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Rundataxin, a novel protein with RUN and diacylglycerol binding domains, is mutant in a new recessive ataxia

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We have identified a novel form of recessive ataxia that segregates in three children of a large consanguineous Saudi Arabian family. The three patients presented with childhood onset gait and limb ataxia, dysarthria and had limited walking without aid into their teenage years. Two patients developed epilepsy at 7 months without relapse after treatment, and mental retardation. Linkage studies allowed us to identify a single locus that segregated with the disease on chromosome 3q28-qter. Mutation screening of all coding sequences revealed a single nucleotide deletion, 2927delC, in exon 19 of the *KIAA0226* gene, which results in a frame shift of the C-terminal domain (p.Ala943ValfsX146). The *KIAA0226* gene encodes a protein that we named rundataxin, with two conserved domains: an N-terminal RUN domain and a C-terminal domain containing a diacylglycerol binding-like motif. The closest paralogue of rundataxin, the plekstrin homology domain family member M1, has been shown to colocalize with Rab7, a small GTPase associated with late endosomes/lysosomes, suggesting that rundataxin may also be associated with vesicular trafficking and signalling pathways through its RUN and diacylglycerol binding-like domains. The rundataxin pathway appears therefore distinct from the ataxia pathways involving deficiency in mitochondrial or nuclear proteins and broadens the range of mechanisms leading to recessive ataxias.

Keywords: Salih ataxia; cerebellar ataxia; epilepsy; rundataxin; RUN domain

Abbreviation: DAG = diacylglycerol; PCR = polymerase chain reaction; PKHM = Pleckstrin-homology, M subfamily

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Introduction

Recessive progressive ataxias represent a large group of rare neurological disorders involving both central and peripheral nervous systems. They can be classified according to the major site of degeneration into three groups, namely cerebellar, spinocerebellar and sensory ataxias. Nevertheless, variable combinations of sites of degeneration and associated symptoms are often observed, as in Friedreich ataxia. Recessive ataxias can also be classified according to the pathological mechanisms causing the disease, since several ataxia genes turned out to encode either mitochondrial proteins or nuclear proteins involved in DNA repair. Five genes coding for mitochondrial proteins (frataxin, ABCB7, polymerase gamma, twinkle and ADCK3) are known to cause recessive ataxias (Campuzano *et al*., 1997; Koutnikova *et al*., 1997; Allikmets *et al*., 1999; Van Goethem *et al*., 2003; Nikali *et al*., 2005; Lagier-Tourenne *et al*., 2008), suggesting that oxidative stress generated by mitochondrial malfunction is the underlying mechanism. This prediction is supported by the fact that vitamin E, which is known to be the major membrane antioxidant of the body, causes progressive ataxia when deficient, as demonstrated in the ataxia with vitamin E deficiency and abetalipoproteinaemia (Narcisi *et al*., 1995; Ouahchi *et al*., 1995), for which vitamin E supplementation represents an efficient therapy (Muller, 1986; Gabsi *et al*., 2001). On the other hand, five genes encoding for nuclear proteins involved in DNA repair (ataxia telangiectasia mutated, MRE11, aprataxin, tyrosyl-DNA phosphodiesterase 1 and senataxin) are also mutated in recessive ataxias (Chen and Lee, 1996; Stewart *et al*., 1999; Moreira *et al*., 2001, 2004; Takashima *et al*., 2002; Taylor *et al*., 2004), indicating that DNA repair is the pathological mechanism in these diseases. Finally, other ataxia genes encode proteins linked to the membrane cytoskeleton (SYNE1 in autosomal recessive cerebellar ataxia type 1) (Gros-Louis *et al*., 2007) or to cytosolic chaperones (sacsin and SIL1 in autosomal recessive spinocerebellar ataxia of Charlevoix-Saguenay and Marinesco-Sjögren syndrome, respectively) (Engert *et al*., 2000; Anttonen *et al*., 2005; Parfitt *et al*., 2009), pointing to multiple progressive ataxia pathways. Here we report a family with three patients affected by a new form of pure recessive cerebellar ataxia and epilepsy who have a mutation in a novel protein with similarities to proteins involved in vesicular trafficking. We suggest naming this new entity 'Salih ataxia'.

