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Navigating the Benefits and Limitations of Current Molecular Testing Options for the Clinical Identification of Disease-Causing *PCDH19* Mutations

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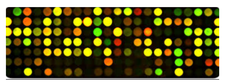
BACKGROUND

- Mutations in the *PCDH19* gene (OMIM: 300460) have been associated with early infantile epileptic encephalopathy type 9 (OMIM:300088).
- *PCDH19*-Associated epilepsy is mostly identified in females and also presents with intellectual disability and behavioral issues.
- The *PCDH19* gene is located on chromosome Xq22.1 and encodes protocadherin-19 protein, which is part of a subclass of protocadherins belonging to the cadherin superfamily.
- Although recent advancements in molecular sequencing technology have provided patients with the opportunity to pursue genetic testing for *PCDH19*-suspicious clinical presentations, benefits and limitations of these testing options remain complex for clinicians and patients to navigate on an ongoing basis

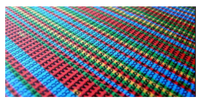
METHODS

- We reviewed the Human Gene Mutation Database (HGMD) and identified 114 mutations described to date in the *PCDH19* gene.
- Of these alterations listed, it was determined by review of the Database which genetic test (gene sequencing vs microarray) was used to identify each variant in PCDH19.

CLINICAL GENETIC TESTING OPTIONS OVERVIEW



Clinical Genomic Microarray utilizes array CGH technology, which was developed for high-resolution, genomic-wide screening of copy number variations. Because of its superior resolution, the 180K Oligo Array can frequently provide a relevant result when the patient has an apparently normal karyotype. Ambry has designed this array to detect aneuploidies, well-characterized microdeletion or microduplication syndromes, and subtelomeric or other unbalanced chromosomal DNA rearrangements. In addition, it may be clinically useful for the detection of microdeletions/duplications in a specific gene(s) in patients who are negative for point mutations or small intragenic aberrations.



Next-Generation Gene Panels are a comprehensive gene sequencing screen for multiple genes associated with a particular condition. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized kit and gene coding exons are targeted through Next-Generation Sequencing. Additional Sanger sequencing may be performed depending upon the technology used and the individual laboratory protocols.

Whole exome sequencing has successfully been used to identify both inherited and *de novo* explanatory gene mutations in a diverse variety of autosomal dominant, autosomal recessive, and X-linked disorders. It is one of the most comprehensive tests available for identifying the underlying genetic cause of a patient's medical condition, and does so in a timely, cost-effective manner.

DISCUSSION

- Of 114 mutations in the *PCDH19* gene described in the HGMD database, over 93% of these cases were identified through gene sequencing. The remaining 7% were identified through chromosomal microarray.
- *PCDH19* associated epilepsy is a very difficult form of female epilepsy to diagnose clinically due to its rarity and phenotypic overlap with other genetic forms of epilepsy. Based on the information available on affected individuals in the literature, it is clear that gene sequencing and chromosomal microarray are the two ideal testing options for genetic diagnosis in these cases. However, limitations of gene sequencing warrant microarray as first-tier option.
- Based on the complex and variable phenotype presented by affected individuals, exome sequencing can be a time and cost-effective way to identify gene mutations in the *PCDH19* gene after a chromosomal microarray has been performed and is found to be negative.
- As Next-Generation sequencing becomes more readily available and utilized for the diagnosis of patients presenting with epilepsy, intellectual disability, and autism spectrum features, more cases of PCDH19 related epilepsy will be recognized adding to further gene characterization.

HGMD REPORTED *PCDH19* MUTATIONS

% Described in HGMD (n=114)	Mutation Type	Clinical Testing
71 (62%)	missense/nonsense point mutations	gene sequencing
3 (~3%)	splicing mutations	gene sequencing
12 (~10%)	small deletions	gene sequencing
17 (15%)	small insertions	gene sequencing
3 (~3%)	small indels	gene sequencing
8 (~7%)	gross deletions	chromosomal microarray

REFERENCES

Stenson, P.D., et al., *The Human Gene Mutation Database: 2008 update*. Genome Med, 2009. 1(1): p. 13.

Ambry Genetics website: www.ambrygen.com

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