Report

Autosomal Recessive Cerebellar Ataxia with Oculomotor Apraxia (Ataxia-Telangiectasia–Like Syndrome) Is Linked to Chromosome 9q34

Andrea H. Németh,^{1,2} Elena Bochukova,¹ Eimear Dunne,¹ Susan M. Huson,² John Elston,³ Mohammed A. Hannan,⁵ Matthew Jackson,⁴ Cyril J. Chapman,² and A. Malcolm R. Taylor⁶

¹Wellcome Trust Centre for Human Genetics, ²Department of Clinical Genetics, The Churchill Hospital, ³Department of Ophthalmology, The Oxford Eye Hospital, and ⁴Department of Neurology, The Radcliffe Infirmary, Oxford; ⁵Department of Biomedical Physics, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; and ⁶CRC Institute for Cancer Studies, University of Birmingham, Edgbaston, Birmingham, United Kingdom

Ataxia with oculomotor apraxia (ataxia-telangiectasia–like syndrome [AOA]; MIM 208920) is an autosomal recessive disorder characterized by ataxia, oculomotor apraxia, and choreoathetosis. These neurological features resemble those of ataxia-telangiectasia (AT), but in AOA there are none of the extraneurological features of AT, such as immunodeficiency, neoplasia, chromosomal instability, or sensitivity to ionizing radiation. It is unclear whether these patients have a true disorder of chromosomal instability or a primary neurodegenerative syndrome, and it has not been possible to identify the defective gene in AOA, since the families have been too small for linkage analysis. We have identified a new family with AOA, and we show that the patients have no evidence of chromosomal instability or sensitivity to ionizing radiation, suggesting that AOA in this family is a true primary cerebellar ataxia. We have localized the disease gene, by linkage analysis and homozygosity mapping, to a 15.9-cM interval on chromosome 9q34. This work will ultimately allow the disease gene to be identified and its relevance to other types of autosomal recessive cerebellar ataxias to be determined.

Ataxia-telangiectasia (AT) is an autosomal recessive disorder characterized by immunodeficiency and neoplasia, with laboratory evidence of chromosomal instability and sensitivity to ionizing radiation. The gene that causes AT, known as “ATM,” has been localized to chromosome 11q22.3 and is a large gene with homology to cell-cycle checkpoint genes in other organisms (Savitsky et al. 1995). The neurological features of AT are characteristic and include early-onset cerebellar ataxia and oculomotor apraxia (slow or absent voluntary eye movements). Later, patients develop conjunctival telangiectases, a progressive neurodegenerative syndrome, and sinopulmonary infections and malignancies.

Some patients have been described who have “variant” AT, with few or no clinical features other than progressive ataxia. These patients may be divided into

three groups. The first have laboratory evidence of AT and have been shown to have mutations in the *ATM* gene on 11q22.3 (McConville et al. 1996; Gilad et al. 1998). A second group of variant AT patients have a progressive cerebellar degeneration (but no telangiectasia) and increased cellular and chromosomal instability, but they do not demonstrate linkage to chromosome 11q22.3 (Hernandez et al. 1993). Recently, some of this latter group of patients have been shown to have mutations in *hMRE11*, a double-strand break-repair gene located on chromosome 11q21 (Stewart et al. 1999). A third group of patients have a neurological disorder with an AT-like syndrome, without laboratory evidence of AT, and have normal or moderately impaired cellular and chromosomal stability. This third condition, which is referred to as “ataxia with oculomotor apraxia” (AOA) or “AT-like syndrome” (ATL) (MIM 208920), was first described in 14 patients, from 10 families, 6 of whom were noted to be consanguineous, suggesting autosomal recessive inheritance (Aicardi et al. 1988). The age at onset of ataxia tended to be in childhood, and, in addition to ataxia of gait, there were also some extrapyramidal movements such as chorea, athetosis, and dys-

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Address for correspondence and reprints: Dr. Andrea H. Németh, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, UK. E-mail: andrea.nemeth@well.ox.ac.uk

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Figure 1 Pedigree with AOA, showing haplotypes along 9q34. Boxes around haplotypes indicate regions of homozygosity by descent in affected individuals. Arrows indicate meiotic recombination events.

tonia. The patients all had severe oculomotor apraxia. Intellectual function was preserved in half the patients and was mildly subnormal in the remainder. There was minimal progression of symptoms after follow-up, although some patients were quite disabled. Results of computed tomography (CT) were normal in six patients and revealed mild cerebellar vermis atrophy in three. Three additional patients, from two consanguineous families, were reported with AOA that presented in early childhood (Hannan et al. 1994; Gascon et al. 1995). Again, detailed investigations of sensitivity to acute and chronic ionizing radiation did not reveal abnormalities typical of AT. On the basis of lack of laboratory evidence for chromosomal instability, it was suggested that AOA was a neurological and genetic entity separate from AT (Aicardi et al. 1988; Hannan et al. 1994; Gascon et al. 1995). However, the families reported were too small to permit linkage analysis, and there has been little progress in determining the genetic basis of AOA/ATL. Until now it has been unclear whether these individuals have mu-

tations in *ATM* or in a related gene or are, in fact, genetically quite distinct.

