# Detection of Mosaicism by Augmented Exome Sequencing

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## Introduction

The contribution of mosaicism to the development of Mendelian disease has been increasingly recognized as techniques sensitive to mosaic detection have been adopted as primary testing strategies. However, routine detection of mosaicism in conventional genome and exome sequencing is hampered by multiple factors. Assumptions inherent to genotyping and filtration strategies utilized by both genome and exome analysis often result in the dismissal or outright failure to call variants at allelic representations consistent with mosaicism. For those variants that are called as mosaic, false positives can be rampant, especially over paralogous sequence regions. Additionally, the overall lower read depths (typically ~30X) achieved in genome sequencing, and the wide variability in positional coverage achieved with conventional exome sequencing make the detection of mosaic variants difficult, especially at lower mosaic fractions. We present two cases of mosaicism identified by augmented exome sequencing with modifications for mosaic detection, and phenotype-driven analysis.

## Methods

The described cases were sequenced using the ACE Research Exome™ (Personalis, Inc.), variants were called and annotated with the Personalis ACE pipeline, and a knowledge-based ranking system approach was utilized to rank variants. The ACE Exome constitutes an exome sequencing platform in which coverage is enhanced for >7800 biomedically relevant genes, providing higher depth of coverage over interpretable content. The platform also incorporates genome-wide structural variant detection. Both cases also underwent high depth genome sequencing (40–60x mean read depth) and that data was similarly analyzed by Personalis.

## Case Study 1:

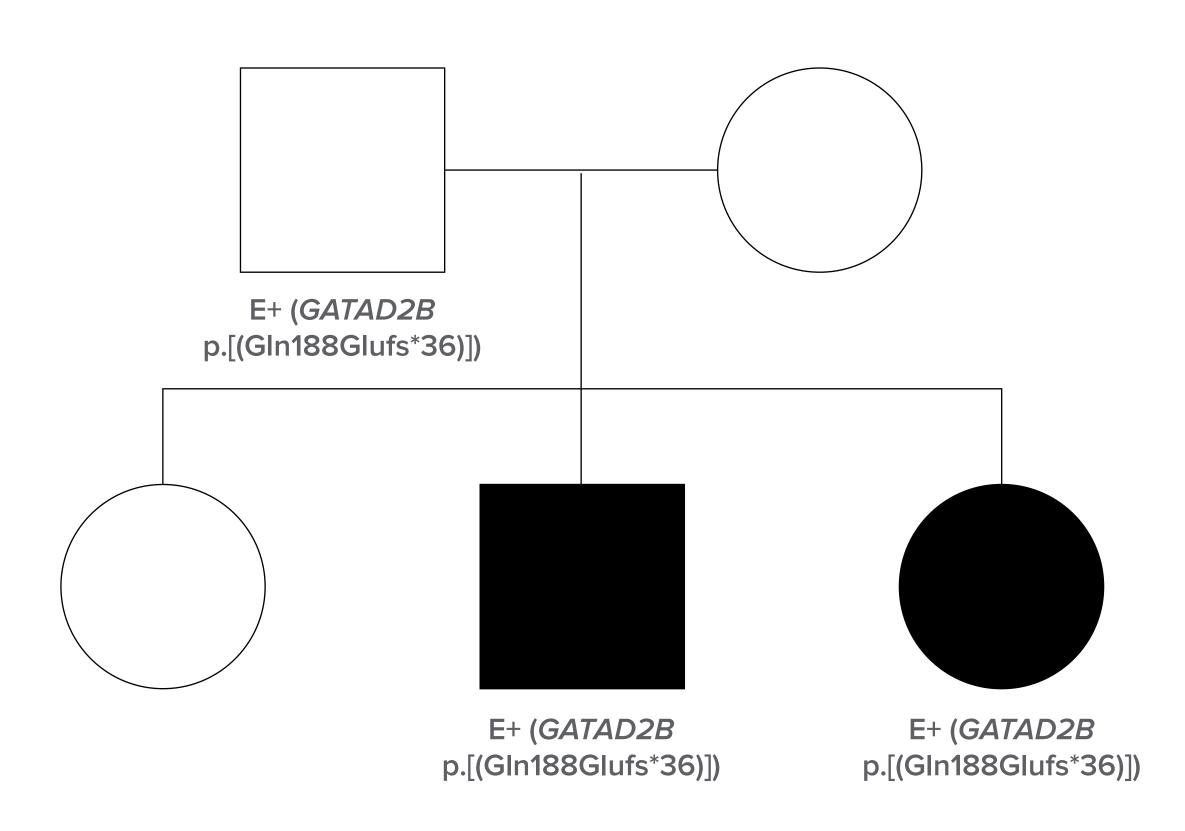### Germline *GATAD2B* Mosaicism Leading to Disease Recurrence within a Family

