A new form of childhood onset, autosomal recessive spinocerebellar ataxia and epilepsy is localized at 16q21-q23

M. Gribaa,1,10,12 M. Salih,2 M. Anheim,1,10,11 C. Lagier-Tourenne,1,10,11 D. H’mida,1,10,12 N. Drouot,1,10 A. Mohamed,3 S. Elmalik,4 M. Kabiraj,5 M. Al-Rayess,6 M. Almubarak,7 C. Bétard,8 H. Goebel9 and M. Koenig1,10,11

1Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Molecular Pathology, CNRS/INSERM/Université Louis Pasteur, Illkirch, France, 2Division of Pediatric Neurology, Department of Pediatrics, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 3Department of Pediatrics, King Fahad Hofuf Hospital, Saudi Arabia, 4Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 5Division of Clinical Neurophysiology, Department of Neuroscience, Armed Forces Hospital, Riyadh, Saudi Arabia, 6Department of Pathology, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 7Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 8Centre National de Génotypage, Evry, France, 9Department of Neuropathology, Johannes Gutenberg-University Medical Center, Mainz, Germany, 10Collège de France, Chaire de Génétique Humaine, Illkirch, France, 11Hôpitaux Universitaires de Strasbourg, France and 12Service de Cytogénétique et de Biologie de la Reproduction, Hôpital Farhat Hached, Sousse – Tunisia

Correspondence to: M. Koenig, Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 rue Laurent Fries BP10142, 67404 Illkirch cedex, France
E-mail: mkoenig@igbmc.u-strasbg.fr

Childhood ataxias are a complex set of inherited disorders. Ataxias associated with generalized tonic-clonic epilepsy are usually included with the progressive myoclonus epilepsies (PME). Five disease entities, Unverricht-Lundborg disease, Lafora’s disease, neuronal ceroid lipofuscinoses, myoclonic epilepsy with ragged red fibres and sialidoses, account for the majority of PME cases. Two rare forms of ataxia plus epilepsy, sensory ataxic neuropathy, dysarthria and ophthalmoaparesis, and infantile onset spinocerebellar ataxia were described recently and found to be caused by defective mitochondrial proteins. We report here a large consanguineous family from Saudi Arabia with four affected children presenting with generalized tonic-clonic epilepsy, ataxia and mental retardation, but neither myoclonus nor mental deterioration. MRI and muscle biopsy of one patient revealed, respectively, posterior white matter hyperintensities and vacuolization of the sarcotubular system. We localized the defective gene by homozygosity mapping to a 19 Mb interval in 16q21-q23 between markers D16S3091 and D16S3050. Linkage studies in this region will allow testing for homogeneity of this novel ataxia-epilepsy entity.

Keywords: ataxia; brain imaging; homozygosity mapping; epilepsy

Abbreviations: GTC = generalized tonic clonic; MERRF = myoclonic epilepsy with ragged red fibres; PME = progressive myoclonic epilepsy


Introduction

Autosomal recessive cerebellar ataxias are a heterogeneous group of rare neurological disorders involving both central and peripheral nervous systems and, in some cases, other systems and organs. Autosomal recessive neurodegenerative ataxias are classified according to the major site of degeneration, which can be the cerebellum or the spinal cord. A third group of disorders involves concurrent cerebellar degeneration and sensorimotor peripheral neuropathy (Koenig, 2003). A few of these ataxias are genetically and molecularly characterized, and the pathogenesis is a ‘loss of function’ of cellular proteins involved in metabolic homoeostasis, DNA repair, chaperone function or of unknown function (Di Donato et al., 2001).

Prominent clinical features are signs of cerebellar ataxia, such as uncoordinated gait and uncontrolled coordination...
of hands, speech and eye movements. Other signs, such as retinal, cardiac, muscle and/or other neurological involvement are less common. The clinical picture is often associated with a large variation in age at onset and disease progression.

The ataxias associated with myoclonus, epilepsy and progressive neurological deterioration are usually included with the progressive myoclonus epilepsies (PME). PMEs are a heterogeneous group of inherited disorders which combine generalized tonic–clonic (GTC) or massive myoclonic jerks, localized myoclonus which may be spontaneous or stimulus-induced and various degrees of neurological and mental deterioration. PMEs differ in clinical features, aetiology and pathogenesis. Five disease entities, Unverricht–Lundborg disease, Lafora’s disease, neuronal ceroid lipofuscinoses, myoclonic epilepsy with ragged red fibres (MERRF) and sialidoses, account for the majority of PME cases.

