

Application of Preimplantation Genetic Diagnosis to Reduce Transmission Risk of Early Onset Hereditary Spastic Paraplegia

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Abstract

We report the use of Preimplantation Genetic Diagnosis (PGD) combined with parental and affected child genetic analysis to identify embryo genotype in a case of discordant parental haplotype and variable expression for Hereditary Spastic Paraplegia (HSP) mutation. The couple presented to care following referral from genetics secondary to their first child being diagnosed with early onset SPG4 hereditary spastic paraplegia. Genetic testing was performed on both intended parents as well as the affected child. Sequencing of the paternal *SPAST* gene revealed an R460C mutation and maternal genetic sequencing revealed a Q490P variant in the *SPAST* gene. Testing of the affected child revealed the presence of both maternal and paternal *SPAST* gene mutations as well as a *de novo* E39K variant in the *ATF2* Gene. The couple underwent In Vitro Fertilization (IVF) treatment with trophoctoderm biopsy for preimplantation genetic diagnosis. Biopsy results revealed presence of homozygous disease and presence of maternal and paternal mutant haplotypes in selected embryos making them unsuitable for transfer. The remaining embryo had only a maternal mutant haplotype of the *SPAST* gene present with a normal paternal haplotype. We describe the first case of *ATF2* gene mutation in *SPAST* as a potential phenotypic modifier and/or causative mutation in HSP that may be contributing to early onset of disease. Using PGD, an unaffected embryo was transferred resulting successful delivery at term of a currently unaffected child.

Keywords: Hereditary Spastic Paraplegia; Preimplantation Genetic Screening; Trophoctoderm Biopsy

Abbreviations

Preimplantation Genetic Diagnosis (PGD); Hereditary Spastic Paraplegia (HSP); Anti-Mullerian Hormone (AMH); Gonadotropin Releasing Hormone (GnRH).

Introduction

Hereditary spastic paraplegias (HSP) are a rare group of diverse neurodegenerative disorders targeting the corticospinal tract that, in their “pure” form, present with progressive spasticity and weakness of the lower limbs. Affected individuals may also display scissoring gait, pyramidal signs in the upper limbs, progressive muscle wasting in the lower limbs, bladder dysfunction, and decreased vibratory sense at the ankles. In “complicated” forms, other possible neurological manifestations may include deafness, cognitive disorders, parkinsonism, cerebellar involvement, neuropathy, epilepsy, or extrapyramidal signs [1].

Mutations in more than 70 genes have been implicated thus far (SPG1-72) in HSP [2]. The most common causes of HSP, accounting for 15 – 40% of cases, are mutations in the *SPAST* gene (previously known as *SPG-4*), located at 2p22.3 [3]. *SPAST* encodes the protein Spastin, a member of the AAA (ATPase associated with various cellular activities) family, where its major role has been described as microtubule-severing activity. This is believed to enable the creation of shorter microtubules with more plus ends available for dynamic axonal growth. However, other roles of Spastin

have been described to account for the spectrum of phenotypes present in HSP, such as modulation of endosomal trafficking or endosomal-microtubule interactions [4]. Proposed mechanisms for pathogenicity of *SPAST* mutations include haploinsufficiency, where decreased microtubule splicing impairs axonal transport; alternatively, mutant Spastin protein may exert a dominant negative or neurotoxic effect as microtubule aggregates accumulate and interfere with cellular functioning. Despite the description of *SPAST* deletions as well as nonsense, missense, and splice site mutations, no correlation has been identified between the severity of the genetic defect and the phenotype or age of onset of disease.

The mean age of onset of classic HSP is estimated to be late in the 3rd decade of life with a wide range of variability and incomplete penetrance [5]. However, rare forms of infantile onset HSP-type disease have been described in the literature. Several mutations have been described in *Atlastin 1* as a known cause of a childhood variant of HSP that may instead present in infancy [6]. In contrast, the classic autosomal dominant HSP associated with *SPAST* mutations very rarely causes infantile-onset HSP. In a family with multiple members previously diagnosed with diplegic cerebral palsy, a *SPAST* mutation (G471D) was identified as the cause of infant-onset HSP [7]. Chinnery, et al. describe an infant presenting with lower limb spasticity and motor delay as evidenced by ability to crawl at 15 months and ability to walk unaided at 30 months [8]. On genetic analysis, the infant inherited two codominant *SPAST* mutations – P631L from an asymptomatic mother and S44L from a father without walking difficulty but with urinary urgency /frequency, hyperreflexia, bilateral ankle clonus and extensor plantar reflexes. On further analysis, the maternal grandfather, also with the P631L mutation, displayed progressive gait disturbance starting at age of 35 with lower limb spasticity. Another case of infantile-onset HSP which was described in a child with the S44L mutation, believed to be a phenotypic modifier that in combination with the D470V mutation resulted in a more severe phenotype presenting in infancy [9].

