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ORIGINAL COMMUNICATION

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Absence of mutations in *ATM*, the gene responsible for ataxia telangiectasia in patients with cerebellar ataxia

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Abstract Ataxia-telangiectasia (AT) is an autosomal recessive multisystent disorder presenting in childhood with progressive cerebellar ataxia, oculocutaneous telangiectasia, immune deficiency, radiosensitivity, and cancer predisposition. The gene for AT, designated *ATM* (AT, mutated) encodes a protein with a carboxy-terminal phosphoinositide-3 kinase domain which is involved in cell cycle checkpoints and other responses to genotoxic stress. Most of the patients with the classical AT phenotype are homozygous or compound heterozygous for severe mutations causing truncation or destabilization of the *ATM* protein. Patients with a milder forms of disease, called AT variants, have been found to be either homozygous for milder mutations or compound heterozygotes for null alleles and mild mutations. In order to define the clinical phenotype of patients homozygous (or compound heterozygotes) for other, milder mutations, we decided to search for *ATM* mutations in patients with either sporadic or familial idiopathic ataxia. Thirty-four patients with idiopathic cerebellar ataxia, aged 3–77 years, were screened for mutations in the *ATM* coding region. There were 12 familial cases. None of the patients had abnormal immunoglobulin or α-fetoprotein levels, and none had mutations in the *ATM* coding region. In this heterogeneous group of patients with cerebellar ataxia we found no mutations in the *ATM* gene. We conclude that mutations in the *ATM* gene are probably not a common cause for cerebellar ataxia other than AT.

Key words Ataxia telanglectasia · *ATM* · Cerebellar ataxia

Introduction

Ataxia-telangiectasia (AT) is a multisystem autosomal recessive disorder with an average worldwide frequency of 1:40,000–1:100,000 live births. The neurological hallmarks of the disease are cerebellar ataxia, oculomotor apraxia, dysarthria, drooling, and choreoathetosis. AT patients are usually wheelchair-bound by the age of 10–15 years. Other features include oculocutaneous telangiectasia, cellular and hummoral immune deficiency, and cancer predisposition. Common laboratory findings are reduced levels of IgA, IgG2, and sometimes IgE, impaired cellular immunity, and increased levels of α-fetoprotein and carcinoembryonic antigen. The disease is relentlessly progressive, and life is compromised usually by the early 20s due to an infectious

complication or malignancy [1]. The cellular phenotype of AT is characterized by radiation sensitivity and defective induction of various damage-induced signal tranduction pathways, most notably cell cycle checkpoints [2, 3].

The responsible gene, *ATM* (AT, mutated), was identified using positional cloning. It spans over 150 kb of genomic DNA and produces a transcript of about 13 kb representing 66 exons [4–6]. The open reading frame of this transcript encodes a 370-kDa protein with a predominant nuclear fraction and a variable cytoplasmic one. *ATM*'s carboxy-terminal region is homologous to the catalytic domain of the signal transduction enzyme phosphatidylinositol-3 kinase, suggesting involvement in signal transduction pathways [6, 7].

ATM mutations in patients with classical AT usually lead to elimination of the *ATM* protein. The vast majority of patients are homozygous or compound heterozygotes for such null alleles [8–12]. One would expect a higher representation of missense mutations in patients since such mutations constitute a significant portion of the molecular lesions in many disease genes [13, 14]. However, point mutations causing amino acid substitutions are relatively rare in AT [8].

There is clinical heterogeneity among classical AT patients, such as variability in recurrent infections, age at onset and rate of progression. The reason might be related to interaction of the *ATM* gene product with some other proteins. Patients with a milder clinical or cellular phenotype designated "AT variants," have been reported and may represent approx. 10–15% of all AT patients [15–22]. They may have a late-onset ataxia [23], a slowly progressive ataxia [16, 17], or a milder cellular phenotype [18–24]. There are also syndromes which partly overlap with the AT phenotype. These patients may exhibit a phenotype lacking one or more of the AT features, such as no telangiectasia [25–29].

Most AT variants have been found to be either compound heterozygotes for a null allele and a milder mutation or homozygous for the milder mutations. Small deletions or short truncations have also been described. In some of these cases the *ATM* gene product is found in small amounts (1–17% of normal levels) in cells [22].

The consequences of gene mutations can range from complete loss or inactivation of the protein to partial or minimal loss of the protein's activity. Genotype-phenotype relationships associated with the *ATM* gene may extend beyond the "classical" AT or variant phenotypes since in both of them mutations of a very mild nature are not observed. Such mutations can lead to a phenotype involving some of the AT features, perhaps to a milder extent. The present study searched for *ATM* mutations in patients exhibiting one of the main AT features, cerebellar ataxia.