Subjects and methods

Subjects

The study includes three children who were referred to the Division of Paediatric Neurology at King Khalid University Hospital, College of Medicine, King Saud University, Riyadh, Saudi Arabia, for evaluation of psychomotor retardation. The age at referral ranged between 8 and 16 years. The parents originated from the North-Western Province of Saudi Arabia. Neurological examinations and follow-up (spanning 8 years) were performed (M.A.S.). 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 of affected patients and their unaffected parents and siblings. Clinical evaluation, blood samples and skin biopsy were obtained, after written informed consent, as defined by the Local Ethical Committee of the College of Medicine, King Saud University, Riyadh.

Linkage analysis

Patients were analysed with the GeneChip Human Mapping 10 K 2.0 Xba Array from Affymetrix. Single-nucleotide polymorphism genotypes were obtained by following the Affymetrix protocol for the GeneChip1 Mapping Array. Homozygous regions shared between the patients were identified with the HomoSNP program (plewniak@igbmc.u-strasbg.fr) that was set to identify regions of 25 or more consecutive homozygous single-nucleotide polymorphisms. Single-nucleotide polymorphisms of the shared homozygous regions were then individually inspected to verify that the same haplotype is shared between the patients and that, apart from recombination events, genotypes are identical beyond the homozygous regions. This was in order to demonstrate identity by descent, as opposed to identity by state.

Microsatellite marker analysis

Microsatellite markers referenced to the UCSC Human genome database (http://genome.ucsc.edu, March 2006 release) were used. Analysis was undertaken on an ABI Prism 3100 Genetic Analyser with allele sizes determined using the ABI PRISM1 Genotyper software package (Applied Biosystems). Microsatellite markers were analysed for parents and healthy siblings to confirm linkage to the regions of homozygosity by descent shared by the three affected children. Parental haplotypes linked to the disease were then defined.

Mutational analysis in the reference family

Mutation screening was performed by polymerase chain reaction (PCR) on genomic DNA and direct sequencing of the coding exons and intronic flanking sequences of all genes of the candidate region for Patients 1 and 2 as well as their father. PCR products were purified on Montage PCR_{96} Cleanup Plates (Millipore), used in Sanger sequencing reactions with the ABI BigDye terminator kit (Applied Biosystems) and then subsequently run on an ABI PRISM 3100 Genetic Analyser. The Seqscape 2.5 software (Applied Biosystems) was used to analyse the sequencing results.

Search for mutations in unrelated families and control individuals

The gene defective in the reference family was subsequently analysed in individuals from 172 families with non-Friedreich ataxia by Lightscanner (Idaho technologies) high-resolution melting studies. This system allows the detection of mutational changes in DNA heteroduplexes by the study of the melting curve of PCR products amplified in the presence of 'LC Green' dye. Heteroduplexes were obtained directly by rapid renaturation after the last PCR denaturation cycle and also after mixing the DNA samples two by two prior to PCR in case

a sample contained a homozygous mutation. Samples that presented an abnormal melting profile were subsequently sequenced by the Sanger method. Primer sequences are available on the Helmoholtz Institute of Human Genetics browser: http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html. Fragments were amplified in the presence of 1.5 mM $MgCl_2$ and with annealing temperatures ranging from 60–62°C for sequencing and 63°C for Lightscanner analysis.

The same strategy was used to search for the presence of the coding variants of the reference family in 622 control chromosomes. Ninety-four control chromosomes were from Saudi Arabian individuals, 60 chromosomes were from non-Saudi individuals from the Middle East (including Turkey), 176 chromosomes were from North African individuals and the remaining chromosomes were from European ancestry.

