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Short communication

Novel homozygous variants in *ATCAY*, *MCOLN1*, and *SACS* in complex neurological disorders

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ABSTRACT

Background: Neurological disorders comprise a large group of clinically and genetically heterogeneous disorders, many of which have a genetic cause. In addition to a detailed neurological examination, exome sequencing is being increasingly used as a complementary diagnostic tool to identify the underlying genetic cause in patients with unclear, supposedly genetically determined disorders.
Objective: To identify the genetic cause of a complex movement disorder in five consanguineous Pakistani families.
Methods: We included five consanguineous Pakistani families with complex recessively inherited movement disorders. Clinical investigation including videotaping was carried out in a total of 59 family members (4–21 per family) and MRI in six patients. Exome sequencing was performed in 4–5 family members per pedigree to explore the underlying genetic cause.
Results: Patients presented a wide spectrum of neurological symptoms including ataxia and/or dystonia. We identified three novel homozygous, segregating variants in *ATCAY* (p.Pro200Profs*20), *MCOLN1* (p.Ile184Thr), and *SACS* (p.Asn3040Lysfs*4) in three of the families. Thus, we were able to identify the likely cause of the disease in a considerable number of families (60%) with the relatively simple and nowadays widely available method of exome sequencing. Of note, close collaboration of neurologists and geneticists was instrumental for proper data interpretation.
Conclusions: We expand the phenotypic, genotypic, and ethnical spectrum of mutations in these genes. Our findings alert neurologists that rare genetic causes should be considered in complex phenotypes regardless of ethnicity.

1. Introduction

Neurological disorders, especially movement disorders such as ataxia or dystonia are clinically heterogeneous and often have a genetic cause. Importantly, phenotypic complexity and overlapping clinical features sometimes make it difficult to reach a final diagnosis solely based on neurological examination. Nowadays, exome sequencing is being increasingly used as a complementary diagnostic tool to identify the underlying genetic cause in patients with unclear supposedly genetically determined disorders. Of note, consanguineous populations are a potential source for the identification of new disease-linked genes and new variants in known genes in recessively inherited disorders. In Pakistan, the rate of consanguineous marriages is above 50% resulting in an increased prevalence of genetic syndromes including neurological disorders in this population [1].

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In the present study, we investigated five consanguineous families with multiple affected individuals from Pakistan. Exome sequencing was instrumental to identify the underlying genetic cause in three of the families, i.e. homozygous mutations in *ATCAY*, *MCOLN1*, and *SACS*. The newly identified variants expand the mutational and phenotypic spectrum as well as the ethnical occurrence of disorders previously associated with these genes. Our findings alert neurologists that rare genetic causes should be considered in complex phenotypes regardless of ethnicity.

2. Methods

The study was approved by the Institutional Review Board at School of Biological Sciences, University of the Punjab, Lahore, Pakistan and written informed consents were obtained from all participants.

2.1. Subjects

Five families with multiple affected individuals in consanguineous marriages were recruited in different regions of the province of Punjab, Pakistan (Fig. 1A). Family and clinical history was obtained by interviews. All affected individuals were videotaped and videos were clinically evaluated jointly by movement disorder experts (N.B., T.B., A.M.). If relevant, patients were re-contacted post-genetic analyses for specific biochemical and neuroimaging tests.

2.2. Molecular studies

Blood samples were collected from all available family members for genomic DNA extraction. Further, RNA was extracted from nine patients of families RDHR-04, RDHM-03, and RDHR-07. Exome sequencing was performed in four to five individuals per family (Fig. 1A) at Centogene AG (Rostock, Germany). We used NimblegenSeqCap EZ Human Exome Library v2.0 for exome capturing and enrichment and sequencing was performed on an Illumina HiSeq 2000 machine with a medium coverage of 100X. Detected variants were filtered to be rare in public and in-house databases (<0.01) and shared by all affected individuals within a family. Protein changing, homozygous variants were prioritized (Supplementary Table S1). Segregation and validation of candidate variants was tested in all available family members by Sanger sequencing. Frequencies of segregating variants were assessed in 200 ethnically-matched controls by Sanger sequencing or fragment analysis.

For families RDHM-01 (SACS) and RDHR-04 (ATCAY), five microsatellite markers spanning the *SACS* and *ATCAY* region, respectively, were genotyped for all available family members (Supplementary Table S2). Two-point parametric linkage analysis was carried out using SuperLink v1.5 in the easyLINKAGE® Plus v5.02 program. Haplotypes of each individual were constructed manually (Fig. 1A).

