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Case study

SLC2A1 mutations are a rare cause of pediatric-onset hereditary spastic paraplegia

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ABSTRACT

SLC2A1 mutations cause glucose transporter type 1 deficiency syndrome, whose phenotypic spectrum is a continuum, ranging from classic to variant phenotypes, the latter accounting for about 10% of cases. Very few *SLC2A1*-mutated patients with a spastic paraplegia phenotype have been reported so far, and they are associated with paroxysmal choreo-athetosis (i.e., DYT9). The authors describe two sporadic children with pure and complex hereditary spastic paraplegia (HSP) without paroxysmal non-epileptic movement disorders harboring heterozygous *de novo SLC2A1* pathogenic variants. These patients have been identified by a targeted panel for HSP among 140 pediatric- and adult-onset unrelated cases with pure and complex HSP, thus indicating an overall prevalence of 1.4% of *SLC2A1* mutations, which increases to 3% if only pediatric-onset patients are considered. The implications of these findings in the diagnostic work-up of HSP patients are discussed.

1. Introduction

Hereditary spastic paraplegia/paraparesis (HSP) are clinical and genetic heterogeneous neurodegenerative disorders with more than eighty causative genes and a percentage of genetically solved cases of about 60%.[1] In the recent years, several variant phenotypes related to defects of the glucose transporter type 1 (Glut1) have been reported.[2] Some of these include isolated paroxysmal exercise-induced dyskinesia and a complex form of progressive spastic paraplegia with paroxysmal choreo-athetosis and (i.e., DYT9). We describe two sporadic children with pure and complex HSP without paroxysmal movement disorders related to heterozygous *de novo SLC2A1* missense mutations and discuss the implications of these findings in the diagnostic work-up of HSP patients.

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2. Materials and methods

One hundred forty index HSP cases were analyzed by massively parallel sequencing by mean of a Nextera Custom Enrichment panel (Illumina, San Diego, Ca) for 113 previously described and candidate HSP genes according to manufacturers' protocols, as recently described.[1] All patients who underwent genetic analysis had received a diagnosis of HSP according to clinical features and supported by instrumental investigations. Patients with spastic paraplegias possibly related to other etiologies (i.e., leukodystrophies, vascular, inflammatory, metabolic, infectious or malformative diseases of the central nervous system, and other neurodegenerative diseases) were not included in the genetic analysis. Patients with pure HSP were 76, complex HSP were 64. Onset of symptoms in pediatric age was reported in 65 cases, while the remaining 75 patients had an adult-onset HSP. Most of the patients (107 out of 140), especially pediatric cases, were sporadic. Informed consent was signed by parents of patients or patients themselves (i.e., adult and cognitively normal patients) for performing genetic analysis. Sanger sequencing was used to confirm the annotated variants of interest and perform segregation analysis. Variants were annotated with reference to the following transcripts: *SLC2A1* (NM_006516.2).

3. Results

Two *de novo* *SLC2A1* missense mutations were identified: c.101A > G p.N34S in patient 1 and c.425T > A p.M142K in patient 2. The p.M142K variant has never been reported in available databases (i.e., dbSNP146, 1000 Genomes, ExAC and GnomAD) while p.N34S variant has been previously described.[2] Both mutations were predicted to be damaging by several bioinformatics tools (SIFT, Provean, Mutation Taster, PolyPhen-2).

Patient 1 is a 12-year-old girl second-born from non-consanguineous Italian healthy parents. Pregnancy and neonatal period were normal. At the age of 20 months she moved her first steps with clumsiness and motor difficulties, and pronounced first words. At the age of 3 years she manifested three generalized tonic-clonic seizures; treatment with valproic acid allowed complete control. Neurological examination revealed a mild ataxic-spastic gait with pyramidal syndrome of lower limbs with mild dystonic posturing of arms. Occipito-frontal circunference at the age of three and half years was 48.5 cm (3–10%); mild cognitive delay was present. Electroencephalogram did not show epileptic discharges. Brain and spine MRI were unrevealing, as well as somatosensory evoked potentials (SSEP) and nerve conduction studies (NCS). Motor evoked potential (MEP) detected increased central conduction time. CSF/blood glucose ratio resulted to be 0.31 (i.e., glycorrhachia 25 mg/dl; glycemia 81 mg/dl). At last follow-up spastic paraplegia rating scale (SPRS) score was 7/52. Symptoms remained stable during a follow-up period of nine years, with no further epileptic seizures or other paroxysmal movement disorders. She did never experience feeding- nor stress-related fluctuations of symptoms. Soon after diagnosis, the patient started ketogenic diet with poor compliance, such that we could not yet evaluate its efficacy.