Reverse transcription PCR analysis

Total RNA from primary human fibroblasts and mouse tissues were extracted using Trizol according to the manufacturer's protocol (Invitrogen). Total RNA was reverse transcribed using the Superscript II kit (Invitrogen). The total human complementary DNA was amplified with four sets of primers generating overlapping fragments: exons 1–7 (forward: 5′-CAGGGACGCCTCTCTTGG-3′; reverse: 5′-GTGCCCTCTGCTTTCTTGAG-3′), exons 7–11 (forward: 5′-TTCTCTAGCCTCCACCAATC-3′; reverse: 5′-TTCAGCTCCTGGATCTCTCT-3′), exons 11–17 (forward: 5′-ATGATGAGCCAGTGCCTAGAG-3′; reverse: 5′-CTTCATGTGACACAGCTGGA-3′) and exons 18–21 (forward: 5′ATAGGAAGGTCAAGCTGCTC-3′; reverse: 5′-TCAGTTCTGCAACAGGTGTG-3′). The murine complementary DNA was amplified with primer exons 4 and 7 (forward: 5′GTGCCACTGCCTCTCAGC-3′; reverse: 5′-CTTCTTGAGGTTGCCAGGAA-3′) and in exons 15 and 17 (forward: 5′-AAGCAGAATTACCGCTGTGC-3′; reverse: 5′-AGGAGCTCTTTGGCCAGTC-3′). Murine *GAPDH* and *36B4* (encoding the acidic ribosomal phosphoprotein P0) transcript analysis of the same reverse transcription reaction served as control for the amount of analysed tissue.

Results

A large consanguineous Saudi Arabian family (Family AR) with five healthy siblings and three sisters affected with childhood onset ataxia was identified. Patients 1 and 3 showed unsteadiness since they started walking, which was delayed (age 42 and 28 months, respectively). Patient 2 had delayed onset of walking (age 22 months) and developed unsteadiness at 7 years of age. The three patients had upper and lower limb and gait ataxia, dysarthria and nystagmus or saccadic pursuit (Table 1). They currently have limited walking without aid but are unable to run. Patient 1 has diminished deep tendon reflexes in both upper and lower limbs while the other two have diminished reflexes only in the upper limbs. They have no motor or sensory deficit, muscle wasting, fasciculation or extrapyramidal symptom and plantar response is flexor in each of them. Patients 1 and 3 developed epilepsy, each at the age of 7 months, which responded well to treatment with no relapse since 3 years of age. In Patient 1, the convulsions started in the form of 5–7 myoclonic jerks per day. She has been treated at the regional hospital on carbamazepine (Tegretol), phenytoin (Dilantin) and phenobarbital, none of which was apparently successful. Treatment was discontinued by the parents at the age of 23 months. She was seen, aged 26 months, at another hospital in Riyadh; clonazepam was started with favourable response. EEG (at 2.5 years) was reported to have shown multifocal spikes and spike and slow wave foci. She was successfully weaned off clonazepam at the age of 4.8 years. The seizures in Patient 3 consisted of sudden flexion of the head and

Table 1 Clinical features of Family AR

	Patient 1	Patient 2	Patient 3
Sex (current age, years)	F (25)	F (19)	F (16)
Age at assessment (years)	16	19	16
Age at onset of first sign	Epilepsy at 7 months	Unsteadiness at 7 years	Epilepsy at 7 months
Outcome of epilepsy after treatment	No relapse since age 3 years	–	No relapse since age 3 years
Motor development	Delayed, walked at 42 months	Delayed, walked at 22 months	Delayed, walked at 28 months
Learning abilities	Delayed, talked at >4 years	Delayed, talked at 3 years	Delayed, talked at 4 years
Onset of unsteadiness	Since walking	at 7 years	Since walking
Onset of dysarthria	Since talking	Since talking	Since talking
Cerebellar ataxia			
Upper limbs	Mild	Mild	Severe
Lower limbs	Moderate	Moderate	Severe
Gait	Moderate	Moderate	Severe
Dysarthria	Moderate	Moderate	Severe
Deep tendon reflexes			
Upper limbs	Diminished	Diminished	Diminished
Lower limbs	Diminished	Enhanced	Enhanced
Abnormal eye movements	Nystagmus (no ocular apraxia)	Saccadic pursuit	Saccadic pursuit
Mental status (onset)	Mental retardation (since early childhood)	–	Mental retardation (since early childhood)