We have recently identified a new family with AOA and here describe our results of a genome screen that has identified linkage and homozygosity by descent. Our results clearly show that AOA in this family is distinct from other AT-like syndromes and will allow the identification of the gene that is mutated in this form of autosomal recessive cerebellar ataxia.

The family consists of five affected brothers and four unaffected siblings (two males and two females) (fig. 1). Their parents originated from a small village in the Mirpur district of Azad Kashmir, Pakistan, which comprised approximately eight families in total, and the parents knew that their great grandfathers were brothers. All the patients developed ataxia during their late childhood or early teens. The disease progressed into adulthood but, by the time the affected family members were in their early twenties, became relatively stable, and further progression had been very slow. Because of their disability,

none of the patients were able to work. On examination, the patients had severe ataxia of gait with mild ataxia of the limbs and trunk. There was mild choreoathetosis with dystonic posturing during walking. The lower-limb deep tendon reflexes were absent and the plantars extensor. The most severely affected brother had a flattened affect and a masklike face. Ophthalmological examination revealed a severe oculomotor apraxia with abnormal smooth pursuit movements, absent optokinetic nystagmus, and a global saccade palsy, affecting the vertical more than the horizontal movements. Brain CT of individual II:5 revealed prominence of several cerebellar sulci, suggestive of cerebellar atrophy, and a large cisterna magna. Brain magnetic resonance imaging of individual II:8 revealed no obvious abnormality. Nerve conduction studies revealed absent sensory action potentials. None of the patients had any dysmorphic features, developmental delay, or obvious learning difficulties. There were no telangiectases and no standard laboratory abnormalities of immune function. The patients were diagnosed initially, on the basis of the ataxia and ophthalmological signs, as having AT. A diagnosis of variant Friedreich ataxia was also considered. The patients' cases were reviewed, the discrepancies between their clinical phenotype and both AT and Friedreich ataxia were noted, and further investigations were performed.

Chromosomes from lymphoblastoid cell lines (LCLs) were prepared according to standard protocols, and lymphocytes were irradiated as described in Taylor et al. (1987). Whole-cell extracts were made from LCLs of patients and controls and were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Western blot analysis was performed using antisera directed against the ATM, hMRE11, hRAD50, and Nbs1 proteins (Stewart et al. 1999). The western blot was also probed for actin, to standardize for protein loading.

After informed consent was obtained from all family members, blood samples were collected and DNA extracted by use of the Nucleon Biosciences DNA-extraction kit. Fluorescently labeled markers from the ABI

Figure 2 Expression of ATM, hRad50, Nbs1, and hMRE11 in patient II:5.

PRISM Linkage Mapping Set, version 2, were used for the initial genome screen. Fine mapping was performed using markers from Dib et al. (1996) and from the report on the Sixth International Workshop on Chromosome 9 (Chadwick et al. 1998) .

Before performing the genome screen, we performed power calculations, using SLINK. This indicated power to detect linkage with a maximum LOD score of 2.91 on the assumption that AOA is a fully penetrant, autosomal recessive disorder with a disease frequency of 1/100,000; allele frequencies for each marker were assumed to be equal. Homozygosity by descent was identified by visual inspection. The fine-mapping genotyping data were analyzed using SIMWALK2 (Sobel and Lange 1996). This program allows extended pedigrees to be analyzed, even in the presence of distant inbreeding loops and in the absence of genotyping information on

Table 1
X-Ray–Induced Chromosome Damage in Lymphocytes from a Family with AOA after Exposure to 1.0-Gray X-Rays at G2

Individual	No. in Individual				
	Cells Analyzed	Chromatid Gaps	Chromatid Breaks	Triradial Chromosomes	Quadriradial Chromosomes
II:3	50	8	4	0	0
II:5	50	10	1	0	0
II:6	50	10	0	0	0
Control 1	50	8	4	0	0
Control 2	50	12	1	0	0
Control 3	50	13	3	0	0
AT patient 1	34	80	14	0	0
AT patient 2	36	65	15	1	0

Figure 3 Multipoint location score in family with AOA

the distant ancestors. In the family we studied, the pedigree must include the great-great-grandparents of the patients (a total of 25 individuals and 17 meioses) for the multipoint computation, although information is available only on the patients, their siblings, and their parents. Such pedigrees are difficult to analyze using other linkage programs, because there are an enormous number of underlying configurations that are consistent with the available data and these greatly increase the computation time and memory required. SIMWALK2 uses the Markov chain–Monte Carlo (MCMC) algorithm to analyze such large pedigrees, because it takes into account the underlying configurations in proportion to their likelihood. Thus, a configuration that is theoretically possible but highly unlikely (probably because of the large number of recombinations that the configuration would require) will often not be considered. Because SIMWALK2 uses the MCMC algorithm, its results are estimates and not exact. However, SIMWALK2's estimates have been found to be in excellent agreement with the exact results when they are known—for example, in the analysis of the *AT* gene (Savitsky et al. 1995; Sobel and Lange 1996). SIMWALK2 uses location scores, which indicate the likelihood of several putative positions for the trait locus, among the different marker loci. These location scores are directly comparable to multipoint LOD scores and are presented in log₁₀ units.