Patient 2 is an 9-year-old Albanian boy with no family history of consanguinity or neurological disorders. Delivery and pregnancy were normal, as well as developmental milestones. At the age of 3, he developed insidious gait difficulties due to clumsiness, creeping and falls, slowly progressing for a couple of years up to stabilization. Neurological examination revealed spastic paraparesis with pyramidal signs at lower limbs; SPRS score was 17/52. Cognitive functions were normal. Occipito-frontal circunference was 53.5 cm (50–75%). Brain and spine MRI, SSEP and NCS were all normal. Increased central conduction time was detected by MEP. CSF/blood glucose ratio was 0.40 (i.e., glycorrhachia 31 mg/dl; glycemia 77 mg/dl). No paroxysmal episodes of neurological dysfunction as well as feeding- nor stress-related fluctuations of symptoms were reported in past medical history, neither in one year of follow-up. Following the diagnosis the patient has not yet been able to start the ketogenic diet.

4. Discussion

The molecular genetic diagnosis of HSP is quite challenging due the wide clinical and genetic heterogeneity; additionally, many HSP genes have been reported only in few patients or families.

SLC2A1 mutations cause Glut1 deficiency syndrome (Glut1-DS), whose phenotypic spectrum is a continuum ranging from classic (e.g., infantile-onset epilepsy, acquired microcephaly, psychomotor delay, complex movement disorders including ataxia, dystonia and spasticity) to non-classic/variant phenotypes (e.g., epilepsy including early onset absences, infantile spasms, myoclonic-atonic seizures; paroxysmal non-epileptic manifestations such as episodic ataxia, stroke-like episodes, alternating hemiplegia, exercise-induced dyskinesia/PED [DYT18], paroxysmal choreo-athetosis and progressive spastic paraplegia [DYT9]).[3] Estimated frequency of *SLC2A1* mutations is reported to be approximately 1:83,000.[4] Variant phenotypes are believed to be observed in about 10% of *SLC2A1*-mutated patients, although this percentage needs to be updated since descriptions of variant phenotypes has been largely increased in the last years due to development of the next generation sequencing. Variant phenotypes are usually milder forms than classic Glut1-DS.[3] Although classic Glut1-DS may show spasticity and/or spastic gait in the context of a complex neurological syndrome,[3] very few patients with a variant HSP phenotype of Glut1-DS have been reported.[5,6] Weber and colleagues identified the R212C mutation in a family with eighteen affected patients of which four had DYT9 and one had pure HSP; onset of spastic paraplegia was in early adulthood.[5] Furthermore, the R126C mutation was detected in a twin pair with DYT9, but *SLC2A1* sequencing was unrevealing in 139 dominant or sporadic HSP patients without PED.[5] The R126C hotspot mutation has been found in heterogeneous phenotypes and, recently, in seven members from a five-generation family exhibiting a combination of infantile-onset non-progressive spastic paraplegia, mild-to-moderate cognitive disability, generalized epilepsy, without further paroxysmal non-epileptic manifestations.[6] The N34S change has been reported in one of the first children with

Table 1 – Main features of reported patients with SLC2A1-related HSP.

Patient (sex, age)	HSP (onset)	PED (onset)	Epilepsy (onset)	Cognitive delay	Other	SLC2A1 mutation	Reference
Family A I1, F, NR	Yes[a] (EA)	Yes (PA)	No	No	No	c.634C > T; p.R212C	Weber et al.
Family A II5, M, NR	Yes[a] (EA)	No	No	No	No	c.634C > T; p.R212C	Weber et al.
Family A III1, M, NR	Yes[a] (EA)	Yes (PA)	No	No	No	c.634C > T; p.R212C	Weber et al.
Family A III6, M, NR	Yes[a] (EA)	Yes (PA)	No	Yes, mild-to-moderate	Ataxia, migraine	c.634C > T; p.R212C	Weber et al.
Family A III17, M, NR	Yes[a] (EA)	Yes (PA)	Single GTC seizure (NR)	Yes, mild-to-moderate	Migraine	c.634C > T; p.R212C	Weber et al.
Family B II1, M, 41 yrs	Yes[a] (LC)	Yes (PA)	No	Yes, mild-to-moderate	Mild UL dystonia	c.376C > T; p.R126C	Weber et al.
Family B II2, M, 41 yrs	Yes[a] (LC)	Yes (PA)	No	Yes, mild-to-moderate	Mild UL dystonia	c.376C > T; p.R126C	Weber et al.
II4, F, NR	Yes[b]	No	No	No	No	NP	Diomedi et al.
III2, M, NR	Yes[b]	No	Yes[c] (20 yrs)	Yes, mild	No	c.376C > T; p.R126C	Diomedi et al.
III6, M, NR	Yes[b]	No	Yes[c] (7 yrs)	Yes, mild	No	c.376C > T; p.R126C	Diomedi et al.
III8, M, NR	Yes (10 yrs)	No	Yes[c] (23 yrs)	Yes, mild	No	c.376C > T; p.R126C	Diomedi et al.
IV7, M, NR	Yes (3 yrs)	No	Yes[c] (3 yrs)	Yes, moderate	Dysarthria	c.376C > T; p.R126C	Diomedi et al.
IV10, M, NR	Yes (4 yrs)	No	Yes[c] (4 yrs)	Yes, moderate	Dysarthria	c.376C > T; p.R126C	Diomedi et al.
V2, F, 8 yrs	Yes (infancy)	No	Yes[c] (infancy)	Yes, moderate	Poor language	c.376C > T; p.R126C	Diomedi et al.
Patient 1, F, 12 yrs	Yes (congenital)	No	Yes (3 yrs)	Yes, mild	Mild UL dystonia	c.101A > G; p.N34S	This paper
Patient 2, M, 8 yrs	Yes (3 yrs)	No	No	No	No	c.425T > A p.M142K	This paper

