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A *de novo* mosaic mutation in *SPAST* with two novel alternative alleles and chromosomal copy number variant in a boy with spastic paraplegia and autism spectrum disorder

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ABSTRACT

Here we report a 12 year old male with an extreme presentation of spastic paraplegia along with autism and dysmorphisms. Whole exome sequencing identified a predicted pathogenic pair of missense variants in *SPAST* at the same chromosomal location, each with a different alternative allele, while a chromosome microarray identified a 1.73 Mb paternally inherited copy gain of 1q21.1q21.2 resulting in a blended phenotype of both Spastic paraplegia 4 and 1q21.1 microduplication syndrome. We believe that the extreme phenotype observed is likely caused by the presence of cells which contain only mutant SPAST, but that the viability of the patient is possible due mosaicism of mutant alleles observed in different proportions across tissues.

1. Introduction

Hereditary Spastic Paraplegia (HSP) is characterized by progressive weakness and spasticity. There are now more than 70 subtypes of HSP with all patterns of inheritance (de Souza et al., 2016). Reports on the prevalence of HSP range from 4.3 to 9.3 per 100,000 births (Coutinho et al., 1999). HSPs are normally divided into two groups: pure/uncomplicated and complex/complicated based on their set of symptoms. Patients with pure HSPs typically show spasticity in the lower limbs whereas patients with complex HSPs have additional system involvement (Coutinho et al., 1999).

Approximately 45% of HSP cases have been linked to the autosomal dominant spastic paraplegia-4 disorder (SPG4, MIM #182601) due to heterozygous mutations in Spastin (*SPAST*) (Fink, 2013; Solowska and Baas, 2015). SPAST is an AAA (ATPase associated with various cellular activities) family protein and is ubiquitously expressed in adult and fetal human tissues, showing slightly higher expression in the fetal brain than in other organs (Angelini, 2014). Patients with SPG4 show variable age of onset and even within families there is clinical heterogeneity with some carriers demonstrating reduced penetrance. A variety of mutations in *SPAST* have been shown to result in SPG4 including nonsense, splice site, and missense variants, deletions and insertions (Álvarez et al., 2010; Fonknechten et al., 2000; Crippa et al., 2006; Sauter et al., 2002; Erichsen et al., 2007; Bertelli et al., 2006; Tang et al., 2004;

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Park et al., 2005).

2. Patient data

The patient is a 12 year-old male, the only child to healthy, non-consanguineous Canadian parents of Quebecois-European (maternal) and Portugese (paternal) descent. Family history is unremarkable for spastic paralysis and neurological disorders; a maternal uncle had Asperger syndrome and a paternal great-grandfather's sister had cerebral palsy (CP)-like symptoms post-fever (developed at 9 months, later confined to a wheelchair). Pregnancy was uncomplicated, no teratogen exposure and normal prenatal ultrasounds. Birth was by spontaneous vaginal delivery at 39 weeks to a ($G_1P_1L_1$) 32 year old mother, with fetal extraction due to fetal distress. No resuscitation was required; Apgars were 9 and 9 at 1 and 5 min and Birth Weight (BW) 3.75 kg (50^{th}-85th centile); Birth Length (BL) 54.5 cm (85^{th}-97th centile) and Occipitofrontal Head Circumference (OHC) 34.5 cm (10^{th}-25th centile). Global developmental delay and floppiness were noted: he could lift up his head and roll at 12 months, and sit unsupported at 32 months. When first seen at 12 months by neurology, he was described as having CP with central hypotonia, increased peripheral tone and motor delay, combined with speech delay. Growth parameters were unremarkable. Dysmorphic facial features included calf atrophy and small hands and feet (<3rd centile); dolichocephaly, hypertelorism and macrostomia. Disordered eye movement and significant drooling were present; the latter treated with glycopyrrolate.