both upper limbs, hip and knee flexions. The frequency was approximately 6 times/day, lasting for 10 s, mostly when she awoke from sleep. She received clonazepam for 1 week at the regional hospital and was referred to another hospital in Riyadh at the age of 10 months. An EEG showed frequent discharges consisting of spikes, polyspikes and slow waves, generalized and focal from the right and left hemispheres. A clinical diagnosis of infantile spasm was made and she received vigabatrin. Clonazepam was added because she continued to have daily seizures. She has been seizure free since the age of 3 years and was successfully weaned off her last anticonvulsant (vigabatrin) at the age of 7 years. Patients 1 and 3 also have moderate mental retardation detected since early childhood; however, no formal IQ was done in either patient. MRI of the three patients was normal at ages 16, 9 and 8 years, respectively, but showed mild cerebellar atrophy and prominent folia in Patient 2 at the age of 18 years (Fig. 1). Metabolic ataxias were excluded by laboratory testing (Supplementary Table 1). The parents and healthy carriers were clinically examined and found to be normal; they did not have cerebellar imaging examinations.

We performed homozygosity mapping on Family AR, which shows first-degree consanguinity as well as several loops of remote consanguinity (Fig. 2), by analysing the three affected individuals with whole genome single-nucleotide polymorphism arrays. Two regions of shared homozygosity between the three patients were identified on chromosomes 3qter and 11p13-q12.3, respectively. Linkage studies on these two regions were then performed on all family members (parents and affected and healthy siblings) with a dense set of microsatellite markers, in order to confirm or exclude linkage. This excluded linkage to the 11p13-q12.3 region, as it revealed that the mother was also homozygous over the smallest shared haplotype, probably due to a remote consanguinity that relates her parents three generations ago. Flanking markers of the 11p15.1-q12.3 region showed that Patient 3 received a maternal haplotype different from the other two patients, who shared the same large homozygous haplotype with two of the healthy siblings (Fig. 2), therefore confirming exclusion of linkage.

Linkage analysis with microsatellite markers of the 3q27.3-qter region on all family members is consistent with linkage to this 10.2 Mb interval (Fig. 2). Despite the fact that the LOD score in favour of linkage to the 3q27.3-qter region is only 2.7, due to the multiple consanguinity loops, the 3q27.3-qter region is the only region that shows linkage to the disease in this family. We then screened for mutations in the patients by sequencing the coding exons and exon–intron boundaries of 49 genes localized in the 10.2 Mb linked 3q interval (Supplementary Table 2). Only six genes of the 10.2 Mb linked interval were not sequenced since they were involved in known and distinct diseases or had a specific expression in tissues not compatible with an ataxic phenotype (Supplementary Table 2). In addition to known polymorphisms, three novel nucleotide changes were identified: two missense and one frame-shift change. The two missense mutations were found in genes *ATP13A4* (c.1091T>C, p.Val364Ala) and *FAM43A* (c.982G>A, p.G328S), respectively, but were ascribed as polymorphisms due to their identification in some of the 622 control chromosomes analysed by Lightscanner high-resolution melting studies. The frame-shift change is a single nucleotide deletion, 2927delC, in exon 19 of the *KIAA0226* gene, which results in the usage of a novel 145-amino-acid reading frame (p.Ala943ValfsX146) that is longer than the normal C-terminal sequence. This change was not identified in the 622 control chromosomes analysed. In total, 172 families with non-Friedreich ataxia, including nine families from Saudi Arabia, were analysed for mutations in *KIAA0226*. No additional mutations were identified. Further analysis of clinically targeted cohorts of patients with ataxia will be important since it may increase the chance to identify additional patients affected by this rare condition.

