Evidence for pathogenicity of variant ATM Val1729Leu in a family with ataxia telangiectasia

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Abstract

Ataxia telangiectasia is a rare autosomal recessive multisystem disorder caused by mutations in the gene of ATM serine/threonine kinase. It is characterized by neurodegeneration, leading to severe ataxia, immunodeficiency, increased cancer susceptibility, and telangiectasia. Here, we discovered a co-segregation of two ATM gene variants with ataxia telangiectasia in an Egyptian family. While one of these variants (NM_000051.4(ATM_i001):p.(Val128*)) has previously been reported as pathogenic, the other one (NM_000051.4(ATM_i001):p.(Val1729Leu)) is regarded as a variant of uncertain significance. Our findings in this family provide additional evidence for causality of the second variant and argue that its status should be changed to pathogenic.

Keywords Ataxia telangiectasia · ATM serine/threonine kinase · Mutation · Pathogenicity · Egypt

Introduction

The rare multisystem disorder ataxia telangiectasia (AT) usually starts in childhood and causes neurodegeneration leading to ataxia, movement disorders, and peripheral neuropathy [1]. Additional characteristics are variable amounts of immunodeficiency, increased susceptibility to cancer, especially of lymphoid origin, telangiectasia, and several additional symptoms [2]. AT is an autosomal recessive disorder known to be caused by variants in the ATM serine/threonine kinase gene (ATM) [3].

In the present study, we analyzed an Egyptian family with AT. The family consisted of non-consanguineous parents and four siblings; three of whom are affected by AT. The brother and youngest sister had presented with ataxia, while another sister had a history of ataxia and died undiagnosed at the age of 6 years. We identified compound heterozygous, most likely causative variants in the ATM gene.

Material and methods

Clinical phenotyping

Ethical approval was obtained by Mansoura University, Egypt (RP/42), and Technical University of Munich, Germany (203/15s). Written informed consent was obtained for all
participants. If the participant was minor or incapable, consent was given by the legal custodian. Neurologists specialized in movement disorders (A.S.S. and G.U.H.) examined affected and unaffected individuals at the Department of Neurology, Ain Shams University, Cairo, Egypt. Affected individuals II:1 and II:4 were examined by standard cranial MRI at 1.5 Tesla with T1, T2, and FLAIR sequences.

Genetic analysis

Exome sequencing was performed in the two living, affected individuals II:1 and II:4. Genomic DNA libraries were captured using the Nextera Rapid Capture Expanded Exome Kit (Illumina, San Diego, CA), and DNA fragments were sequenced on an Illumina HiSeq2000 system with an average coverage of 80×. Variants were identified by a standard analysis pipeline and annotated using the ANNOVAR software [4]. Since the parents are not known to be related, we assumed autosomal recessive inheritance and compound heterozygosity or homozygosity of the causative variant(s). We discarded the sex chromosomes and variants with a minor allele frequency (MAF) > 0.01 in gnomAD (Genome Aggregation Database) “all” (https://gnomad.broadinstitute.org) exome as well as genome data. We discarded variants without an annotated exonic or splicing function and variants with a CADD score below 15 [5]. Next, we filtered for variants present in both exomes in the hetero- or homozygous state in the genes with the HUGO (Human Genome Organization) official symbols ATM, MRE1, APTX, SETX, and PNKP implicated in the pathogenesis of AT and its differential diagnoses [2]. This resulted in two variants in the ATM gene which were confirmed and tested for segregation in the whole family by Sanger sequencing. Primer sequences are available on request. We detected no variants fulfilling the abovementioned criteria in any of the 4 other genes. The effects of amino acid substitutions on protein function were predicted using MutationTaster [6], PolyPhen-2 [7], and CADD. We searched the public version of the Human Gene Mutation Database [8], ClinVar [9], and the Leiden ATM mutation database [10] for the identified variants.