The development of positional cloning strategies based on homozygosity mapping of consanguineous families and on the development of the human genome project has allowed the study of rare forms of recessive ataxias. In consanguineous families, affected individuals are likely to have inherited both copies of a mutated gene from a common ancestor and are thus identical by descent not only at the trait locus, but also at neighbouring marker loci.

We describe here the assignment of a new disease locus to a 19 Mb interval on chromosome 16q21-q23 in a consanguineous Saudi Arabian family with childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia associated with epilepsy and mental retardation.

### Material and Methods

#### Patients

The study includes four children who were referred to the Division of Pediatric Neurology at King Khalid University Hospital, Riyadh, Saudi Arabia for the evaluation of psychomotor retardation and epilepsy. Age at referral ranged from 4 to 13 years. The parents are first degree cousins and originate from a village in the Eastern Province of Saudi Arabia. Neurological examinations and follow-up (spanning 6 years) were performed by one of us (MS). The most recent clinical evaluation for each patient was entered in a standardized diagnostic form for spinocerebellar degeneration which includes the salient symptoms and signs of the disease, as well as scores for assessing the severity. The latter included cerebellar gait score, dysarthria score, modified Ashworth score (for assessing muscle tone), ambulatory score and ‘PATA’ test (for dysarthria evaluation). DNA was isolated from peripheral blood lymphocytes from affected patients and their unaffected parents and siblings (Fig. 1). Blood sampling was performed with the approval of the ethical committee of the King Khalid University Hospital, College of Medicine, Riyadh. Muscle biopsy was obtained from the vastus lateralis of Patient II3 at the age of 12 years.

#### Pathology

Muscle was frozen in isopentane cooled in liquid nitrogen. Cryostat sections of 10 μm were prepared for histological and histochemical examination using standardized methods (Duboitz, 1985; Pears, 1985). Stains used included haematoxylin and eosin (H&E), modified Gomori trichrome, myofibrillar ATPase at routine (9.4), reversed (4.3) and intermediate (4.6) pH; NADH-tetrazolium reductase (NADH-TR), succinic dehydrogenase (SDH); periodic acid Schiff (PAS), with and without diastase; and sudan black. Another portion of muscle was fixed in 2% glutaraldehyde solution in cacodylate buffer and processed for electron microscopy.

#### Genotyping

For the whole genome screen, 400 fluorescently labelled microsatellite markers selected from the Genethon human linkage map with an average spacing of 10 cM and an average heterozygosity of 75% were tested (Lagier-Tourenne et al., 2003). This Linkage Mapping Set was developed and commercialized by Applied Biosystems (ABI Linkage Mapping Set version 2, medium density set MD10). Additional CA/TG microsatellite markers from the Genethon human linkage map were amplified with a universal fluoresceinated primer (Lagier-Tourenne et al., 2003). They were amplified using 100 ng of genomic DNA in 25 μl PCR reactions containing 5 μl PCR buffer, 1.5 mM MgCl2, 20 mM primer pair mix and 1 U AmpliTaq DNA polymerase (Promega). Amplification conditions were 5 min at 95°C followed by 35 cycles of 30 s at 95°C; 30 s at 55°C and 30 s at 72°C; and a final extension for 20 min at 72°C.

PCR products were pooled in panels and loaded on an ABI 3100 automated DNA analyser. Data were analysed using the GeneScan software (Applied Biosystems).

#### Linkage analysis

Two-point analyses including the consanguinity loop were performed using the MLINK program of the FASTLINK package. Homozygosity information was taken into account by considering the non-recombinant haplotype as a single locus (Ben Hamida et al., 1993). Marker order and physical distances were obtained from the UCSC Genome Browser. LOD scores were calculated assuming ataxia in this family is an autosomal recessive disorder and assuming a gene frequency of 0.001 that certainly represents an upper limit for this rare condition.