KIAA0226 encodes a 972-amino-acid protein, with two conserved domains (Fig. 3): an N-terminal RUN domain and a C-terminal domain containing a diacylglycerol (DAG) binding-like motif. We therefore named this new protein rundataxin (RUN-DAG-ataxin, RDTX). The 2927delC change of *KIAA0226* causes a frame-shift that removes the DAG binding-like motif of rundataxin, which is the most conserved domain of the protein (in vertebrates and invertebrates), strongly suggesting that the 2927delC change is pathogenic, presumably by a loss of function mechanism. Reverse transcription PCR analysis of *KIAA0226* on human fibroblast RNA showed that exons 2, 8 and 12b can be alternatively spliced out (data not shown). This alternative splicing does not change the reading frame and does not affect the conserved domains. Reverse transcription PCR analysis on adult

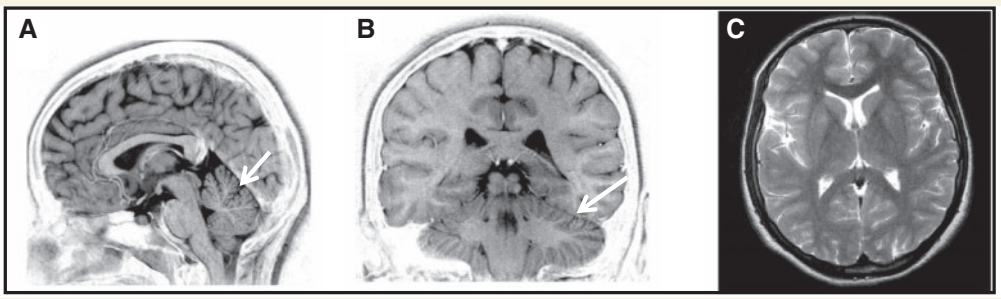
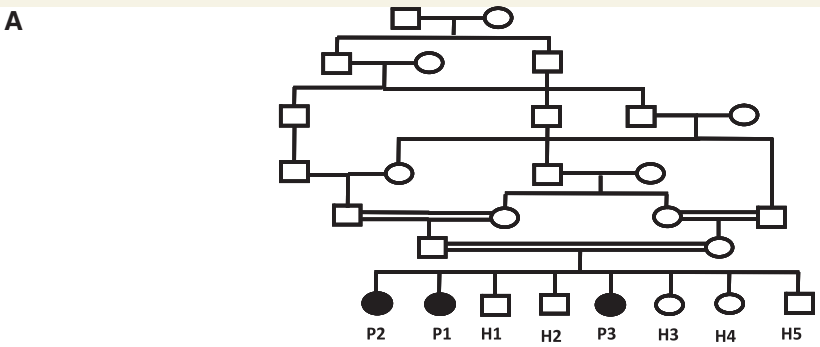
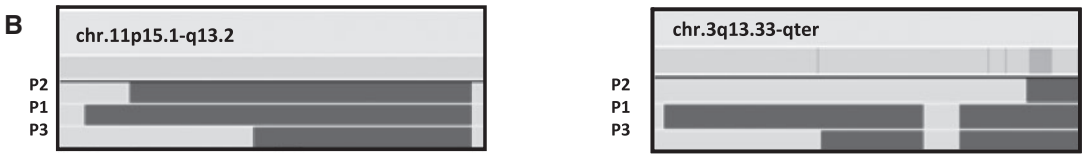


Figure 1 Brain MRI of Patient 2 (aged 18 years; at 9 years, brain imaging was essentially normal). Arrows point to dilated interfoliar sulci. (**A**) Sagittal inverted T_2-weighted image showing moderate cerebellar vermis atrophy. (**B**) Coronal inverted T_2-weighted image showing mild atrophy of the cerebellar hemispheres and prominent foliae. (**C**) Transverse T_2-weighted image at the level of the basal ganglia. There are no hyperintense signals or white matter alterations.





