel potentiator, XEN496, is currently in development as a precision medicine for KCNQ2-DEE
- The objective of this analysis is to determine the molecular diagnostic yield of neonatal patients with unprovoked seizures, identified through a sponsored, targeted testing program for children suspected to have genetic epilepsy

METHODS

- The testing program used a next-generation sequencing panel with simultaneous exonic sequence and copy number variant detection in up to 302 genes
- Ordering clinicians provided a brief medical history including seizure type, age of seizure onset, family history, developmental delays, and use of antiseizure medications
- Patients who had an unprovoked seizure were eligible for testing (at no charge) and were 0–48 months (Feb 2019–Jan 2020) and 0–96 months of age (Jan 2020–Jan 2022)
- A positive molecular diagnosis (PosMD) was defined as a patient harboring a pathogenic or likely pathogenic variant(s) with the expected inheritance pattern match for the gene condition
- Variants of uncertain significance (VUS) were also returned on clinical reports
- For the first part of the study, evaluations were in patients reporting seizure onset at <1 month of age. This is the youngest age grouping accessible. Data on seizure onset at <1 week of age are not available

Figure 1. Positive Molecular Diagnoses in Patients With Reported Seizure Onset Aged <1 Month

ΙΝΥΙΤΛΕ

Genetic Burden of KCNQ2 in Neonates With Epilepsy

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RESULTS

• Of the 1276 patients with a seizure onset reported in the first month of life, 24% (309/1276) had a PosMD

The most common genetic etiology was KCNQ2; seen in 13.6% (174/1276) of all individuals tested and 56.3% (174/309) of the PosMD for this seizure-onset age group (Figure 1)



Note: Patients were <96 months of age at the time of testing but had a seizure onset of <1 month of age reported on their test requisition form.

In patients with seizure onset in the first month of life, PosMD in KCNQ2 were approximately 8 times more frequent than PosMD in the next most common gene SCN2A (6.8%)

• The other more common genes with a PosMD in this seizure-onset age group were *KCNQ3* (3.6%), *STXBP1* (3.6%), and *CDKL5* (2.6%)

• To confirm age of onset trends, a separate analysis was also performed for patients who were tested in the first month of life who presumably presented with seizures before testing (Figure 2)



Figure 2. Positive Molecular Diagnoses in Neonates Aged <1 Month at Testing

Note: Patients were <1 month of age at the time the test was ordered.

- Of the 801 patients tested <1 month of age, 29% (233/801) had a PosMD</p>
- PosMD in KCNQ2 predominated and were seen in 19.1% (153/801) of all individuals tested and 65.7% (153/233) of the PosMD, followed again by SCN2A (7.7%)
- Together, these data confirm that KCNQ2 is the most common molecular diagnosis in both patients with seizure onset in the first month of life and in patients receiving genetic testing in the first month of life
- ~40% of KCNQ2 PosMD had seizure onset recorded after 1 month of age

KCNQ2 Variant Types

- A total of 174 KCNQ2 unique variants were identified in the neonates with seizure onset <1 month and 101 were identified in neonates aged <1 month at testing
- The most common variant types were missense, followed by copy number deletions (Figure 3)



CNV, copy number variants.

KCNQ2 VUS Interpretation

- VUS resolution testing was offered to solve the interpretation of a VUS in KCNQ2 in 262 patients who did not have a PosMD in another gene
- Family member testing was performed for 37% (97/262) of patients aged 0–96 months, who harbored a KCNQ2 VUS
- -27 (28%) had seizure onset <1 month
- -70 (72%) had seizure onset ≥1 month
- 63% (17/27) of patients with seizure onset <1 month had VUS reclassified to</p> pathogenic or likely pathogenic (**Table 1**)
- –In contrast, only 11% (8/70) of patients with seizure onset ≥1 month had a *KCNQ2* VUS reclassified as likely pathogenic or pathogenic
- 74% of reclassifications of unique VUS (17/23) were the result, at least in part, of family testing; the majority of reclassified variants (15/23, 65%) were determined to have occurred de novo via family testing

Table 1. Unique VUS Reclassifications (All Ages)

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HGVS Variant Nomenclature	Effect	Classification Change	Reason for Reclassification
NM_172107.2:c.380A>G	p.Tyr127Cys	VUS>LP	Family testing, de novo
NM_172107.2:c.578C>T	p.Ala193Val	VUS>P	Family testing, de novo
NM_172107.2:c.649A>G	p.Thr217Ala	VUS>P	Functional modeling
NM_172107.2:c.667T>C	p.Ser223Pro	VUS>P	Family testing, de novo
NM_172107.2:c.680C>T	p.Ala227Val	VUS>LP	Family testing, segregation
NM_172107.2:c.684C>A	p.His228Gln	VUS>LP	Case reports, family testing, segregation, functional modeling
NM_172107.2:c.686G>T	p.Ser229lle	VUS>P	Family testing, de novo
NM_172107.2:c.689A>T	p.Lys230Met	VUS>LP	Family testing, de novo
NM_172107.2:c.734T>C	p.Leu245Pro	VUS>P	Family testing, de novo
NM_172107.2:c.827C>A	p.Thr276Asn	VUS>P	Family testing, de novo
NM_172107.2:c.970C>T	p.His324Tyr	VUS>LP	Family testing, de novo
NM_172107.2:c.1025C>T	p.Ser342Leu	VUS>LP	Family testing, de novo
NM_172107.2:c.1064A>G	p.Asp355Gly	VUS>P	Family testing, de novo
NM_172107.2:c.1065C>A	p.Asp355Glu	VUS>LP	Family testing, de novo
NM_172107.2:c.1067T>A	p.Leu356Gln	VUS>P	Family testing, de novo
NM_172107.2:c.1270C>T	p.Pro424Ser	VUS>LB	Functional modeling
NM_172107.2:c.1619T>G	p.lle540Ser	VUS>LP	Family testing, de novo
NM_172107.2:c.1627G>A	p.Val543Met	VUS>P	Additional clinical information
NM_172107.2:c.1639C>T	p.Arg547Trp	VUS>LP	Additional clinical information
NM_172107.2:c.1757A>C	p.Gln586Pro	VUS>P	Functional modeling, additional case report
NM_172107.2:c.1906_1908del	p.Lys636del	VUS>LP	Family testing, de novo
NM_172107.2:c.2266G>A	p.Gly756Ser	VUS>LB	Functional modeling
NM_172107.2:c.2486_2487del	p.Lys829Serfs*35	VUS>P	Family testing, de novo

HGVS, Human Genome Variation Society; LB, likely benign; LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.

CONCLUSIONS

- KCNQ2 molecular diagnoses were observed in approximately one-fifth of all individuals tested in the first month of life and accounted for almost two-thirds of the PosMD in this age group
- Missense variants (~40%) were the most common variant type in KCNQ2-positive patients with reported seizure onset <1 month
- Maximizing the diagnostic yield in neonates by access to genetic testing early in the diagnostic pathway, including VUS resolution testing, is important as a molecular diagnosis is vital to facilitate early intervention, avoid unnecessary specialist evaluations, and to inform treatment strategies and prognosis, including access to potential precision therapies currently in clinical development

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