#### Results

Clinical and laboratory features of the patients are reported in Table 1 and in the supplemental table. The four affected siblings presented with a similar clinical picture. The onset was defined by the appearance of epilepsy at ages ranging between 9 and 12 months. Ataxia was first noticed when the children started to walk, which was delayed until the age of 2–3 years. The epilepsy was of GTC type in all patients. Seizures were controlled in patient II3 by phenobarbitone, which has been initiated following his first atonic seizure at the age of 9 months. For patient II4, they were relatively well-controlled on valproate and phenobarbitone, and occurred once every 10 months.
Fig. 1 Pedigree of the family with autosomal recessive ataxia and epilepsy. Markers are indicated on the left and are organized from top to bottom in centromeric to telomeric order. The marker order is based on the UCSC Assembly of Human Genome browser (May 2004 update). For each marker, the position on the genome is indicated between brackets in megabases. Parental haplotypes linked with the disease are boxed. The region of homozygosity by descent is highlighted in grey. Dotted lines indicate the centromeric and telomeric boundaries of our locus defined, respectively, by a paternal recombination in patient II4 and heterozygosity in patients due to an ancestral recombination (arrows).
Table 1 Clinical features of family H

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ill</th>
<th>Ii2</th>
<th>Ii3</th>
<th>Ii4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Age at onset of first sign</td>
<td>Epilepsy at 1 year</td>
<td>Epilepsy at 9 months</td>
<td>Epilepsy at 9 months</td>
<td>Epilepsy at 9 months</td>
</tr>
<tr>
<td>Delayed motor development</td>
<td>Delayed, talked at 2 years</td>
<td>Delayed, talked at 3 years</td>
<td>Delayed, talked at 2 years</td>
<td>Delayed, talked at 3 years</td>
</tr>
<tr>
<td>Delayed learning abilities</td>
<td>Diminished</td>
<td>Diminished</td>
<td>Diminished</td>
<td>Diminished</td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Gait</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Reflexes</td>
<td>Flexor</td>
<td>Flexor</td>
<td>Flexor</td>
<td>Flexor</td>
</tr>
<tr>
<td>Plantar reflex</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Sphincter disturbances</td>
<td>No ocular apraxia</td>
<td>No ocular apraxia</td>
<td>No ocular apraxia</td>
<td>No ocular apraxia</td>
</tr>
<tr>
<td>Eye movements</td>
<td>Nystagmus (on lateral gaze)</td>
<td>Nystagmus (on lateral gaze)</td>
<td>Nystagmus (on lateral gaze)</td>
<td>Nystagmus (on lateral gaze)</td>
</tr>
</tbody>
</table>

Patient II1 continued to have GTC seizures during sleep (about once/month) despite adequate therapy with valproate. Also seizures were not controlled in her younger sibling (II2) with combined phenobarbitone and valproate therapy (occurring once or twice a week). The four patients also presented with psychomotor delay and learning disabilities. Deep tendon reflexes were diminished in both upper and lower limbs. Limb ataxia was moderate but patients II1, II2 and II4 had severe dysarthria, whereas patient II3 had moderate dysarthria. Ophthalmic examination revealed nystagmus on lateral gaze without apraxia. No other neurological signs or symptoms were found except equivocal plantar response and absence of sphincter control in patient II4 at 10 years of age. Routine laboratory examinations, alpha-fetoprotein and very long chain fatty acids levels were normal. EEG was normal for patients II1 and II4 at age 18 months and 4 years, respectively, but was not investigated subsequently. Patient II2 showed, at 13 years, several bursts of discharges of spikes, multiple spikes and slow waves at 4–5 Hz without clinical manifestation (Fig. 2). The frequency of the generalized discharges was exaggerated by hyperventilation. The background was 9–10 Hz and no photosensitivity was demonstrated. Patient II3 had 3–4 Hz occipital paroxysms, suggestive of benign occipital epilepsy with fixation-off sensitivity. Brain MRI was performed only for patients II3 and II4 and showed mild cerebellar vermis atrophy (Fig. 3). In addition, Patient II3 had abnormal signal intensity involving the terminal zone of myelination. Nerve conduction studies were normal in all patients. Two patients (II1 and II3) had EMG which also revealed normal results. Muscle biopsy of patient II3 (Fig. 4) showed mild variation in myofibre size but no evidence of myofibre necrosis, basophilic fibres, interstitial fibrosis or inflammatory infiltrates. There was no evidence of ragged red fibres or nemaline rods. The normal mosaic pattern was preserved with no specific fibre type atrophy, hypertrophy or grouping. The most conspicuous finding was the presence of irregular vacuolar changes in several fibres. Under electron microscopy, these appeared along the longitudinal axis of the myofibrils suggesting vacuolation of the sarcotubular system.

