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Title:

Novel homozygous missense mutation in *NT5C2* underlying Hereditary Spastic Paraplegia
SPG45

Running title:

Novel *NT5C2* mutation in SPG45

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Abstract

SPG45 is a rare form of autosomal recessive spastic paraplegia associated with mental retardation. Detailed phenotyping and mutation analysis was undertaken in three individuals with SPG45 from a consanguineous family of Arab Muslim origin. Using whole-exome sequencing, we identified a novel homozygous missense mutation in *NT5C2* (c.1379T>C; p.Leu460Pro). Our data expand the molecular basis of SPG45, adding the first missense mutation to the current database of nonsense, frameshift and splice site mutations. *NT5C2* mutations seem to have a broad clinical spectrum and should be sought in patients manifesting either as uncomplicated or complicated HSP.

Keywords: Hereditary spastic paraplegias; SPG45; Exome sequencing; *NT5C2*

INTRODUCTION

Hereditary spastic paraplegias (HSPs) comprise a group of genetically heterogeneous neurodegenerative disorders presenting with progressive spasticity in the lower limbs. The prevalence is between 3 and 10 per 100,000 people [de Souza et al., 2017]. Pathological findings show axonal degeneration resulting in loss of corticospinal tract function. Clinically, HSPs are divided into two major entities, uncomplicated and complicated, the latter including additional manifestations such as intellectual disability, epilepsy, optic atrophy, deafness, peripheral neuropathy, ataxia and skin abnormalities. HSPs also vary genetically with autosomal dominant, autosomal recessive and X-linked modes of inheritance. Autosomal recessive inheritance is generally associated with complicated HSPs.

Autosomal recessive spastic paraplegia (SPG45) with intellectual disability was mapped to 10q24.3-q25.1 by Dursun *et al.* in 2009. Some five years later, in those same affected subjects, Novarino *et al.* identified a homozygous truncating mutation in *NT5C2* [Novarino et al., 2014]. Their report also described four additional families with SPG45 in whom homozygous mutations were also found in *NT5C2*. The function of the 5'-Nucleotidase Cytosolic II protein encoded by *NT5C2* involves maintenance of a constant composition of intracellular purine/pyrimidine nucleotides in cooperation with other nucleotidases, preferentially hydrolyzing inosine 5-monophosphate and other purine nucleotides [Camici et al., 2010]. *NT5C2* has also been implicated in schizophrenia, leukemia and hypertension [Aberg et al., 2013; Meyer et al., 2013; Kelly et al., 2013], but the loss-of-function (frameshift, nonsense, splice site) mutations identified by Novarino *et al.* establish this as the causative gene in SPG45. Underscoring this association, a further autosomal recessive splice site mutation in *NT5C2* has also been reported in two more siblings with SPG45 [Elsaid et al., 2017].

We herein describe the clinical, radiologic and genetic findings in three individuals of Arab Muslim origin with SPG45 belonging to a consanguineous family who harbor a new mutation in *NT5C2*, the first missense mutation in this disorder. The patients manifested a phenotype of complicated HSP with moderate intellectual disability, attention deficit disorder and each individual had significant corpus callosum and white matter pathology. We compare our findings to the previously described six families.

SUBJECTS AND METHODS

Three affected individuals born to parents of Israeli Arab Muslim descent with known consanguinity and originating from the same village presented with motor, developmental, and intellectual abnormalities (pedigree is shown in Fig. 1a). Assessments and investigations were made between 14 and 19 years of age. Informed consent was obtained from all 14 members of the family included in the study. The study was approved by the Helsinki Committee in Rabin Medical Center.

There was no history of any complications in pregnancy. Delivery was normal in two subjects, although case IV:8 suffered from meconium aspiration. According to the parents, all three affected individuals (IV:7, IV:8 and IV:10) developed normally until 6, 9 and 12 months of age, respectively. Because of delayed milestones the children were referred to physiotherapy and at that time shortening and tension in the Achilles' tendons bilaterally was noticed in addition to lower limb spasticity. Subject IV:8 achieved standing at the age of 2 years and examination at that time showed spastic tone with scissoring, such that she could only stand on her toes. The children (IV:7, IV:8 and IV:10) achieved independent walking at 3 and 1/2 years, 3 years and 4 and 1/2 years, respectively. Language acquisition was also delayed; words were first spoken around 2 years. Currently, they all have moderate intellectual disability and

attention deficit disorder with hyperactivity and receive treatment with methylphenidate. However, they can all walk independently using specially adapted crutches, although all have undergone several tendon release operations. Case IV:8 also had eye surgery for strabismus. Individuals IV:7 and IV:10 are toilet trained, but case IV:8 has ongoing primary nocturnal enuresis.

Regarding neurological features, individuals IV:7, IV:8 and IV:10 were examined at the age of 16 and 1/2, 14 and 19 years, respectively. All were normocephalic (25th, 45th and 30th head circumference percentile). Cranial nerves, light and deep sensation, muscle strength, cerebellar functions, vibration and proprioception were all normal. However, hypertonia was noted in the lower limbs, the patellar reflexes were very brisk and clonus was elicited. Achilles' tendons were tight. Babinski's sign was extensor bilaterally in all subjects. There was spastic gait with crouch and circumduction as well as foot inversion with flat feet but no contractures were present. All three subjects underwent brain MRI and in each case the corpus callosum was thin all along but more so in the posterior aspect of the body and splenium, and abnormal signal intensities were observed in the periventricular white matter (Fig. 1b). Case IV:7 also had a normal spinal MRI (not performed in the others).

