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Autosomal recessive ADCY5-Related dystonia and myoclonus: Expanding the genetic spectrum of ADCY5-Related movement disorders

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ABSTRACT

Introduction: ADCY5-related hyperkinesia encompasses a heterogeneous group of phenotypes, including paroxysmal chorea, myoclonus, and dystonia. The disease is attributed to mutations of *ADCY5*, which encodes an adenylate cyclase enzyme. The disease can occur in a sporadic or familial pattern. With exception of one study, all reports on familial ADCY5-related hyperkinesia were associated with an autosomal dominant inheritance. Herein, we describe a native Arabian Bedouin family with an autosomal recessive ADCY5-related disorder and expand the genotypic and phenotypic spectrum of this disorder.

Methods: The pedigree included 4 generations of a family with 6 affected individuals. The patients were examined clinically and radiologically. Homozygosity mapping and Whole Exome Sequencing (WES) were used to identify a variant, predicted to be pathogenic, which segregated with disease in this family.

Results: All patients presented with early-onset dystonia and myoclonus. The patients had delayed motor and language milestones, axial hypotonia, severe anxiety, social phobia, and isolation. One patient had dilated cardiomyopathy. WES of one affected individual revealed a novel homozygous missense mutation (c.1762G > A, p.D588N) of *ADCY5*, that segregated with disease in an autosomal recessive manner, and was absent in more than 1000 ethnically-matched chromosomes. The mutation replaces a highly conserved nucleotide and is predicted to be deleterious.

Conclusion: This study reports the second family with autosomal recessive childhood-onset ADCY5-related disorder and expands our understanding of phenotype/genotype correlations of this disorder.

1. Introduction

ADCY5-related hyperkinesia encompasses a heterogeneous group of movement disorders including paroxysmal chorea, myoclonus, dystonia, and oculomotor apraxia with normal brain imaging [1–3]. These movements have an early-onset and typically involve the limbs, neck, and/or face. Additionally, patients usually have axial hypotonia and delayed motor and/or language milestones. The movements tend to occur during sleep and in response to stressful stimuli [1–3]. The disease was first described in 2001 as familial dyskinesia and facial myokymia (OMIM 606703) [4]. However, further reports elucidated that these facial movements are not myokymic but in fact are myoclonic/dystonic movements documented by electromyographic (EMG) findings [5–7]. Studies have also delineated the genotypic mechanisms underlying the disease and expanded our understanding of associated phenotypes [4–22].

The hyperkinetic movements are attributed to mutations of *ADCY5* on chromosome 3q21.1 [1,9]. To date, there are more than 70 cases (familial and sporadic) of genetically confirmed *ADCY5* mutation leading to hyperkinesia [5–22]. ADCY5-related diseases occur in either sporadic (*de novo mutation*) or familial pattern. Except for one study [14], all reports on familial ADCY5-related hyperkinesia depicted an autosomal dominant inheritance pattern. Herein, we describe a native Bedouin Arabian family with an autosomal recessive ADCY5-related disorder and expand the genotypic and phenotypic spectrum of this disorder.

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Table 1
Clinical features of the patients.

Patient	Age (yrs)/ Gender	Movements onset (yrs)	Motor delay	Language delay	Weight loss	Axial hypotonia	Facial/ oral twitching	Dysarthria	Initial intermittent movements	Dystonia	Myoclonus	Abnormal gait	Tremor	Cognitive function	Cardiomyopathy	Thyroid manifestation	Psychiatric manifestation
IV-2	20/F	3	+	+	+	+	+	+	+	+	+	+	–	Intact	+	+ hypothyroid	+ social phobia
IV-3	18/F	4	+	+		+	+	+	+	+	+	+	–	Intact	–	+ hypothyroid	+ social phobia and irritability
IV-5	15/M	10	+	–	–	+	+	+	+	+	–	+	+	Intact	–	–	+ social phobia and isolation
IV-6	12/M	8	–	–	+	+	+	+	+	+	–	–	–	Intact	–	–	+ social phobia and anxiety
IV-7	8/F	5	–	–	+	+	+	+	+	+	+	+	–	Intact	–	–	–
IV-8	6/M	2	+	+	+	+	+	+	+	+	–	–	–	Intact	–	–	–