A total of 400 markers of the ABI PRISM Linkage Mapping Set were tested with this family. Given the close consanguinity of the parents and the close spacing of the markers, we selected the regions for which at least one marker was homozygous in all four affected individuals and we analysed flanking markers to test for homozygosity by descent. Two regions, one on chromosome 3 and one on chromosome 16, appeared to be homozygous for more than 10 consecutive markers. The three healthy siblings were sampled subsequently and allowed to eliminate linkage at chromosome 3 since subject II7 was homozygous for the same alleles as his affected brother and sisters at this locus (not shown).

On chromosome 16q21-q23, the consecutive markers D16S516 and D16S515 of the ABI PRISM Linkage Mapping Set were homozygous in all patients. The study of a dense set of microsatellite markers from this region confirmed linkage at this locus, since the four patients were homozygous for at least 18 consecutive markers and the healthy siblings were heterozygous for 17 of these markers (Fig. 1). LOD score calculation, including the consanguinity loop, gave a value of 3.3 at a recombination fraction θ of 0 (Table 2), demonstrating linkage between the disease and...
the 16q21-23 haplotype. On the telomeric side, a paternal recombination in patient II4 excluded marker D16S3091 from linkage. On the other side, marker D16S3050 and further centromeric markers were heterozygous in all patients, indicating the occurrence of an ancestral recombination. The recombinant markers define a 19 Mb interval containing the gene defective in this syndrome. The giant axonal neuropathy gene (GAN), which is localized in this region, was sequenced and no mutation in the coding and flanking intronic sequences was identified, therefore excluding an atypical presentation of this rare disorder. The set of 16q21-23 microsatellite markers was tested in one consanguineous Algerian family having ataxia associated with epilepsy. Linkage of this family to 16q21-23 was excluded since the three patients did not share the same haplotypes and were not homozygous at this locus.

**Discussion**

The present study reports the identification of a new locus on chromosome 16q21-q23 for an autosomal recessive ataxia associated with epilepsy and psychomotor retardation. The identification of this locus was based on

---

**Fig. 2** Sleep/sedated EEG of Patient II2 at the age of 13 years. The background is 9–10 Hz in addition to a mixture of beta and theta activity. There is a burst of generalized discharges of spikes, multiple spikes and slow waves at 4 to 5 Hz, with greater voltage on the left. The discharges lasted for more than a second without clinical manifestation.

**Fig. 3** Patient II3 (aged 12 years). (A) Axial T2-weighted image and (B) axial FLAIR image demonstrating asymmetry in the appearance of the terminal zone of myelination posterior to the lateral ventricle trigona with the right side showing more hyperintense signal that extends toward the subcortical white matter (arrows). (C) Sagittal TI-weighted brain MR image showing mild cerebellar atrophy. (D) Sagittal TI-weighted MR images of Patient II4 (aged 4 years) showing mild cerebellar atrophy and normal corpus callosum.
homozygosity of patients in a consanguineous family, leading to a LOD score in favour of linkage of 3.3. Consistently, this was the only locus that showed linkage with the disease in this family.

The four patients share stereotyped clinical features. Clinical presentation started between 9 months and 1 year of age with seizures partially controlled by medical treatment. Later, psychomotor delay and mental retardation associated with limb ataxia, dysarthria and nystagmus appeared in all four patients. These clinical features may suggest the diagnosis of any form of recessively inherited progressive myoclonus epilepsy, since about 50% of cases have in fact tonic–clonic seizures (Koskiniemi et al., 1974a; So et al., 1989; Minassian, 2001). The differential diagnosis of our patients includes Unverricht–Lundborg disease (EPM1), Lafora disease (EPM2), the neuronal ceroid lipofuscinoses (CLN1 to 8), sialidoses and the sensory ataxia, neuropathy, dysarthria and ophthalmoplegia (SANDO) syndrome (Van Goethem et al., 2003). The differential diagnosis of MERRF should also be taken into account since maternal inheritance of a new mutation cannot be excluded. However, onset of epilepsy occurred