Results

Case reports

The detailed clinical findings of both living patients are presented in Table 1, and the pedigree is shown in Fig. 1. Patient 1 (II:1) presented to us at age 23 with gait and limb ataxia, spastic paraparesis with pyramidal signs in the presence of length-dependent peripheral neuropathy. He showed dystonia of the upper limbs as well as the neck, severe dysarthria, oculomotor abnormalities, and cognitive impairment. At age 14, he developed focal seizures. Patient 2 (II:4) is a sister of patient 1 and presented to us at age 11 with a very similar clinical picture but with less prominent dystonia and without epileptic seizures. Both patients showed cerebellar atrophy on 

| Demographic/clinical features                  | Patient #1 (II:1) | Patient #2 (II:4) |
|-----------------------------------------------|-------------------|-------------------|
| Age at onset (y)                              | 1.5               | 1.6               |
| Age at first examination (y)                   | 23                | 11                |
| Age at last follow-up (y)                      | 27                | 14.5              |
| Sex                                           | Male              | Female            |
| Eye movement abnormalities                     | Nystagmus, oculomotor apraxia | Nystagmus, oculomotor apraxia |
| Bulbar abnormalities                           | Dysarthria        | Dysarthria        |
| Upper motor neuron                             | Spastic paraparesis, pyramidal signs | Spastic paraparesis, pyramidal signs |
| Lower motor neuron                             | Absent ankle jerk, sensory loss, and wasting of small foot muscles | Absent ankle jerk, sensory loss, and wasting of small foot muscles |
| Movement disorders                             | Limb ataxia, dystonia of upper limbs and neck | Limb ataxia, chorea |
| Age at wheelchair dependence (y)               | 15                | 10                |
| Epileptic seizures                             | Focal seizures    | None              |
| Cognitive impairment                           | Moderate to severe | Moderate to severe |
| Telangiectasia                                 | Conjunctival      | Conjunctival      |
| Skin changes                                   | Few vitiligo-like patches | Prominent vitiligo-like |
| Alpha-fetoprotein (AFP)                        | 521 ng/ml at age 22 (strongly elevated) | 457 ng/ml at age 10 (strongly elevated) |
| Brain MRI                                      | Cerebellar atrophy | Cerebellar atrophy |
cranial MRI. Both patients suffered from recurrent infections and showed conjunctival telangiectasia (Fig. 2a, b) as well as hypopigmented skin patches. Progressive ataxic gait noticed at the age of around 1.5 years was the first symptom in both siblings. Increased levels of alpha-fetoprotein (AFP) were found in both affected siblings. Another sister (II:3) died at age 6 with a similar clinical picture. She suffered reportedly from hemolytic anemia and was treated with blood transfusions and steroids. The third sister (II:2) and the parents were neurologically healthy.