2. Methods

2.1. Patients inclusion

We performed a retrospective review of the electronic medical records and charts of this Arabian family to obtain demographic and clinical data for the entire family. Six affected siblings were enrolled for this study under an IRB-approved protocol (RAC# 2070005). All patients or their legal guardians provided written consent for the publication of their videotapes and photos, in both the print and online modalities. All subjects were examined in the Department of Neurosciences, King Faisal Specialist Hospital and Research Centre. History was obtained and clinical examination performed on the affected individuals and their family members. Magnetic resonance imaging (MRI) was obtained for the affected individuals.

2.2. Genetic studies

2.2.1. Homozygosity mapping

All participating individuals (affected and unaffected) were genotyped using the Affymetrix Axiom array (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol (http://www.affymetrix.com/support/technical/manuals.affx). Resulting genotypes were analyzed for shared runs of homozygosity (ROH) using autoSNPa (http://dna.leeds.ac.uk/autosnpa/).

2.2.2. Exome sequencing and analysis

Briefly, 100 ng of each DNA was amplified in 12 separate wells using Exome Primer Pools, AmpliSeq HiFi mix (Thermo Fisher, Carlsbad, CA, USA) and 10 amplification cycles. All 12 PCR pools were combined in one well and subjected to primer digestion by incubation with FuPa reagent (Thermo Fisher, Carlsbad, CA, USA). Amplified Exome targets were ligated with Ion P1 and Ion Xpress Barcode adapters. After purification, libraries were quantified using qPCR with the Ion Library Quantification Kit (Thermo Fisher, Carlsbad, CA, USA). The prepared exome library was further used for emulsion PCR on an Ion OneTouch System and templated Ion Sphere particles were enriched using Ion OneTouch ES, both procedures following the manufacturer's instructions (Thermo Fisher, Carlsbad, CA, USA). The template-positive Ion PI Ion Sphere particles were processed for sequencing on the Ion Proton instrument (Thermo Fisher, Carlsbad, CA, USA). Reads were mapped to UCSC hg19 (http://genome.ucsc.edu/) and variants identified using the Ion Torrent pipeline (Life Technologies, Carlsbad, CA, USA). The resultant variant caller file (vcf) was filtered to include only homozygous variants within the pre-determined ROH (shared by affected individuals only) that were absent from dbSNP, 1000 genomes, approximately 3000 Saudi exomes. Included variants were further selected based upon pathogenicity predicted by Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

DNA Sanger sequencing: Coding regions of *ADCY5* were sequenced using BigDye Terminator kit (Applied Biosystems, Foster City, CA) and run on an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA). SeqScape v.2.6 software (Applied Biosystems, Foster City, CA) was used to align sequence data against the relevant reference.

This family has been followed for several years and when the variant allele was first identified, next generation sequencing (NSG) data from ethnically-matched chromosomes was not available. Thus, Sanger sequencing of a single amplicon encompassing the allele of interest was undertaken in 1000 ethnically-matched chromosomes and demonstrated absence of the variant, which was then regarded as a disease-related mutation. The absence of this allele was then reconfirmed in 3000 ethnically-matched WES samples to further support the variant allele as being a disease-related mutation.