Spastic paralysis was first observed at 1 year, initially as spastic diplegia with normal upper-extremity function. His condition progressed to a state of ascending spastic tetraplegia with significant accompanying contractures. Patient has minimal finger movement and poor head control. He uses a wheelchair fulltime for mobility, and has significant paralytic scoliosis. At age 10 years, he underwent surgical reduction of a dysplastic hip with a varus derotation osteotomy of proximal femur with tendon release; he subsequently experienced significant developmental regression with loss of oral motor skills (eating), fine motor skills and communication function. Hardware removal occurred at 11 years of age for pain reduction. Two years postoperatively he had recovered his non-verbal communication skills. Nasogastric tube feeds started at 3 years to supplement oral eating and gastrostomy tube insertion at 5 years. He suffers chronic gastro-esophageal reflux disease (GERD), chronic non-obstructive constipation and there is a history of recurrent urinary tract infections requiring multiple antibiotics. At 8 years of age for precocious adrenarche; there was mildly elevated 17-hydroxyprogesterone but no further hormonal abnormalities. At 10 years, he developed supraventricular tachycardia (SVT) following surgery and is currently maintained on propranol with subsequent electrocardiograms (ECGs) showing normal sinus rhythm. He has severe obstructive sleep and central apnea, as determined by polysomnography at 11 years.

At school, he is on a modified education program and is accompanied by a full-time aide. He is followed by occupational therapy, physiotherapy, speech therapy and receives in-home nursing support. He has been diagnosed with Autism Spectrum Disorder (ASD) and anxiety. He is nonverbal but comprehends, and is becoming increasingly emotional and aware of his situation. Communication takes place with arm lifting movements.

An electroencephalogram (EEG) was performed due to head-dropping at 4 years 2 months showing bi-frontal and right posterior temporal slowing, with no epileptiform discharge or seizure activity. A computerized tomography (CT) scan at 3 years was unremarkable as was magnetic resonance imaging (MRI) brain/spine scan with spectroscopy. Magnetic resonance spectroscopy (MRS) at 8 years was unremarkable, and brain and spine were previously unremarkable at age 3. CT scan at 3 years was also normal. Hearing and vision were tested and normal.

The following investigations yielded unremarkable results: liver and kidney function parameters, complete blood count, cortisol and an adrenocorticotropic hormone (ACTH) stimulation test, plasma amino acids, homocysteine, ammonia, lactate, uric acid, vitamin B12, acylcarnitine profile, plasma very long chain fatty acids, transferrins iso-electric focussing, purines and pyrimidines, organic acids, biotinidase activity, oligosaccharides, beta-galactocerebrosidase activity, arylsulphatase A activity, hexosaminidase A&B activity, cerebrospinal fluid (CSF) neurotransmitters, glucose and lactate. Muscle biopsy during orthopedic surgery revealed reductions in complex 2 and 4 activities, however not indicative of respiratory chain defect. Electron microscopy showed non-specific pathological changes in skeletal muscle as clusters of inflammation in the formalin fixed paraffin embedded muscle, however not consistent with inflammatory myopathy. Molecular testing for Fragile X, Angelman syndrome, subtelomeric fluorescence in situ hybridization (FISH) and *MECP2* were all negative.

3. Methods

Ethics:Parents provided informed consent for the publication of this report. Index and parents were enrolled in the TIDEX study (H12-00067), approved by the Research Ethics Board of BC Children's and Women's Hospital, University of British Columbia, Vancouver, Canada.

Sample collection and DNA extraction: Initial blood samples were collected for whole exome sequencing (WES) from mother, father and index. Genomic DNA was extracted from blood, muscle, buccal, fibroblasts, urine and saliva by standard methods (Tarailo-Graovac et al., 2016).

WES and Bioinformatics: WES was performed using the Agilent V4 51 Mb kit and Illumina HiSeq 2000 (Perkin-Elmer, USA). All annotations provided are based on the Human Feb. 2009 (GRCh37/hg19) Assembly on the UCSC Genome Browser (http://genome.ucsc.edu/index.html). Data was processed and prioritized through a published semi-automated pipeline (Tarailo-Graovac et al., 2016). Prioritized variants were confirmed with Sanger sequencing according to standard methods.