Abbreviation NR not reported; EA early adulthood; LC (later childhood); PA pediatric age (1–15 years); GTC generalized tonic-clonic; UL upper limbs; NP not performed; yrs years.

[a] Described as slowly progressive.

[b] Described as "minimal evidence of pyramidal tracts involvement".

[c] Electro-clinical features of generalized epilepsy.

Glut1-DS without epilepsy.[2] Compared with cases reported above (Table 1) our patients did not manifest PED, which does not seem to be an obligatory feature in *SLC2A1*-related HSP. Patient 1 had characteristics similar to those described in Diomedi et al.[6] To the best of our knowledge, patient 2 is the first reported case of pure pediatric-onset HSP related to *SLC2A1* mutations.

Our two patients have been identified among 140 pediatric- and adult-onset unrelated cases with pure and complex HSP, thus revealing an overall prevalence of 1.4% of *SLC2A1* mutations in our cohort, which increases to 3% if only pediatric-onset patients are considered. Of note, since the variant "spastic paraplegia" phenotype is somewhat difficult to relate to Glut1-DS, especially if paroxysmal movement disorders or other clinical clues are absent, a diagnosis of *SLC2A1*-related HSP is primarily linked to the execution of genetic analysis. In our current experience, targeted NGS panel for HSP-related genes is considered the first step in the genetic work-up of HSP patients.[1] This point implies that *SLC2A1* screening must be included in targeted panels designed for approaching both pediatric and adult patients with a HSP phenotype. Considering that a causing mutation in *SLC2A1* frequently consists in a single heterozygous variant in a given sporadic affected individual, a 100% coverage of coding regions should be warranted as in our panel. Although this may not be enough to identify intragenic deletions or duplications, which account for up to 10–14% of all Glut1-DS patients,[3] genotype–phenotype correlations indicate that exon or whole gene deletions occur predominantly in severe Glut1-DS while missense mutations are mainly related to milder forms including variant phenotypes.[3] In order to avoid any chance of diagnostic error, a lumbar puncture for evaluation of CSF/blood glucose ratio should be considered in each HSP patient with no pathogenic variants identified by targeted NGS panel for HSP gene. This diagnostic work-up in HSP patients would make it possible not to miss a diagnosis of a potentially treatable (i.e., by mean of ketogenic diet) condition such as *SLC2A1*-related HSP.

Main limitations of this study are the heterogeneity of HSP, as encountered in our sample, which may have influenced the result of the putative prevalence of *SLC1A2* mutation in HSP patients, as well as the absence of functional studies for further confirmation of the pathogenicity of mutations.

5. Conclusion

Our experience suggests that pure and complex pediatric-onset HSP without paroxysmal non-epileptic features may rarely be caused by mutation in the *SLC2A1* gene and that these patients may lack typical features of Glut1-DS. *SLC2A1* analysis remains mandatory in all HSP patients with familiar and sporadic spastic paraplegia with or without paroxysmal movement disorders. Given the low prevalence of *SLC2A1*-related HSP in our series, lumbar puncture for CSF/blood glucose ratio seems to have little utility as first step in the work-up of most HSP patients and it should be limited for cases with unrevealing targeted NGS panel and/or incomplete coverage of the *SLC2A1* coding regions.

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Conflicts of interest

None.

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