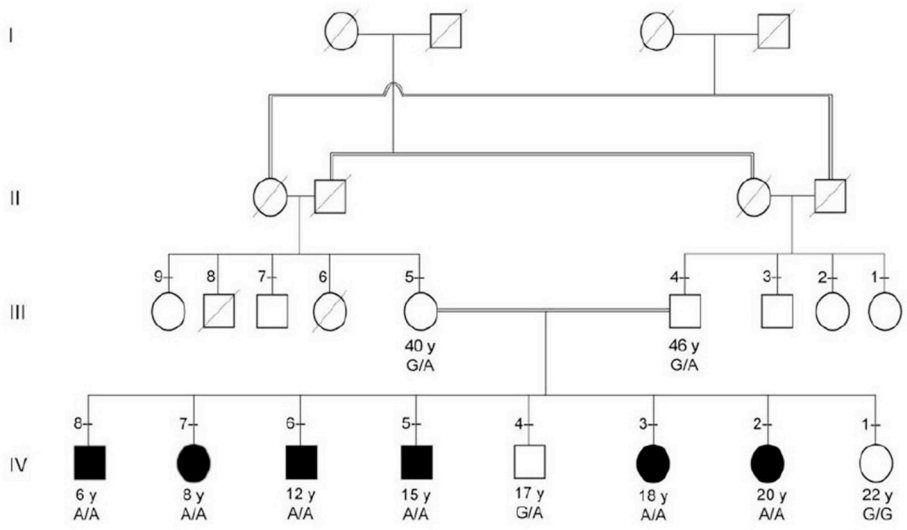

Fig. 1. Pedigree of Saudi family with autosomal recessive ADCY5-related dystonia and myoclonus.

3. Results

3.1. Clinical data

The family studied is of native Bedouin Arab origin from the Northern Province of Saudi Arabia. The parents are first cousins and double-loop consanguinity was noted both maternally and paternally. The sibship consisted of 3 affected males, 3 affected females, 1 unaffected male, and 1 unaffected female (Table 1; Fig. 1; Supplementary material 1&2).

3.2. Genetic analysis

Given consanguinity in this extended family and the apparent autosomal recessive inheritance, we hypothesized that the responsible genetic defect originated in a common ancestor with homoallelic transmission to affected individuals. Therefore, homozygosity mapping was used to identify a common critical interval (chr3: 118,208,800-123,923,000) with which disease segregated (Fig. 2b). Whole exome sequencing of the index case (IV:3) identified 35,720 variants relative to hg19, with 1686 variants on chromosome 3. These were further filtered to exclude all variants present outside our defined ROH (chr3: 118,208,800-123,923,000) within which we identified 81 variants. By focusing on only non-synonymous homozygous changes, splicing variants, frameshift insertions or deletions, and nonsense variants, we decreased the number of candidate variants to 30. By excluding previously reported variants (present in dbSNP, 1000 genomes and 2000 Arab exomes), the list was narrowed to 1 (Supplementary material 3). It was a G to A change in exon 6 of *ADCY5* (c.1762G > A).

This mutation was confirmed by Sanger sequencing which was performed on all family members and found to co-segregate with the phenotype (Fig. 2a). Mutation Taster and CADD_phred predicted this variation to be pathogenic with a variant score of 0.999 and 37, respectively. This variant results in the substitution of aspartic acid at position 588 of *ADCY5* by asparagine (p.Asp588Asn), a highly conserved amino acid.

4. Discussion

Mutations in the *ADCY5* gene have been linked to various disorders including benign hereditary chorea, familial dyskinesia and facial myokymia, essential hereditary chorea, paroxysmal dyskinesia, oculomotor apraxia with nocturnal ballistic bouts, and chorea-dystonia disorders [1–22].

Various mutations described in the literature have been implicated in ADCY5-related disease (Supplementary material 4). In fact, an attempt to elucidate the genetic contributions to the disease and delineate certain genotype-phenotype correlations was proposed by Chen et al. [11]. The authors found that p.Ala726Thr is associated with a milder phenotype compared to p.Arg418Trp which is associated with a more severe form of the disease. Additionally, somatic mosaicism has been suggested to result in a milder phenotype. Interestingly, as depicted in Supplementary material 4, there seems to be a mutational hot spot area in the Arginine 418 region, accounting for the increased frequency of mutations in this region. Our patients demonstrated a novel mutation in the *ADCY5* gene, expanding the genotypic spectrum of this disorder.

Many authors have suggested that *ADCY5* mutations (especially missense mutations) result in gain-of-function mechanism, leading to hyperactive adenylate cyclase activity and altered striatal dopamine signaling, resulting in these hyperkinetic movements [6,9,11]. However, in Carapito et al. [10] study, the authors reported a novel c.2088+1G > A variant affecting a donor splice site, which resulted in absence of mRNA activity from the mutant allele on functional studies, suggesting a loss of function mechanism. The mutation described in our study affects the first catalytic (C1) domain of the protein and, using different in-silico tools, is predicted to be damaging for the encoded protein. Based on the mode of inheritance, this would represent a biallelic loss of normal protein function, similar to what Carapito et al. [10] observed. Therefore, it seems that ADCY5-related disease can result from either a loss-of-function or gain-of-function mechanism. This difference in mutation mechanism warrants further investigation to delineate the link between *ADCY5* dysfunction and various associated phenotypes.