Pyrosequencing: Pyrosequencing was used to quantitatively establish the percentage of the reference (G) and alternative alleles (C or T). Primer sequences and reaction conditions are provided in Supplementary Table S1 with additional details in Supplementary Figure S1.

Cellular Genotype models: The mathematical viability of three cellular genotype models were explored and are outlined in Supplementary Figure S2. Where mathematically possible, the resulting percentage of cells with each genotype are also listed. Only Model 1 was mathematically possible in all six index tissues.

Copy number variants: The presence of copy number variants (CNV) was assessed using the CytoScanHD chromosomal microarray.

4. Results

After bioinformatics processing of the trio of blood WES data, a total of 33 potential candidate variants in 22 genes were identified: homozygous (*SLC39A8*), compound heterozygous (*ANKK1, BMPER, E2F2, HMCN2, MYH13, NFXL1, PIGC, PPL, RYR3, UNKL, ZAN*), X-linked hemizygous (*CACNA1F, CCDC22, CYBB, DCAF8L2, FAM9A, GUCY2F, IDS)* and *de novo* (*EXPH5, PLEKHD1, SPAST*). Manual visual inspection revealed that three different alleles were present in WES data for the *de novo SPAST* variant (chr2:32,362,241 hg19, ClinVar Accession

SCV000540926 and SCV000540927) a reference (G) and two different alternative alleles (C and T). The two alternative alleles (NM_014,946.3:c.1477G > C/NP_055,761.2:p.Asp493His and NM_014,946.3:c.1477G > T/NP_055,761.2:p.Asp493Tyr) were both predicted to be damaging by all computational tools (i.e., SIFT, PolyPhen, CADD see Supplementary Table 2) (Wang et al., 2010; McLaren et al., 2016) and are classified as "Likely Pathogenic" [PS2, PM2, PM5 (Crippa et al., 2006), PP3] (Richards et al., 2015). Sanger validation confirmed the presence of all three alleles in blood and urine of the index, however no alternative T allele was detectable in his saliva (Fig. 1A). Multiple tissues from both the index and parents were examined by an additional quantitative technique, Pyrosequencing, to determine the overall reference to alternative allele frequencies in each sample. Pyrosequencing quantitatively determines the percentage of each allele in a sample, though it cannot directly be used to determine in what combinations the alleles are found in individual cells. All parental samples were homozygous reference (G/G) based on Pyrosequencing, with no detectable alternative alleles above background. Six samples from the affected index were found to have varying degrees of both alternative alleles (Fig. 1B). In all index tissues there was a higher percentage of alternative C alleles compared to alternative T alleles.

Chromosomal microarray (array platform: CytoScanHD) did not detect any copy number variants (CNV) involving *SPAST* (2p22.3) however, a paternally inherited copy gain (1.73 Mb) (arr [hg19] 1q21.1q21.2 (146,105,170–147,823,369)x3 pat) was found. This region contains 2 OMIM genes (*GJA5* and *GJA8*) and 15 RefSeq genes and overlaps the recurrent 1q21.1 microduplication syndrome (MIM #612475) which has been associated with variable penetrance and variable phenotypes including autism, intellectual disability and dysmorphic features (Van Dijck et al., 2015; Brunetti-Pierri et al., 2008). In keeping with previous reports of variable penetrance and variable expressivity, the father did not show any signs of ASD, nor did he present with any dysmorphisms. This CNV was determined to likely contribute to the ASD and cognitive difficulties in the patient.

5. Discussion

The patient described here is remarkable for two reasons. First, he suffers two distinct genetic conditions. Untangling the interaction between a CNV and SNV is always challenging; however, in this case, the progressive spastic paraparesis and atypical CP are most likely explained by the *SPAST* mutation, while the 1q21.1 CNV likely contributes to the ASD, behavioural disturbances, cognitive impairment, and dysmorphisms. Variable rates of coexisting monogenic conditions (Yang et al., 2014) (Li et al., 2016) and phenotypes due to a CNV and a SNV are also known to occur (Yang et al., 2014).