**Genetic evaluation**

We performed exome sequencing in both living patients (II:1, II:4, Fig. 1). Only the two heterozygous variants in the ATM gene shown in Table 2 withstood the filtering process as described in materials and methods. Variant 1 is a one base pair deletion leading to an immediate truncation of the large (3056 aa) ATM serine/threonine kinase after amino acid 128. This variant almost certainly results in a loss of function of the ATM protein and has previously been reported as pathogenic (Table 2) [11–13]. Variant 2 is a missense variant that has a CADD score of 23.1 which is nearly identical to variant 1 (23.3) but is classified as benign by PolyPhen-2 and deleterious by MutationTaster (Table 2). Analysis of the data deposited in ClinVar shows that AT-causing variants are relatively evenly distributed over the whole ATM protein, including the region which contains variant 2 (data not shown). Variant 2 has previously been reported as variant of uncertain significance (VUS) or benign because it has also been observed in healthy controls (Table 2) [14]. However, an allele frequency of 1.03e-04 in gnomAD does not argue against causality for a recessive disease. In addition, Coutelier et al. found this variant in the homozygous state in one patient and reported it to be most likely causative [15]. Both variants segregate with disease and are located in trans in the examined family. They are the only variants in ATM and genes causative for related phenotypes which were identified in our exome data using a fairly relaxed filtering approach (CADD score > 15 and MAF < 1% and affecting protein sequence or splice sites). Therefore, our data suggest that both variants together cause AT in this family and that variant 2 should be regarded as causative.
We show co-segregation and trans-positioning of two putatively causative \textit{ATM} variants in an Egyptian family with AT. The clinical findings, elevated AFP, and inheritance pattern are typical for AT [2]. Elevated AFP serum levels are also found in ataxia with ocular apraxia types 2 and 4 (AOA2, AOA4). These are differential diagnoses of AT caused by variants in the senataxin (\textit{SETX}) and the polynucleotide kinase 3′-phosphatase (\textit{PNKP}) genes [16]. However, patients with AOA2/4 do not exhibit telangiectasia, and our patients did neither carry putatively causative \textit{SETX} nor \textit{PNKP} variants. While variant 1 (NM\_000051.4(\textit{ATM\textunderscore i001}):p.(Val128*)) has already been regarded as causative, variant 2 (NM\_000051.4(\textit{ATM\textunderscore i001}):p.(Val1729Leu)) has been classified as VUS or benign. Our data argue that this variant 2 is causative. Segregation, position in trans, low MAF in gnomAD, high CADD score, and the fact that we found no other putatively causative variants for AT or genes implied in the differential diagnoses of AT all point to a pathogenicity of the variant 2. Additionally, this variant in the homozygous state has previously been reported to be causative in a single AT patient [15]. In summary, we conclude that variant 2 is causative. Limitations of our study are the relatively small size of the family and a lack of functional data.

\section*{Discussion}

We show co-segregation and trans-positioning of two putatively causative \textit{ATM} variants in an Egyptian family with AT. The clinical findings, elevated AFP, and inheritance pattern are typical for AT [2]. Elevated AFP serum levels are also found in ataxia with ocular apraxia types 2 and 4 (AOA2, AOA4). These are differential diagnoses of AT caused by variants in the senataxin (\textit{SETX}) and the polynucleotide kinase 3′-phosphatase (\textit{PNKP}) genes [16]. However, patients with AOA2/4 do not exhibit telangiectasia, and our patients did neither carry putatively causative \textit{SETX} nor \textit{PNKP} variants. While variant 1 (NM\_000051.4(\textit{ATM\textunderscore i001}):p.(Val128*)) has already been regarded as causative, variant 2 (NM\_000051.4(\textit{ATM\textunderscore i001}):p.(Val1729Leu)) has been classified as VUS or benign. Our data argue that this variant 2 is causative. Segregation, position in trans, low MAF in gnomAD, high CADD score, and the fact that we found no other putatively causative variants for AT or genes implied in the differential diagnoses of AT all point to a pathogenicity of the variant 2. Additionally, this variant in the homozygous state has previously been reported to be causative in a single AT patient [15]. In summary, we conclude that variant 2 is causative. Limitations of our study are the relatively small size of the family and a lack of functional data.

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\section*{Author contribution}

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by ASS, GK, TWR, FH, MP, MS, GUH, and SHM. Medical examinations were carried out by ASS, GUH, FH, and GK. The first draft of the manuscript was written by GK, ASS, and TWR, and all authors revised and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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\section*{Data availability}

Required data are stored and available upon request.

\section*{Code availability}

Not applicable.

\section*{Declarations}

\subsection*{Ethics approval}

The present study was conducted in accordance with the principles of the Declaration of Helsinki and approved by local Ethics Committee of Mansoura University (RP/42), Egypt and Technical University of Munich (203/15s), Germany.

\subsection*{Consent to participate}

Written informed consent was obtained for all participants. If the participant was minor or incapable, consent was given by the legal custodian.

\subsection*{Consent for publication}

All participants gave their consent for publication.

\subsection*{Conflict of interest}

The authors declare no competing interests.
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