ADCY5-related hyperkinesia occurs in either sporadic (*de novo mutation*) or familial pattern. Since the initial description, the familial pattern was thought to be inherited in an autosomal dominant fashion (heterozygous) [4]. Further reports followed documenting this pattern of inheritance as the sole model of disease transmission with almost 100% penetrance rate [5–13,15–22]. However, a recent study by Barrett et al. [14] reported the first familial autosomal recessive inheritance pattern in 2 affected siblings with generalized dystonia and myoclonus and phenotypically normal parents. The patients had a maternally inherited frameshift variant (c.409_428del20; p.G137Cfs*184) and a paternally inherited missense variant (c.3037C > T; pR1013C) confirmed on Sanger sequencing. Both of these variants were required for the disease to appear. Therefore, an autosomal recessive model, as a second inheritance pattern for the disorder, was suggested. In fact, multiple inherited movement disorders have shown a dual inheritance pattern being either autosomal dominant or recessive, including *THAP1* and *GNAL*

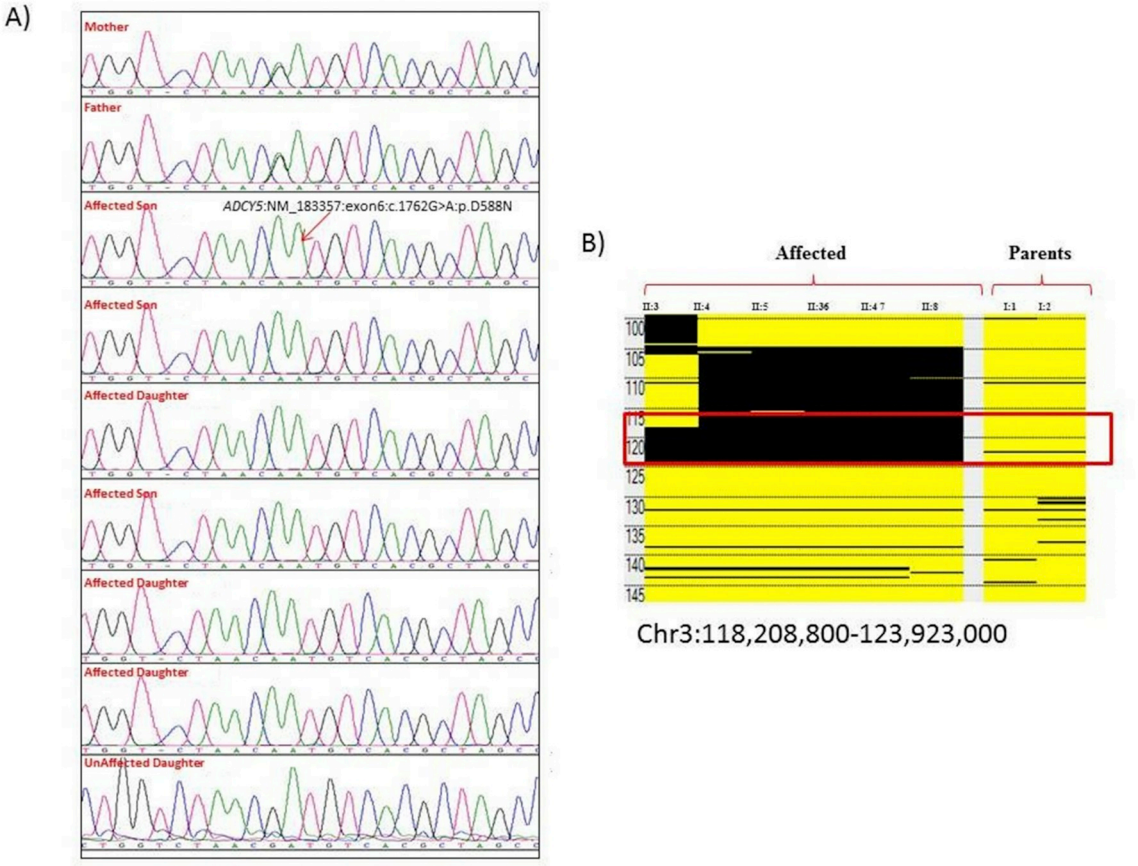

Fig. 2. Identification of disease locus on chromosome 3: a) DNA electropherogram with the G > A change in ADCY5:NM_183357:exon6:c.1762G > A:p.D588N. b) AutoSNPa output for chromosome 3 reveals an ROH (boxed in red) shared among affected members (IV:2, IV:3, IV:5, IV:6, IV:7 and IV:8) and not present in unaffected parents (III:4, III:5). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mutations [23,24]. Our series represents the second family with an autosomal recessive ADCY5-related disorder. The affected individuals had a homozygous mutation c.1762G > A, p.D588N, while their asymptomatic parents were heterozygous for this mutation.

In our patients, there was a mild clinical overlap between the different affected individuals. Generally, the lower facial, oromandibular, and tongue movements started initially then progressed to the neck, axial, and limb muscles. Our patients had variable degrees of posturing and twisting, as well as periodic and patterned features, which sometimes can be jerky or tremulous. These movements were initially intermittent and became constant later on during the course of the disease, as previously reported. Axial and limb posturing were found in all cases and striatal upgoing toes were seen in the two youngest patients (IV-7 and IV-8). Interestingly, one of our patients (IV-6) had decreased intrauterine movements, which was not evident in any of the other patients. Additionally, we did not find myokymic discharges on EMG in any of our six cases (Table 1; Supplementary material 1).