C Chr.11p15.1-q13.2

Mb		F	P2	P1	H1	H2	P3	H3	H4	H5	M
17.0	D11S4160	295 265	265 295	295 295	265 265	295 295	295 265	265 265	265 295	ND	265 295
20.0	rs1822272	ND	BA	AA	ND	ND	AA	ND	ND	ND	ND
20.6	rs953115	ND	BB	BB	ND	ND	BA	ND	ND	ND	ND
20.6	rs951199	ND	BA	AA	ND	ND	AB	ND	ND	ND	ND
22.3	rs956349	ND	BB	BB	ND	ND	BA	ND	ND	ND	ND
23.9	rs1406314	ND	BB	BB	ND	ND	BA	ND	ND	ND	ND
34.8	D11S4200	139 139	139 139	139 139	139 139	139 139	139 139	139 139	139 139	139 139	139 139
35.0	rs353599	ND	BB	BB	ND	ND	BA	ND	ND	ND	ND
35.1	rs2421826	ND	AA	AA	ND	ND	AB	ND	ND	ND	ND
35.3	rs3847621	ND	AA	AA	ND	ND	AA	ND	ND	ND	ND
35.7	D11S4203	239 239	239 239	239 239	239 239	239 239	239 239	239 239	239 239	239 239	239 239
35.9	D11S935	218 218	218 218	218 218	218 218	218 218	218 218	218 218	218 218	218 218	218 218
36.0	D11S4185	165 165	165 165	165 165	165 165	165 165	165 165	165 165	165 165	165 165	165 165
36.0	rs1499511	ND	BB	BB	ND	ND	BB	ND	ND	ND	ND
36.4	D11S4083	206 207	206 206	206 206	206 206	206 206	206 206	207 206	207 206	206 206	206 206
36.7	D11S4102	166 182	166 166	166 166	166 166	166 166	166 166	182 166	182 166	166 166	166 166
40.9	D11S905	243 227	243 243	243 243	243 243	243 243	243 243	227 243	227 243	243 243	243 243
46.1	D11S1344	306 306	306 306	306 306	306 306	306 306	306 306	306 306	306 306	306 306	306 306
48.1	D11S1350	224 224	224 224	224 224	224 224	224 224	224 224	224 224	224 224	224 224	224 224
59.2	D11S4075	226 226	226 226	226 226	226 226	226 226	226 226	226 226	226 226	226 226	226 226
59.2	D11S1335	197 197	197 197	197 197	197 197	197 197	197 197	197 197	197 197	197 197	197 197
60.5	D11S1765	260 262	260 260	260 260	260 260	260 260	260 260	260 260	262 260	260 260	260 260
62.6	rs1944043	ND	AA	AA	ND	ND	AA	ND	ND	ND	ND
62.6	rs1944042	ND	BB	BB	ND	ND	BB	ND	ND	ND	ND
62.7	rs1944086	ND	AA	AA	ND	ND	AB	ND	ND	ND	ND
62.9	D11S4205	218 218	218 220	218 220	218 218	218 220	218 218	218 218	218 218	218 220	218 220
63.0	rs1404501	ND	BA	BA	ND	ND	BB	ND	ND	ND	ND
64.2	rs490192	ND	AA	AA	ND	ND	AB	ND	ND	ND	ND
65.6	D11S913	243 243	243 241	243 241	243 239	243 241	243 239	243 239	243 239	243 241	239 241
68.5	D11S4113	261 252	261 279	261 279	261 252	261 279	261 252	252 252	ND	261 279	252 279