Patients and methods

Patients

The study population consisted of patients with an idiopathic cerebellar syndrome. Most patients were recruited by referral from neurologists or pediatric neurologists throughout Israel. Others were identified through hospital records. We excluded patients with possible "symptomatic" ataxia (e.g., demyelinating disease), other etiological or nosological cases (e.g., diagnosed Friedreich's ataxia), prominent extrapyramidal or autonomic features, and familial cases in which the inheritance is probably autosomal dominant. Patients were invited for an interview, examination, and blood test, after signing an informed consent form. Detailed patient history was taken, concerning disease onset and progression, with all results of imaging, neurophysiological, and other studies documented. Detailed family history, ethnic background, consanguinity, neurological disorders, malignancy, recurrent infections, and endocrinological problems in the patient or family members were documented. The patients underwent a complete neurological examination, and telangiectasias were looked for. A blood sample was drawn for molecular studies and measurement of immunoglobulin (IgA, IgG, IgM) and α-fetoprotein levels.

Mutation analysis

Mutation analysis was based on reverse transcription polymerase chain reaction of *ATM*'s mRNA, followed by restriction endonuclease fingerprinting analysis. The method is described in detail elsewhere, and its sensitivity for detection of deletions, insertions, and base substitutions has been documented [24]. Total RNA was extracted from whole blood, and the open reading frame of the *ATM* transcript was amplified by eight partly overlapping polymerase chain reaction fragments. The amplified DNA was digested separately with five restriction endonuclease combinations, and the fragments were end-labeled. Electrophoresis was performed on polyacrylamide gels which were then dried and subjected to autoradiography. In this system a sequence alteration is observed as an abnormal band pattern.

Results

Thirty-four patients with progressive ataxia were screened for *ATM* mutations. Consanguinity was documented in 2 cases, where 12 other patients from 7 families had an affected sib. Twelve of the patients were Ashkenazi Jews and 22 Sephardic Jews. There were 17 women and 17 men. The patients were clinically heterogeneous. The ages at examination ranged between 3 and 77 years (mean 34). The age at onset of neurological symptoms ranged between infancy (presenting with delayed motor milestones) to 63 (average 22): 19 had onset before 20 years, 8 between 20 and 39, and 7 at the age of 40 or older. The course of disease was rather diverse. Most patients had a relentlessly progressive disease, although some had periods of stability. The clinical features ranged from a pure cerebellar syndrome to a mixed syndrome including other features. The patients' characteristics are shown in Table 1. Three of the patients were thought to have ocular telangiectasia. Three others had recurrent respiratory tract infections. There were seven patients with a first-degree relative affected by cancer and seven with a first-degree relative affected by diabetes mellitus. One of the patients was diabetic.

All patients had immunoglobulin and (α-fetoprotein levels within the normal range. No mutations were identified in the *ATM* coding region.

Table 1 Patient characteristics

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Men/women	17/17
Mean present age (years; range)	34 (3–77)
Mean age at onset (years; range)	22 (infancy to 63)
Sephardic/Askenazi	22/12
Familial occurrence	12
Consanguinity	2
Limb ataxia	34
Truncal ataxia	30
Dysarthria	29
Nystagnms	16
Other extraocular abnormalities	18
Hypertonus	14
Hypotonus	13
Pyramidal signs	17
Sensory loss	12
Sphincteric involvement	7
Intellectual impairment	11
Ambulation	
Normal	3
Independent	16
Assisted	7
Bilateral assistance	3
Wheelchair	4
Brain imaging	
Cerebellar atrophy	25
Normal	4
Not available	4
Other	2

Discussion

In our heterogeneous group of patients with cerebellar ataxia we could not implicate *ATM* mutations as the cause of the disease. Possible explanations for our findings are that mutations in the *ATM* gene are not a common cause for cerebellar ataxia other than either classical AT or the AT variant phenotype. Since in AT variants *ATM* protein levels were found to range from 1% to 17% of the normal level [22], perhaps higher amounts of the protein are sufficient to avoid neurodegeneration. It is possible that individuals with certain missense mutations are symptomatic and do not manifest cerebellar ataxia but rather other features found in AT, such as immune deficiency or predisposition to malignancies. It is also possible that our mutation detection method failed to identify certain *ATM* mutations. Although our restriction endonuclease fingerprinting proved to be efficient in finding several point mutations (many of which caused stop codons) in the coding region of the *ATM* transcript, it cannot detect mutations outside the open reading frame in regulatory sequences. Such mutations may lead to instability or reduced production of *ATM*'s mRNA, In addition, our series was relatively small and quite diverse in terms of age at onset and rate of progression, and included both sporadic and familial cases.

The question of the spectrum of *ATM* mutations can further be addressed by analysis of a larger series of patients, with emphasis on familial ataxias and patients exhibiting other features of the AT phenotype. Thanks to the recent development of antibodies to *ATM*, the cellular amount of this protein can now be estimated, possibly in peripheral blood lymphocytes. This may serve as a simple diagnostic test for *ATM*-related disease, leaving the mutation search for further investigation.

Addendum

Since the search for *ATM* mutations was negative, as soon as it became available, we recently screened all the patients' DNA for expanded GAA repeats in the first intron of the *X25* gene, which is the common mutation found in Friedreich's ataxia. Two patients not previously diagnosed (neither was familial or with consanguineous parents) were found to have Friedreich's ataxia; one had a rather classical phenotype and the other a late onset disease with atypical features.

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