#### HISTORY

- Two affected siblings, no other family history
- Shared features: intellectual disability/developmental delay, macrocephaly, hypotonia, delay in early milestones, esotropia and dysphagia
- Additional features in male: mild hearing loss, high arched palate, small cyst near eye (by MRI)
- Additional features in female: sleep apnea, failure to thrive, laryngomalacia, anisocoria, small optic nerves

#### PRIOR TESTING

- **Male:** Negative Fragile X, X-linked panel: VUS not segregation consistent, arrayCGH intronic duplication (VUS)
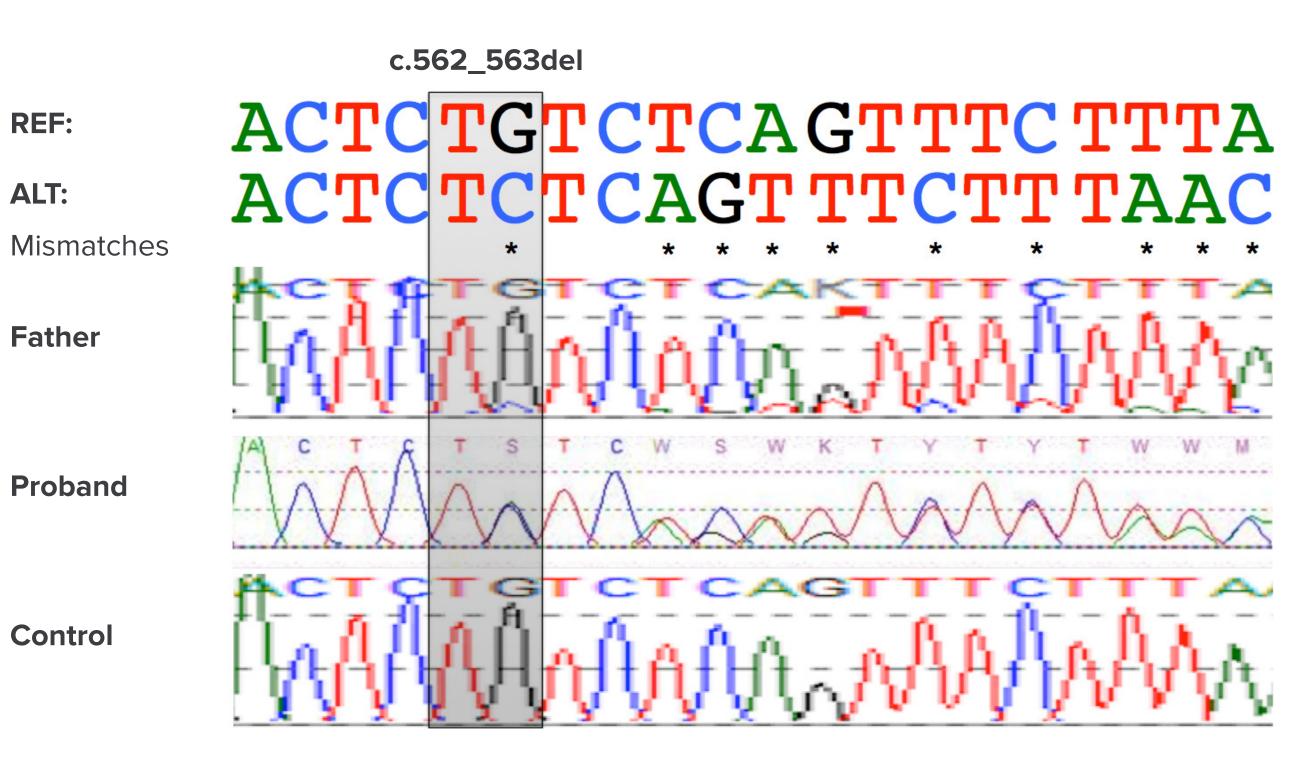- **Female:** arrayCGH negative