Truly unique is the identification of two predicted damaging *SPAST* alternative variants at the same location causing a progressive early onset HSP phenotype. CNVs within *SPAST* are a known cause of SPG4 (Boone et al., 2011) however in this patient, no CNVs involving *SPAST* were detected suggesting that only two of the three different alleles (C, G, and T) are present at one time in a cell and that cellular mosaicism is present in the index and involves different combinations of genotypes. The simplest, and we believe most likely, biological model would involve two separate mutation events in the index resulting in mosaicism across tissues. We assumed there were no homozygous alternative cells (C/C or T/T) in the index as would require an addition mutational event. Thus, for a given cell in the index, four potential genotypes are possible: homozygous reference (G/G), heterozygous reference/alternative (G/C or G/T), heterozygous bi-alternative (C/T). Given that both parents have the homozygous reference (G/G) genotype, three models involving different combinations of cellular genotypes were compared: Model 1 (G/G, G/C and C/T cells), Model 2 (G/G, G/C and G/T cells) and Model 3 (G/G, G/T and C/T cells). We hypothesized that in order for a model to be biologically possible, the allelic percentages established by Pyrosequencing (Fig. 1B) would need to translate mathematically into corresponding cellular genotypes in all tissues examined, only Model 1 meets this criteria (further details in Methods and Supplementary Figure S2). The process by which Model 1 could have arisen is outlined in Fig. 2A. While Pyrosequencing established the overall percentage of each allele (C, G or T) is it not as biologically meaningful as knowing the combination and percentage of each cellular genotype. Therefore, Fig. 2B breakdowns the percentage of homozygous reference (G/G), heterozygous reference/alternative (G/C) and heterozygous bi-alternative (C/T) cells in each of the six examined index tissues, based on Model 1.

We cannot exclude the possibility that one parent may be mosaic for one of the alternative alleles (C or T). This may be of importance as at least one case of a somatic mosaic patient who showed much later disease onset than any of his other relatives has been reported (Depienne et al., 2007). We choose to examine parental DNA extracted from blood, urine and saliva as these are easily obtainable and non-invasive samples to collect. Further testing, including the testing of sperm was declined by the family. However, none of the biological models results from one parent carrying either alternative allele are mathematically possible in all tissues, suggesting that the model we propose (Model 1) where two mutation events occurred in the index is still the most likely. This site is not part of a CpG, thus we could eliminate a C to T deamination event a possible explanation for the presence of both the C and T alternative allele. Within this model we proposed that a G to C mutation occurred before the G to T mutation because the alternative C allele is more frequent than the alternative T allele in all tissues examined. However we cannot exclude the possibility that the G to T mutation occurred first and the difference is due to a growth advantage or disadvantage of some cellular genotypes over others. As multiple tissues show both mutations, these must have arisen early in development, *i.e.* prior to differentiation of somatic lineages from the inner cell mass of the blastocyst. This study also supports the finding that Sanger sequencing cannot detect low levels of mosaicism whereas Pyrosequencing can (<10%), as was observed in the DNA extracted from the index's saliva (Freed and Pevsner, 2016).

The extreme phenotype in this patient (ascending spastic paralysis beginning at 12 months of age resulting in regression and complete wheelchair bound immobility in early childhood), may be explained by the predicted presence of double mutant cells (C/T). Typically a single damaging variant is enough to cause autosomal dominant SPG4. The mosaic bi-alternative variant reported here, along with nearly all identified missense SNVs, are within the AAA cassette which is located at residues 342 to 599 (based on NP_055,761.2). This variant occurs at the same residue (c.1478 A > G/p.Asp493Gly) as a missense variant previous reported by Crippa et al. (Crippa et al., 2006). Disease causing SNVs in *SPAST* are believed to function through a loss-of-function mechanism and disrupt the ability of Spastin to function normally with respect to membrane trafficking and microtubule dynamics (Lo Giudice et al., 2014). Recent work suggests that *SPAST* mutations may result in neurodegeneration due to decreased efficiency of peroxisome transport (Wali et al., 2016). We hypothesize that although the presence of double mutant cells is likely highly damaging, mosaicism within tissues allows for viability. Somatic mosaicism of a damaging *SPAST* variant has been previously detected in an

