# A novel gene not so novel anymore: personalized *in vitro* model and further evidence for association of *DNM1* with early infantile epileptic encephalopathy through clinical exome sequencing

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## BACKGROUND

- The role of dynamin, a GTPase involved in synaptic vesicle endocytosis, has been extensively studied both *in vitro* and in mouse models of epilepsy. However, no human diseases had been associated with alterations in the encoding gene *DNM1* until recently.
- Our report of a non-recurrent heterozygous *de novo* alteration in the *DNM1* gene comes concurrently with the EuroEPINOMICS-RES/Epi4K consortia's report of *DNM1* alterations in five unrelated patients with early infantile epileptic encephalopathy.
- Patient: a 10-month old boy of South Asian descent with infantile spasms, hypotonia, severe global developmental delay, and secondary microcephaly. Although the brain MRI was normal, the patient's electroencephalogram revealed frequent generalized and multifocal epileptiform activity, suggestive of hypsarrhythmia and supportive of an underlying epileptic encephalopathy.

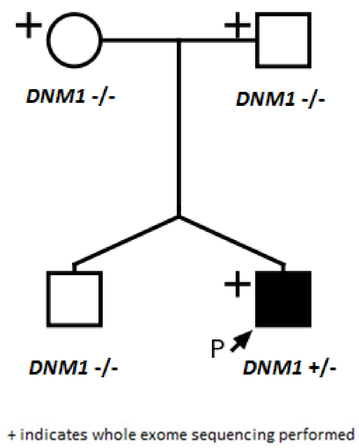
## METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing and data analysis:** Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) or SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA). Data analysis were performed as previously described (Farwell et al. 2014).

## RESULTS

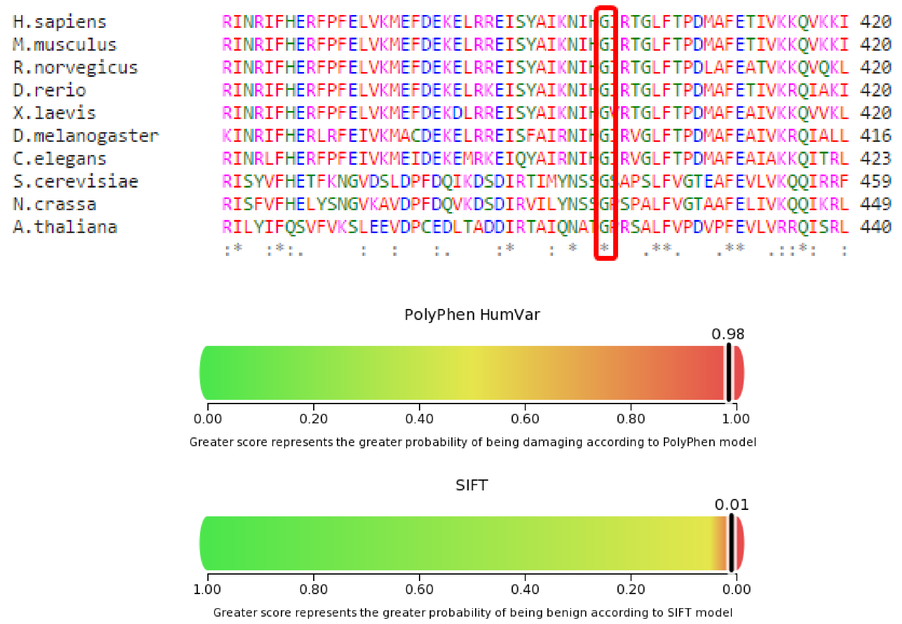
- We identified a c.1190G>A (p.G397D) alteration through proband-parents trio whole exome sequencing.
- This novel alteration co-segregated with the phenotype in the family since it was absent in the unaffected parents and the proband's unaffected dizygotic twin brother (figure 1).
- The glycine at this position is highly conserved through evolution and the alteration to aspartic acid is predicted to be deleterious by *in silico* methods (figure 2).
- The p.G397D alteration is located in the middle domain of the dynamin protein, which is thought to interact with adjacent dynamin molecules to form super-helical structures that use GTP hydrolysis to pinch off vesicles during endocytosis and membrane remodeling, a crucial function at the synaptic vesicle (figures 3 and 4).
- Coincidentally, mutant rat dynamin expressed in E. coli containing the orthologous G397D alteration was previously reported to be deficient in assembly-dependent GTP hydrolysis, thus exerting a dominant negative effect on the function of the wild type protein by inhibiting endocytosis and normal synaptic vesicular function (figure 5). Thus, this mutant rat protein provides a ready-made personalized *in vitro* model for this specific patient.
- In addition, 'fitful' mice, carrying a spontaneous missense alteration (A408T) in the same middle domain of dynamin, are afflicted with seizures due to defective synaptic transmission, providing an animal model for this gene which may be used to test potential pharmacologic treatments.