3. Results

3.1. Clinical and neuroimaging findings

Table 1 summarizes the clinical features of the affected individuals in the five families.

In family RDHM-01 (SACS) (Fig. 1A), the patients had a disease onset at about 1.5 years. The video evaluation revealed evidence of spasticity, spastic-ataxic gait, bradykinesia including hypomimia, mild dystonic postures of upper limbs, and muscular atrophy. In some patients, supranuclear gaze palsy was suspected but not formally tested. Brain MRI of individual IV:8 showed severe vermal and paravermal cerebellar atrophy, thinning of the corpus callosum, global subcortical atrophy, diffuse hypointense strips in the central pons (FLAIR), hyperintensity of the lateral pons (T2, FLAIR) and a large posterior fossa retro-cerebellar cyst communicating with the 4th ventricle with partial agenesis of the inferior part of vermis representing Dandy Walker's variant. Sylvian fissures appeared relatively prominent bilaterally (right > left) (Fig. 1B).

In family RDHR-04 (ATCAY) (Fig. 1A, Video 1), disease onset was in infancy. On examination, all patients had severe gait ataxia. Some patients also had strabismus, possible ocular apraxia, mild bibrachial dystonia, bradykinesia, and distal muscular atrophy predominantly in the feet. All patients had pes planus bilaterally. The serum ceruloplasmin level of V:6 was low but within the normal range (0.20 g/l; reference: 0.20–0.60 g/l). Brain MRI in Patient V:6 showed marked atrophy of cerebellar vermis with expansion of the cisterna magna, communicating with the 4th ventricle. There was also atrophy of cerebellar hemispheres with prominent cerebellar folia (Fig. 1B).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2018.02.005.

In family RDHM-03 (MCOLN1) (Fig. 1A, Video 2), both patients had adolescent onset generalized dystonia, mild ataxia and bradykinesia. The serum ceruloplasmin level of IV:1 was in the lower normal range (0.20 g/l). After the genetic diagnosis became clear (see below), the serum gastrin level of IV:1 was tested and found to be normal (85.5 pg/ml, reference: 13–115 pg/ml). Due to claustrophobia in IV:3 and tremor in IV:1, MRI scanning was declined.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2018.02.005.

In family RDHR-02 (Fig. 1A), perinatal onset was reported. None of the patients could walk and two of them moved by creeping on the ground. Despite these similarities, the phenotypic spectrum was broad and included spasticity, ataxia, bradykinesia, hypomimia, dystonia, strabismus, pendular nystagmus, and stereotypies. Cranial MRI was available in two patients: Findings of schizencephaly with pachygyria in IV:4 were consistent with a neuronal migration disorder, whereas IV:5 showed severe hydrocephalus supplied with ventricular shunt possibly due to bilateral open lip schizencephaly.

In family RDHR-07 (Fig. 1A), the onset of symptoms was reported as 3–6 years. IV:9 and IV:10 could only walk with support; the other three had an unsteady gait. Other symptoms included spasticity, weakness, ataxia, hypomimia, and dystonic posturing. One subject presented with generalized dystonia (V:5) and no abnormalities in brain MRI.

3.2. Genetic findings

Focusing on rare, homozygous variants shared by all affected but not the unaffected family members we found pathogenic or likely pathogenic mutations in three of the five families. In Family RDHM-01, we identified a frameshift mutation in exon 10 of *SACS* (sacsin molecular chaperone, NM_014363.5; c.9119dupA; p.Asn3040-Lysfs*4). In family RDHR-04, a novel frameshift mutation in *ATCAY* (caytaxin, NM_033064.4; c.599_605del; p.Pro200Profs*20) was identified. Finally, in family RDHM-03, a novel missense variant in *MCOLN1* (mucolipin 1), affecting a highly conserved amino acid (NM_020533.2; c.551T > C; p.Ile184Thr) was detected (Supplementary Figs. S1A and B) and considered as the most plausible cause among four candidate variants with a CADD score (http://cadd.gs.washington.edu/score; v1.0) of >15 (Supplementary Table S4).

The variants in *ATCAY*, *SACS*, and *MCOLN1* (Supplementary Table S3) fully segregated with the disease phenotypes in the families (Fig. 1A). While two of the mutations were absent in 400

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