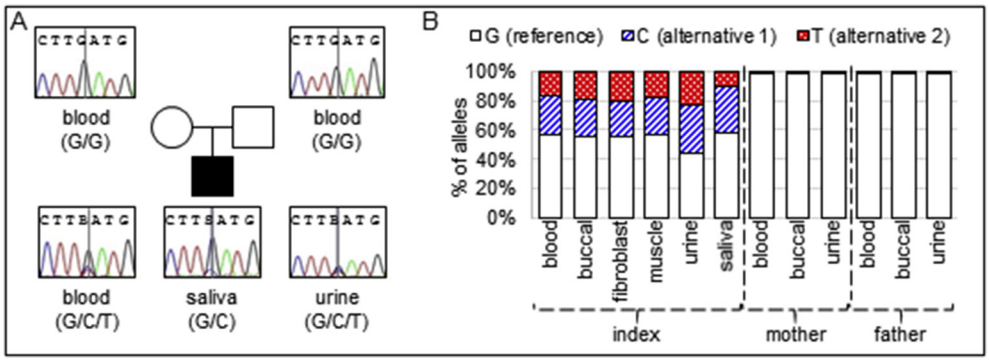

Fig. 1. A) Pedigree (affected index and unaffected parents) and Sanger validation of *de novo* mosaic bi-alternative *SPAST* variant (chr2:32,362,241 hg19) identified by WES of index and parental blood. Parental blood genotypes were determined by Sanger sequencing to be homozygous reference (G/G). Three index tissue samples were tested via Sanger Sequencing, and blood and urine were found to carry all three alleles (G/C/T) whereas only two alleles (G/C) were detectable in saliva. B) Pyrosequencing of mosaic bi-alternative *SPAST* variant across index and parental tissues. The percentage of background alternative alleles (C + T) was 1–3% in ten control homozygous reference (G/G) samples. A standard curve with varying mixtures of G, C and T alleles established the accuracy of the assay (Supplementary Figure S1, $R^2 > 0.87$).

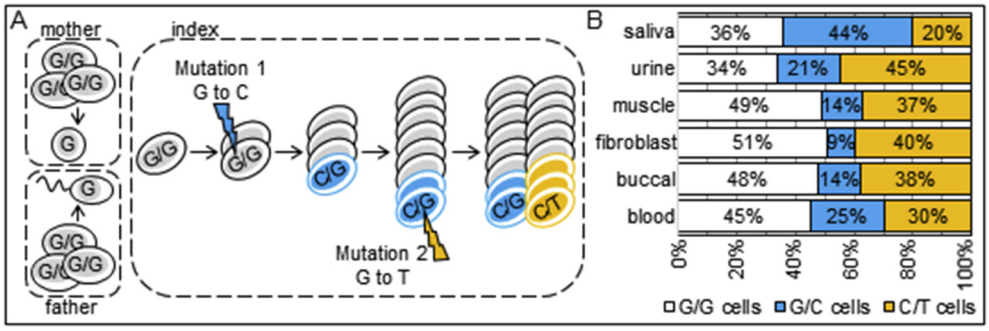

Fig. 2. A) Proposed biological mechanism to explain presence of bi-alternative *SPAST* variant in index with varying mosaicism across tissues. Note, the stage in development at which the mutations occurred and in which order is unknown. Additionally, although not detected, it is possible that one parent may carry a germline mutation for one of the alternative (C or T) alleles. B) Percentage of homozygous reference (G/G), heterozygous reference/alternative (G/C) and heterozygous bi-alternative (C/T) cells across six index tissues as mathematically determined based on Model 1 using Pyrosequencing allele percentages (further details in Supplementary Figure S2).

unaffected parent (Aulitzky et al., 2014) and in a patient with late onset HSP (Depienne et al., 2007). To our knowledge, this is the first patient reported with varying degrees of mosaicism objectively quantified across several different tissue types of specific embryonic origins. Finally, we cordially invite the readers to propose alternative biological mechanisms to explain this unique form of mosaicism in our patient.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2017.07.015.

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