“n=1” Cases in GeneMatcher: Reporting Alterations in Truly Novel Disease Genes

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BACKGROUND

- Mendelian disease gene discovery efforts have gained momentum with the use of whole exome sequencing (WES) for identifying pathogenic alterations in novel candidate genes.
- These efforts were aided to a large extent by data sharing and collaborations between referring physicians, diagnostic and academic laboratories.
- Since 2011, we have reported alterations in candidate genes in 6% (131/2223) of patients referred to our laboratory for diagnostic WES (DES).
- Fully vetted candidate gene scoring criteria were used for evaluating the evidence to support the reporting of such alterations.[1]
- In March 2016, we began submitting candidate gene alterations to GeneMatcher[2] and have matched with submissions spanning 97 genes. Matches have yet to be established for 13 genes.
- Here we present a case with compound heterozygous alterations in the *NMNAT2* gene for which we have yet to establish a match with additional patients despite strong evidence for gene-disease association from experimental studies of the gene function and its disruption in animal models.

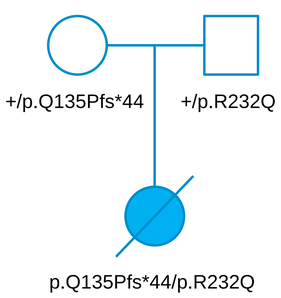
CLINICAL HISTORY

- Proband sample from a stillborn fetus delivered at 27 weeks gestation.
- Prenatal findings included ventriculomegaly, flexion contractures and oligohydramnios.
- Fetal MRI identified brain abnormalities including enlarged ventricles, deficient cerebellum and dysgenesis of the corpus callosum.
- Additional findings on autopsy included complete absence of muscle tissue, lung hypoplasia, contractures, micrognathia, cleft palate, and dysmorphic features.
- Family history is negative for similarly affected individuals.

METHODS

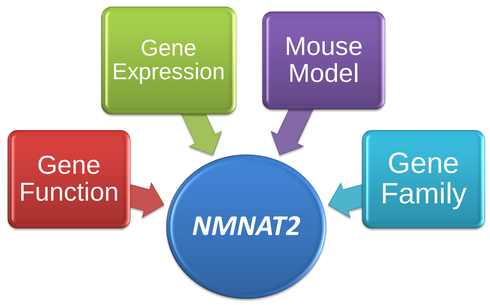
- Diagnostic WES (DES) was performed on genomic deoxyribonucleic acid (gDNA) isolated from sample from the proband and both parents.
- Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described.[3]
- For cases with negative or uncertain findings in characterized genes, analysis and interpretation of novel candidate gene findings were performed using criteria reported previously.[2]
- Targeted Sanger sequencing of the identified alterations was performed for confirmation of DES results and for co-segregation analysis.
- Structural variant analysis was performed using PyMol (The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC).
- Alterations in reported novel candidate genes were submitted to GeneMatcher.

RESULTS



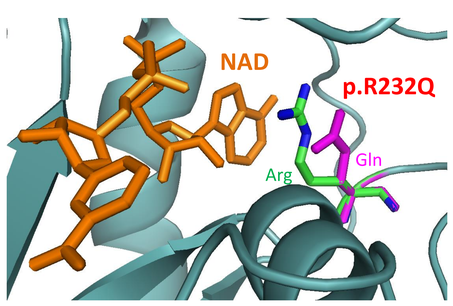
- Analysis of **Characterized Genes** did not prioritize any relevant findings.
- Analysis of **Novel Candidate Genes** identified compound heterozygous alterations in the *NMNAT2* gene (OMIM: 608701) that encodes nicotinamide nucleotide adenylyl transferase 2 (NMNAT2), an enzyme that converts nicotinamide mononucleotide (NMN) to nicotinamide dinucleotide (NAD) in the presence of ATP.[4]
- The maternally inherited c.403dupC (p.Q135Pfs*44) variant is absent in ExAC[5] but is observed at a frequency of 4.1e-6 (1/244224 alleles) in gnomAD.[5] No individuals homozygous for this variant or any other loss-of-function variants are present in either database.
- The paternally inherited c.695G>A; p.R232Q variant is rare and occurs at a frequency of 2.5e-5 (3/121352 alleles) in ExAC[5] and 1.22e-5 (3/246230 alleles) in gnomAD.[5] No individuals homozygous for this variant are present in either database.

NOVEL CANDIDATE GENE ANALYSIS



- **Gene Function**:
  - Plays an essential role in axon growth and survival during embryonic development.[6]
  - Involved in delaying or preventing Wallerian (injury-induced) axon degeneration or Wallerian-like axon degeneration seen in neurodegenerative disorders such as peripheral neuropathies and amyotrophic lateral sclerosis.[7]
- **Gene Expression**:
  - Expressed in the brain with high levels in all the sub-regions of the brain tested, lower expression in heart and skeletal muscle.[4,8]
  - Localized to the Golgi complex, membranes of vesicles and synaptic terminals.[9,10]
- ***In vivo* – mouse model**:
  - Homozygous *Nmnat2* null mice die at birth, likely due to failure of respiratory function with a collapsed lung structure, a severely underdeveloped diaphragm, and a significant decrease in the overall skeletal muscle mass along with a drastic reduction in the numbers of motoneurons and sensory neurons of the dorsal root ganglia.[11]
- **Gene Family**:
  - Biallelic mutations in *NMNAT1* (OMIM: 608700) cause Leber congenital amaurosis 9 (LCA9), a severe neonatal neurodegeneration of the central retina and early-onset optic atrophy (OMIM: 608553).
  - Homozygous *Nmnat1* knockout mouse exhibits prenatal lethality[12], similar to the *Nmnat2* knockout mouse.[11]
  - Functional studies of *NMNAT1* mutations indicate that NMNAT1 function is significantly reduced but not completely abolished in LCA9 patients.[13]

STRUCTURAL VARIANT ASSESSMENT



- NMNAT2 p.R232Q lies in the NAD-binding pocket as determined from the structure of the highly homologous NMNAT3.[14]
- Structural overlap places p.R232Q in direct contact with the bound adenine group of NAD.
- The interaction is a stacking interaction of the guanidinium group of Arg and the base of adenosine.
- The observed variant does not have the large size and positive charge of Arg indicating it would not be able to recapitulate these wild type features and have a deleterious impact on adenosine binding and NAD formation.

DISCUSSION

- Reporting candidate gene findings to patients and families, and data sharing via avenues such as GeneMatcher assist in the potential identification of additional patients with the same altered candidate gene.
- We came across two submissions in GeneMatcher for our *NMNAT2* case: one was a phenotype mismatch – two sisters with polyneuropathy and intense pain in the feet; the second submission was from a mouse researcher – no patient.
- While matches made via GeneMatcher have been instrumental in the characterization of several new gene-disease associations, and increased the confidence of others, variants in certain rare disease genes might only be identified in n=1 cases.
- There is clinical utility in reporting variants in novel candidate disease genes for healthcare management and family planning.

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