Materials and Methods

A 30-year-old woman and her 32-year-old husband presented to the Wake Forest Center for Reproductive Medicine, Winston-Salem, North Carolina, as a referral from their geneticists secondary to their first child being diagnosed with SPG4 Hereditary spastic paraplegia. The affected child was free of symptoms at birth but began to manifest spasticity, dystonia, and global developmental delay at age one. Following initial misdiagnosis of cerebral palsy, the child was eventually diagnosed with hereditary spastic paraplegia (HSP). The diagnosis of HSP is based on a clinical history of progressive spastic paraparesis, neurologic exam showing hyperreflexia, spasticity, extensor plantar responses and evidence of a genetic mutation in a *locus* compatible with a phenotype of HSP previously described in the literature [10]. Both partners had no neurologic symptoms at the time of presentation to care. Family history was significant

for a history of dystonia in the paternal grandfather. Consanguinity was denied.

In view of the desire to offer preimplantation genetic diagnosis, in vitro fertilization with ICSI and PGD was recommended and accepted. The infertility work-up of the female partner included a normal SIS, and an AMH of 4.77 ng / mL. Male factor fertility testing was deferred. Additionally, samples from the affected son and both parents were submitted for variant segregation analysis by whole exome sequencing.

Samples were sent to Genesis Genetics (Plymouth, MI), where the Agilent Sure Select XT2 All Exon V4 kit was used to target the regions of the target genome. These targeted sequences were sequenced using the Illumina HiSeq 2000 sequencing system with 100bp paired-end reads. The DNA sequence was then mapped and comparisons were made with the published human genome build UCSC hg19 reference sequence. The Xome Analyzer was used to evaluate sequence changes comparing the patient to both parents. All reported sequence variants were confirmed by conventional di-deoxy DNA sequence analysis.

Results

The intended mother underwent controlled ovarian stimulation with GnRH agonist protocol. She was triggered on stimulation day 11 with a peak estradiol level of 1485 pg/mL. A hCG trigger of 10,000 IU was given 36 hours before transvaginal ultrasound-guided retrieval. Twenty oocytes were retrieved and 15 mature metaphase II oocytes underwent ICSI. The following morning, 14 injected eggs exhibited two pronuclei and were cultured to blastocyst stage. Poor embryo development was noted. Only four grade I and II blastocysts underwent trophectoderm biopsy on day 5 or 6 after retrieval. After the biopsy, trophectoderm biopsies were placed in microtubules for shipping and the blastocysts were collapsed and vitrified.

The four biopsy specimens were sent to Genesis Genetics (Plymouth, MI), for comparative genome whole exome sequence analysis. Prior to transport of biopsied cells, maternal and paternal buccal swabs were sent for variant segregation analysis by whole exome sequencing and targeted probe creation. Sequencing of the paternal *SPAST* gene revealed an R460C mutation and maternal genetic testing revealed a Q490P variant in the *SPAST* gene. The paternal R460C mutation in the *SPAST* gene has been previously reported in an individual with autosomal dominant spastic paraplegia with onset at age 30 [11]. Missense mutations at the same residue site have been reported in the Human Gene Mutation Database in association with spastic paraplegia [12]. These findings lend evidence towards the R460C mutation found on paternal analysis to a disease causing mutation. The maternal Q490P variant in the *SPAST* gene has not published as a mutation. The Q490P variant is a non-conservative amino acid substitution which *in silico* analysis predicts is likely to impact secondary protein structure. Given that mutations in this region of the *SPAST* gene [12], the Q490P variant is a good candidate for a disease causing mutation, however the possibility of it being a rare benign variant cannot be excluded.