**FIGURE 1:** Case Study 1 Family History



E+ (*GATAD2B* p.[(Gln188Glufs*36)])

E+ (*GATAD2B* p.[(Gln188Glufs*36)])

E+ (*GATAD2B* p.[(Gln188Glufs*36)])

## Case Study 1 (cont.)

Augmented exome sequencing of the affected siblings and their parents identified a novel indel in *GATAD2B*, c.562_563del (p.Gln188Glufs*36), present heterozygously in the siblings and at ~10% variant fraction in the blood of the father (11 of 119 reads by augmented exome, 8 of 63 reads by high depth genome). Sanger confirmation was performed and was consistent with the exome and genome results (**FIGURE 2**).

**FIGURE 2:** Sanger Validation of *GATAD2B* Variant



Loss-of-function *GATAD2B* variants have been described in the literature in association with hypotonia, intellectual disability, speech delay, and a characteristic pattern of dysmorphic facial features. These individuals represent the 6th and 7th reported cases of *GATAD2B*-associated intellectual disability and further characterize the molecular mechanism and phenotypic spectrum of this disorder. Two of the five previously described variants are indels occurring within or adjacent to the CR1 domain, as is the variant described in this case, and result in premature truncation at nearly the same position (p.Gln190Alafs*34 and p.Asn195Lysfs*30). A case of maternal germline mosaicism for one of these indels, p.Gln190Alafs*34 (Willemsen *et al.*, 2013) has been previously described, while all other variants have apparently occurred *de novo*.

## Case Study 2:

### Somatic *SCN5A* Mosaicism Leading to Perinatal Long QT Syndrome

#### HISTORY

- Female affected newborn, no family history
- Delivered prematurely after prenatal detection of 2:1 AV block
- Multiple episodes of torsade de pointes on day of life 1, QT prolongation (740 msec)
- ICD placement on day of life 2
- Heart transplant at 6 months
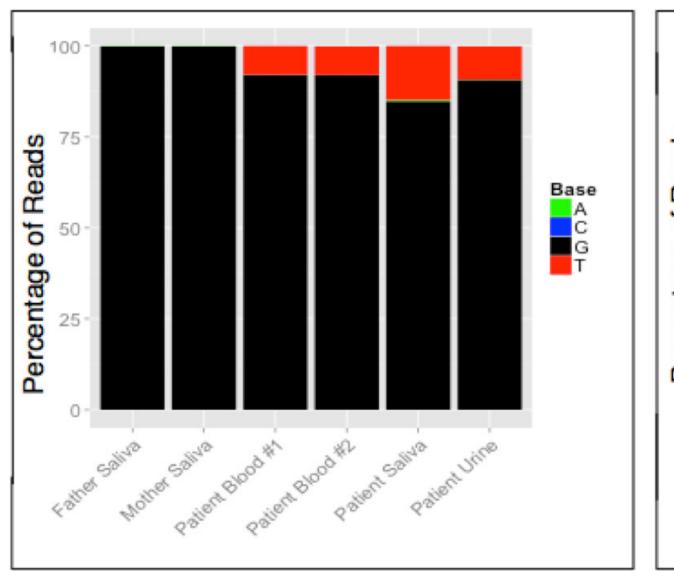
#### GENETIC TESTING