We performed exome sequencing in nine individuals from this pedigree (Fig. 1a), two of whom are affected (IV:8 and IV:10). Variant calling was performed with a previously published in-house pipeline [Lee et al., 2017]. More than 4.9 Gb of sequence was generated per sample, such that >94% of the target exome was present at >20-fold coverage, and >98% was present at 5-fold coverage (Supplementary Tables 1 and 2). Because both affected individuals are offspring of consanguineous marriages, we evaluated variants consistent with a model of rare autosomal-recessive inheritance, focusing on homozygous predicted protein altering substitutions and indels that were shared by both affected members. We filtered variants to retain variants that had a minor allele frequency <0.005 in each of the 1000 Genomes

Project, ExAC and our in-house database of in excess of 6000 exomes. We further filtered their variant profiles by excluding all homozygous variants that were not shared by their parents in a heterozygous state, and all homozygous variants that were present in their unaffected siblings. Variants highlighted from exome analysis were confirmed by Sanger sequencing.

RESULTS

Our stepwise filtering strategy revealed a single homozygous missense variant in *NT5C2* (c.1379C>T; p.Leu460Pro), which is predicted to be “deleterious” by the CADD pathogenicity software tool (CADD score 32) (Supplementary Table 3). Segregation analysis of the c.1379C>T substitution in all available members of the extended pedigree, including an additional affected individual (IV:7), confirmed the recessive inheritance of the variant.

To the best of our knowledge, we report the 7th family with SPG45 with *NT5C2* gene pathology, with our report containing the first description of a missense mutation (p.Leu460Pro). Details of our patients and those previously published are shown in Supplementary Table 4.

DISCUSSION

Dursun *et al.* reported a consanguineous Turkish family in which five individuals had a form of complicated spastic paraplegia with mental retardation. Novarino *et al.* studied the family reported by Dursun *et al.* and eight individuals from four additional families with SPG45. Recently, Elsaid *et al.*, reported two additional brothers of a consanguineous Qatari family harboring a novel homozygous *NT5C2* splice site mutation. They were unique to all other patients reported so far with SPG45 in that they displayed persistent early truncal

hypotonia, dysarthria and variable-sized patches of skin brownish discoloration that appeared at 6 years of age.

Our family is similar to the family reported by Dursun *et al.* in that both families exhibit a phenotype of complicated hereditary spastic paraplegia due to the intellectual disability manifesting in all the affected individuals. However, our patients manifested at an earlier age. All of them had ADHD and were treated by methylphenidate and all had pathological brain MRIs as opposed to the normal MRI reported in one of the individuals described by Dursun *et al.* The patients reported by Novarino *et al.* had a phenotype of pure uncomplicated hereditary spastic paraplegia because they had normal intelligence; however, their MRIs displayed significant pathology in the structure of the corpus callosum similar to our patients. Elsaid's patients display a phenotype of complicated HSP [Elsaid et al., 2017].

Pathologies of the corpus callosum are found in many HSPs (Supplementary Table 5). The most prevalent is autosomal recessive SPG11 – hereditary spastic paraplegia with thin corpus callosum. Next in frequency is SPG15. Ruano *et al.* reported the prevalence of autosomal dominant HSP to range from 0.5 to 5.5/10(5) and that of autosomal recessive HSP from 0.0 to 5.3/10(5) with pooled averages of 1.8/10(5) and 1.8/10(5), respectively. They surveyed 22 prevalence studies, reporting on 14,539 patients from 6 countries [Ruano et al., 2014]. These patients display complicated HSP phenotype. Both SPG11 and SPG15 proteins interact with a 4-protein complex designed AP5 (adapter protein 5), which localizes to small intracellular membrane-bound vesicles named endosomes [Hirst et al., 2011; Hirst et al., 2013]. Although AP5's function is not fully understood, it probably acts to remove membrane cargoes away from the endosomal compartment [Hirst et al., 2011; Hirst et al., 2013]. Twenty-one of the HSPs involve thin corpus callosum, thus reflecting an important association between central and peripheral nervous system pathology.

NT5C2 encodes a downstream cytosolic purine nucleotide 5' phosphatase. Purine nucleotides are neuroprotective and have a major role in the pathogenesis of brain ischemia and the developing brain [Thauerer et al., 2012]; thus, changes in their levels could sensitize neurons to stress and insult.

In conclusion, we have presented the 7th family with a novel mutation in *NT5C2* causing SPG45. This disorder may manifest either as pure or complicated HSP with or without typical MRI findings.

Fig. 1 Pedigree structure and Brain MRI scans. **a** Segregation analysis of the c.1379T>C (p.Leu460Pro) mutation. **b** Brain MRI Scans of three affected individuals. *Subject IV:7*. Sagittal T1 Weighted Imaging (T1WI) demonstrates a thin corpus callosum (*left panel*). On axial T2WI (*middle panel*) and FLAIR (fluid attenuation inversion recovery) abnormal periventricular perioccipital signal intensities are seen (*right panel*). *Subject IV:8*. Sagittal T1 Weighted Imaging (T1WI) demonstrates a thin corpus callosum (*left panel*). On axial T2WI abnormal periventricular signal intensities are seen (*right panel*). *Subject IV:10*. Sagittal T1 Weighted Imaging (T1WI) demonstrates a thin corpus callosum (*left panel*). On axial T2WI abnormal periventricular perioccipital signal intensities are seen (*right panel*).

Supplementary Table 1. Exome sequencing coverage and mapping statistics

Supplementary Table 2. Variant calling for exome sequenced individuals

Supplementary Table 3. Summary of whole exome filtering process

Supplementary Table 4. Phenotype of HSP patients with mutations in candidates linking to nucleotide metabolism network

Supplementary Table 5. HSPs with Corpus Callosum pathologies

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical compliance: The research was prospectively reviewed and approved by a duly constituted ethics committee.

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