Analysis of the affected patient confirmed that the son was compound heterozygous for the R460C mutation and the Q490P variant in the *SPAST* gene. Additionally, the patient was found to be heterozygous for the *de novo* E39K variant in the *ATF2* gene. Following this data collection, PGD was performed via whole genome amplification with targeted maternal and paternal probes on the four trophectoderm biopsies. The results of genetic testing are shown in table 1. Two embryos returned with both of the identified maternal and paternal haplotypes present (homozygous disease). Another embryo had the maternal haplotype present with a normal paternal haplotype being present which was considered heterozygous disease status. The last embryo had a mutant maternal haplotype with no mutant paternal haplotype detected. Additionally this biopsy specimen had insufficient quality to amplify thus raising the possibility of aneuploidy. Re-biopsy of this embryo was recommended if further testing was desired.

After counseling the family, the embryo harboring the mutant maternal and normal paternal haplotype (heterozygous disease) was transferred after endometrial preparation with three weeks of twice daily oral estradiol and estradiol transdermal patches. The embryo transfer occurred on the sixth day of intramuscular progesterone injections resulting in a singleton intrauterine pregnancy. Estradiol supplementation was continued through eight weeks of pregnancy and progesterone supplementation was continued through ten weeks of pregnancy.

Discussion

As stated previously, mutations in the *SPAST* genes are typically associated with autosomal dominant inheritance although at least one family with an autosomal recessive inheritance pattern has been reported in the literature [13]. The identification of the paternal R460C mutation and the maternal Q490P variant in the *SPAST* gene may represent a rare instance of autosomal recessive inheritance. Additionally, the paternal R460C mutation that was identified may be responsible for the spasticity present in the affected child and may play a role in the reported dystonia in the paternal grandfather. The male partner may be presymptomatic or unaffected due to reduced penetrance.

Genetic analysis of the affected child also revealed, in addition to the inherited *SPAST* gene mutations, a *de novo* E39K variant in the *ATF2* Gene. While the function of the *AFT2* gene mutation has not been fully elucidated, the *ATF2* gene plays a role in the development of the respiratory, reproductive, immune, renal, skeletal, and central nervous system by activating transcription factor that mediates the transcription of a variety of genes that regulate cellular processes such as differentiation, proliferation, and apoptosis [14].

While exact function of the *AFT2* gene is not known, mutations present in the animal kingdom may give a clue as to its potential role in the affected child's phenotype. A homozygous missense mutation in the *AFT2* gene has been reported in standard poodle puppies with neonatal encephalopathy with seizures (NEWS). Affected puppies often die in the first week of life but those that survive longer develop ataxia, tremor, and seizures and have

Embryo Number	Spastic Paraplegia Genetic Result	R460C mutation	Q490P Variant	Interpretation
1	Mutant alleles observed	+	+	Mutant maternal and paternal haplotype present – homozygous disease
2	Mutant alleles observed	+	+	Mutant maternal and paternal haplotype present – homozygous disease
3	Mutant alleles observed	-	+	Mutant maternal and normal paternal haplotype present – heterozygous disease
4	Partial data obtained	unknown	+	Paternal haplotype not detected; Mutant maternal allele present – possible affected and/or possible aneuploidy; unsafe genetically for transfer

Table 1: Testing Results from Preimplantation Genetic Diagnosis (PGD) of biopsied embryos from Genesis Genetics.

dysplastic and small cerebella [14]. Mice that are homozygous for an ATF2 mutation have ataxia, hearing defects, hyperactivity, and skeletal defects and have similar cerebellar abnormalities [15].

After discussion with the family regarding the PGD results of their biopsied embryos, the family opted to transfer the embryo carrying the maternal haplotype as she was not symptomatic and there had not been any case reports of her variant being associated with HSP unlike the paternal haplotype. They declined to re-biopsy the embryo that returned with partial data.

Our case further supports the concept of the role of co-dominant *SPAST* mutations, where a mutation of unknown pathogenicity inherited from one side of the family may serve as a phenotypic modifier of HSP derived from the other side of the family (maternal Q490P variant and paternal R460C mutation). Furthermore, we describe the first case of ATF2 gene mutation in *SPAST* as a potential phenotypic modifier and / or causative mutation in HSP. As approximately 6% of individuals carrying a *SPAST* mutation are completely asymptomatic [16], this case highlights the necessity of pursuing genetic analysis of both parents in the setting of family planning, even after the identification of one implicated mutation of HSP in an affected parent.

Conflicts of Interest

There are no conflicts of interests to report for any of the authors.

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