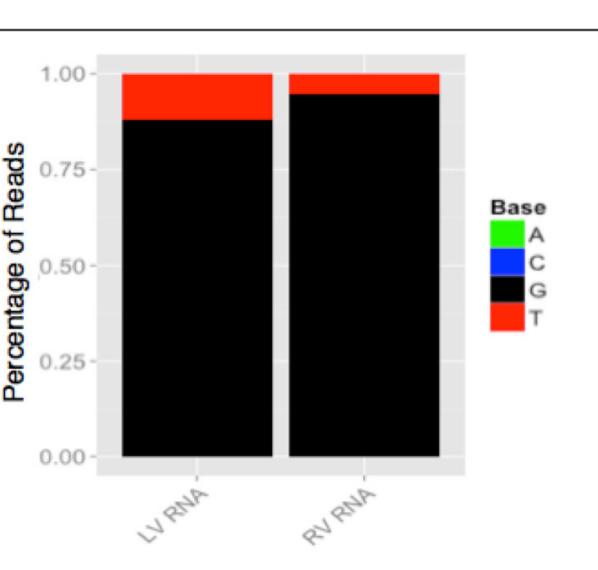- Clinical long QT gene panel on day of life 1 (negative)

## Case Study 2 (cont.)

Rapid whole genome sequencing was performed on the patient on day of life 3 and a novel missense variant in *SCN5A*, c.5284G>T (p.V1762L), was called by some genotyping pipelines, but not others. Those pipelines that did not call the variant presumably failed to do so due to the low fraction of variant alleles represented (8 of 34 reads). Augmented exome sequencing of the entire trio was pursued which revealed that the *SCN5A* variant was present as a *de novo* mosaic variant in the patient (17 of 227 reads). Manual re-mapping of the reads confirmed optimal mapping to *SCN5A* and quantitative PCR copy number assays confirmed that the 28th exon of the *SCN5A* gene existed in diploid copy number, supporting the conclusion that the variant was mosaic in the affected patient.

The p.V1762L variant occurs within a highly conserved transmembrane helix and a p.V1762M variant has been previously described in association with perinatal LQTS (Chang *et al.*, 2004). Single cell genotyping, direct Sanger sequencing from single cell clones, next-generation sequencing from single cell clones from multiple lineages including blood, saliva, and urine, and RNA sequencing in cardiac tissue were all consistent with the exome result of ~10% mosaicism across all developmental lineages (**FIGURE 3**). Further functional characterization of the detected variant by single cell patch clamping demonstrated that the variant results in delayed and incomplete inactivation compared to wildtype, consistent with a highly pathogenic LQTS variant. Clinical LQTS panel testing failed to detect the mosaic variant.

**FIGURE 3:** Minor Allele Fraction in DNA from Multiple Tissues (left), Minor Allele Fraction in RNA from Cardiac Tissue (right)



## Discussion

Next generation sequencing technology can facilitate the detection of biomedically interpretable mosaicism, but achieving sensitivity for the detection of germline or somatic mosaicism by clinical exome and genome sequencing requires the implementation of specialized approaches. In order to routinely detect mosaicism, sequence coverage must be consistently deep, and variant-calling algorithms appropriately adjusted to recognize mosaic variants. We present two cases that demonstrate that the use of augmented exome sequencing with modification for mosaic detection allows for the sensitive detection of mosaicism. In one of these cases we demonstrate that this exome approach was more sensitive to mosaic detection than both clinical panel testing and some genome-based approaches.

**References:**
- Willemsen *et al.*, J Med Genet. 2013 Aug;50(8):507-14.
- Chang *et al.*, Cardiovasc Res. 2004 Nov 1;64(2):268-78.

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