### Figure 1. Co-segregation data




+ indicates whole exome sequencing performed

### Figure 2. Conservation and *in silico* data




### Figures 3. & 4. Domain data

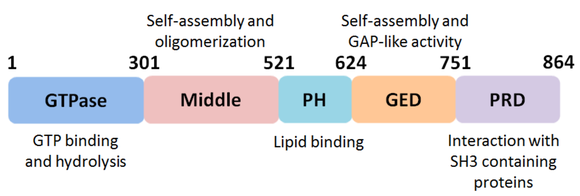



Fig.3. Domain organization of dynamin (Adapted from Chappie 2010).

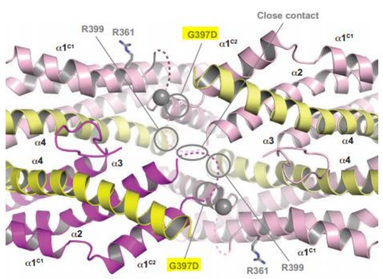



Fig.4. Part of the helical bundle in the middle domain showing the location of G397D, which disrupts multimerization (Ford, 2011).

### Figure 5. Functional data

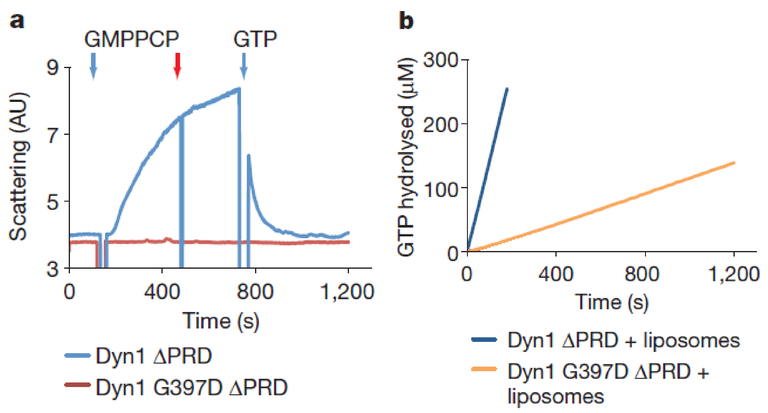



Fig.5. a. Self-assembly of wild-type and G397D dynamin measured by light-scattering. b. GTP hydrolysis kinetics of the G397D mutant relative to wild-type (Adapted from Ford, 2011).

## TAKE-HOME POINTS

- Based on this evidence, we propose that the *DNM1* alteration seen in our proband is causative of his epileptic encephalopathy and adds to the number of *de novo DNM1* mutations reported by the EuroEPINOMICS-RES/Epi4K consortia.
- Functional *in vitro* studies of the orthologous *DNM1* alteration had already been done and revealed deficient protein function, thus adding to the evidence of pathogenicity for this variant.
- A mouse model with a similar mutation is available, enabling the study of potential treatments for this type of *DNM1* mutations.

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