The phenotype of myoclonus-dystonia due to *ADCY5* mutation was previously encountered by Barrett et al. [14] and Douglas et al. [16]. Interestingly, the elder member of our family had an asymptomatic young-onset cardiomyopathy without a clear etiology despite extensive workup. Similarly, there were five affected individuals of a single German family in which one case presented with progressive dilated cardiomyopathy diagnosed in the fourth decade of his life and four cases of congestive heart failure reported by Chen et al. [8]. This observation is of paramount importance, as *ADCY5* is highly expressed in cardiac myocytes, and long-term follow-up of the reported cases may elucidate an important association between cardiomyopathy and ADCY5-associated disorder. Additionally, psychiatric symptoms are not commonly encountered in ADCY5-related disorder. In Waalkens et al. [22] reported family, only one patient experienced episodes of obsessive-compulsive behavior, anxiety, and phobias. Similarly, Chen et al. [11] reported 2 cases of psychosis in their series. All of our patients had severe anxiety, social phobia, and isolation that significantly impacted their lives. Further studies are therefore important to delineate the frequency of these psychiatric symptoms among these patients.

Clinical comparison of individuals with autosomal dominant and autosomal recessive ADCY5 mutations is currently challenging, due to clinical variability of this condition and the scarcity of reports on autosomal recessive inheritance. Further reports are therefore warranted.

The management of ADCY5-related disorder remains challenging. Various therapeutic modalities have been employed in the literature with variable success rates [4–22]. In our patients, high-dose (7.5–15 mg/daily) anticholinergic therapy (trihexyphenidyl) with clonazepam (0.5–2.0 mg/daily) provided the best control of patients' movements without controlling the psychiatric features. Emerging studies on deep brain stimulation of the globus pallidus interna have shown promising results in the management of this condition, especially among patients with p.R418W variant [12,17]; however, further studies are needed to document its efficacy and safety profile.

In conclusion, we present the second family with an autosomal recessive childhood-onset ADCY5-related disorder characterized by generalized dystonia and myoclonus. Further studies are needed to expand our understanding on the genotype/phenotype correlation of this syndrome and elucidate implicated disease mechanisms.

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Disclosure

The authors have no personal financial or institutional interest in any of the materials or devices described in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.02.039.

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