The results of the chromosome analysis revealed an absence of translocation chromosomes in unirradiated

lymphocytes and no evidence of sensitivity to ionizing radiation (table 1). Western blotting revealed normal expression of *ATM*, as well as *hRad50*, *Nbs1*, and *hMRE11* (Petrini 1999) (fig. 2). Analysis with polymorphic markers close to the *ATM* gene was not consistent with linkage to this locus. These investigations demonstrated that the patients did not have *AT* or a related disorder of chromosomal instability. A diagnosis of Friedreich ataxia was considered; however, the patients did not have the GAA expansion in the *FRDA* gene, which is found in the majority of patients with Friedreich ataxia (Campuzano et al. 1996). Linkage analysis to the *FRDA* locus was performed in case the patients had a rare homozygous mutation in the *FRDA* gene, but linkage to 9q13 was also excluded. Therefore, a genome screen was initiated.

The results of two-point linkage analysis revealed the maximum possible LOD score of 2.91, with marker D9S164, at zero recombination. Inspection of haplotypes revealed homozygosity by descent in 10/10 chromosomes in all affected individuals at marker D9S164 and in 9/10 chromosomes in all affected individuals at marker D9S290 (fig. 1). A total of 17 markers were informative and used for fine mapping in distal 9q34. Multipoint linkage analysis of these markers using SIMWALK2 revealed a maximum location score of 5.32 at markers D9S1861, D9S179, D9S1793, and D9S164 (fig. 3). Examination of haplotypes in affected and unaffected siblings revealed critical recombinations between D9S1863 and D9S1861,

Figure 4 Ideogram of 9q34, illustrating the location of markers, approximate genetic distances, and overlap between the AOA locus and the Joubert syndrome locus.

placing the disease gene distal to D9S1863, and between D9S114 and D9S67, placing the disease gene proximal to D9S67 (fig. 3). This distance is estimated to be ~15.9 cM (Murrell et al. 1995; Dib et al. 1996).

Several neurodegenerative conditions map to 9q34, including one form of Joubert syndrome (Saar et al. 1999). Joubert syndrome is also an autosomal recessive condition characterized by ataxia and oculomotor apraxia, mental retardation, abnormal breathing, and aplasia/hypoplasia of the cerebellar vermis (Chance et al. 1999; Sztriha et al. 1999; Tusa et al. 1999). Occasional patients have also been reported to be dysmorphic (Maria et al. 1999). The patients in the family we studied do not have mental retardation or dysmorphic features, but their neurological features are reminiscent of those found in Joubert syndrome. Homozygosity mapping suggests that the gene mutated in Joubert syndrome is telomeric to D9S164, which overlaps with the AOA locus (fig. 4). However, further studies of both families

with AOA and families with Joubert syndrome will be necessary to determine whether AOA and Joubert syndrome are allelic or not.

Other neurodegenerative conditions mapping to the same region include autosomal dominant juvenile-onset amyotrophic lateral sclerosis, which has been mapped between D9S1831 and D9S164 (Chance et al. 1998), and one form of Leigh disease, which is caused by mutations in SURF1, located near D9S164. However, neither of these conditions has a phenotype that includes cerebellar ataxia. The gene for autosomal recessive microcephaly has recently been mapped to 9q34, but the locus is more proximally located and the phenotype is also quite different (Moynihan et al. 2000).

Approximately 100 known genes and anonymous ESTs map to 9q34, and some of these are interesting candidates for AOA. These include the gene for synaptotagmin VII, which is intimately involved in the regulation of neurotransmitter release by neurons, because

it is the major Ca^{2+} sensor for Ca^{2+} -regulated exocytosis at the synapse (reviewed by Schiavo and Stenbeck 1998). Another interesting candidate gene is *Barhl1*, a novel homeobox gene that maps to 9q34 and has been suggested to be a candidate for Joubert syndrome, as it is expressed in the developing nervous system (Bulfone et al. 2000). The expression pattern of this gene also makes it an interesting candidate for AOA.

In summary, we have identified a novel locus for a primary autosomal recessive cerebellar ataxia whose features resemble those of AT. The clinical features in the family we studied are very similar to those of other patients with AOA described in the literature (Aicardi et al. 1988; Hannan et al. 1994; Gascon et al. 1995). However, it is not yet clear whether AOA is genetically homogeneous; this will require the analysis of additional families. The identification of the disease gene will determine whether Joubert syndrome and AOA are allelic and whether other forms of autosomal recessive cerebellar ataxia are caused by mutations at this locus. It will also allow further understanding of the genetic mechanisms underlying autosomal recessive cerebellar ataxias.

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Electronic-Database Information

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