**Fig. 4** Muscle biopsy from the vastus lateralis of Patient II3. (A) Histology (H&E, ×200) revealed mild variation in myofibre size and the presence of vacuolar changes in a few fibres (arrows). (B) The intermyofibrillar sarcoplasmic architecture also showed focal decreased staining (arrows, NADH-TR, ×400). (C) Electron microscopy delineated the vacuoles which appeared scattered along the longitudinal axis of the myofibrils (magnification ×30 000). (D) Subsarcolemmal vacuoles of irregular shape containing loosely arranged glycogen granules were also seen (magnification ×24 000). (E) The vacuoles appear scattered or in small groups, empty or containing some hazy material of unknown type (magnification ×12 000). (F) Enlarged area showing the grouped vacuoles being delineated by membranes (magnification ×37 500).
much earlier in our patients than in EPM1, EPM2, SANDO, MERRF or type I sialidosis. Also, background activity on EEG is usually slow in these conditions (Koskiniemi et al., 1974b; So et al., 1989; Delgado-Escueta et al., 2001) while it was normal in our family. CLN1 is particularly interesting since age at onset ranges from 8 to 18 months. However, this is followed by rapid mental deterioration and ataxia. Myoclonic jerks appear during the second year of life and there is progressive microcephaly. Blindness, due to optic atrophy and macular and retinal changes, is a feature of the disease (Seehafer and Pearce, 2006). There is also progressive slowing of the EEG and loss of amplitude leading to an isoelectric tracing during the third year of life (Santavuori et al., 1973; Santavuori, 1988).

The disease produces a marked degree of brain atrophy as revealed by MRI (Vanhanen et al., 1995). Type II sialidosis may also have infantile onset. While this disease may present with MRI findings and EEG background activity similar to those found in our family (Engel et al., 1977; Palmeri et al., 2000), clinical features are distinct, with coarse facial features, corneal clouding, hepatomegaly, skeletal dysplasia, learning disability and myoclonus.

It is noteworthy that the association of epilepsy, ataxia and mental retardation is often found in the case of mitochondrial (MERRF and SANDO) or lysosomal (CLN and sialidoses) dysfunction. Muscle biopsy of patient II3 showed sarcotubular system vacuolization but no structural changes in mitochondria or the lysosomes. Nevertheless, that sarcotubular system vacuolization has been observed in miscellaneous cases of myopathy including hypokalemic periodic paralysis channelopathy (Carpenter and Karpati, 2001). It may also be seen in conditions where focal necrosis has recently occurred, or as an artefact when there is acute focal swelling following ingress of sodium into a fibre (Carpenter, 2001). However, neurophysiologic testing revealed no features of myotonia, myopathy or neuropathy in the present family. Biopsies from other siblings could not be obtained. It is therefore not possible to conclude whether this finding is a characteristic feature of the disease affecting this family, like Lafora bodies, ragged-red fibres and autofluorescent lipopigments are in Lafora disease, MERRF and CLN, respectively.

In conclusion, the patients of this family presented with an autosomal recessive disorder with a clinical picture similar to PME diseases. Age at onset, absence of myoclonus and dementia, the presence of spikes, multi-spikes and slow waves on EEG, abnormal signal intensities on MRI and muscle vacuoles, as well as linkage analysis excluded these disorders and allowed us to define a new clinical entity related to the 16q21-q23 locus. The identification of other families with a similar phenotype and linked to the same interval should allow to reduce the size of this locus and to search for mutations in candidate genes located in the critical interval. This interval contains 183 genes, five of which encode for mitochondrial proteins: thymidine kinase 2, tyrosine aminotransferase, glycine cleavage system H protein, cytochrome b5B and dihydroorotate dehydrogenase isofrom 1. The first three proteins are mutant in mitochondrial DNA depletion.
myopathy, tyrosinemia type II and non-ketotic hyperglycinemia, respectively, and another isoform of cytochrome b5B, cytochrome b5A, is mutant in methemoglobinemia type IV. Atypical mutations of these genes, such as partial loss of function or recessive gain of function, might be the cause of the disease in the present family.

Acknowledgements
We are indebted to S. Vicaire, E. Troesch, and I. Colas for excellent technical help. Studies were supported by funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Hôpitaux Universitaires de Strasbourg (PHRC régional), the Agence Nationale pour la Recherche–Maladies Rares (ANR-MRAR) and the College de France. M.G. was supported by an exchange program fellowship ‘Charcot’ from the French Ministry of Foreign Affairs (MAE) and D.H. by the Tunisian Ministry of Health.

References