D Chr.3q27.3-qter

Mb		F	P2	P1	H1	H2	P3	H3	H4	H5	M
184.9	rs262980	ND	BA	BB	ND	ND	BB	ND	ND	ND	ND
185.0	rs1829515	ND	BA	BB	ND	ND	BB	ND	ND	ND	ND
189.1	rs763342	ND	BA	BB	ND	ND	BB	ND	ND	ND	ND
189.4	rs719872	ND	AA	AA	ND	ND	AA	ND	ND	ND	ND
189.7	rs726711	ND	AA	AA	ND	ND	AA	ND	ND	ND	ND
190.0	D3S1580	176 166	176 176	176 176	166 176	166 170	ND	166 170	ND	176 170	176 170
191.7	D3S1288	265 257	265 265	265 265	257 265	257 269	265 265	257 269	257 269	ND	265 269
193.3	D3S3663	231 231	231 231	231 231	ND	231 247	231 231	231 247	231 247	231 247	231 247
197.0	D3S1265	238 254	238 238	238 238	254 238	238 238	238 238	254 238	254 238	238 238	238 238
198.5	D3S1311	162 166	162 162	162 162	166 162	162 168	162 162	166 168	162 168	162 168	162 168
198.4	rs1073726	ND	BB	BB	ND	ND	BB	ND	ND	ND	ND
199.3	rs2363306	ND	AA	AA	ND	ND	AA	ND	ND	ND	ND

Figure 2 Pedigree and linkage analysis of Family AR. (**A**) Pedigree of Family AR showing first-degree consanguinity as well as several loops of remote consanguinity. (**B**) Single-nucleotide polymorphism array results of the three patients for the regions 3q27.3-qter and 11p15.1-q13.2. Shared regions of homozygosity are visualized by the HomoSNP software, which displays one patient per line. Regions with more than 25 consecutive homozygous single-nucleotide polymorphisms are in dark grey. Regions of heterozygosity are in light grey. The three affected siblings share regions of homozygosity by descent on chromosomes 3q27.3-qter and 11p13-q12.3. (**C**) Microsatellite marker analysis at chromosome 11p15.1-q13.2 in all family members. Markers and their position along the chromosome (in Mb) are

Continued

mouse tissues showed that *Kiaa0226* is ubiquitously expressed (Fig. 3).

In order to gain insights on rundataxin, phylogenetic studies were undertaken. With respect to the DAG binding-like domain, humans have five rundataxin paralogues: Pleckstrin-homology, M subfamily (PKHM) proteins M1, M3 and M4, DEFI8 and C13ORF18 (Fig. 3). Only rundataxin, PKHM1 and DEFI8 have an orthologue in *Drosophila*. PKHM3/PKHM4 and C13ORF18, which are truncated paralogues of PKHM1 and rundataxin, respectively, appear to be of more recent origin (Fig. 3). Only rundataxin and PKHM1 possess both the RUN and the DAG binding-like domains, PKHM1 having two pleckstrin homology domains in place of the intermediate rundataxin domain with the predicted coiled-coil structure.

Discussion

In this study, we report the identification of a family with three children affected with a new form of recessive ataxia, which we suggest naming 'Salih ataxia', and of a frameshift mutation of *KIAA0226* that segregates with the disease. The disease is an early childhood-onset, slowly progressive pure cerebellar ataxia associated with epilepsy and mental retardation in two of three patients. It is currently not known whether epilepsy and mental retardation are due to a mutation distinct from that causing ataxia which segregated by chance with ataxia in the two patients, or whether they represent clinical variability of this new entity. Identification of additional patients with mutations in *KIAA0226*, by further analysis of clinically targeted cohorts of ataxia patients, will be important as it will help to solve this issue. The demonstration that the *KIAA0226* homozygous 2927delC mutation is indeed causing recessive ataxia is established by the fact that the 3q27.3-qter region is the only region that segregates with the disease in this family, and that the 2927delC mutation is the only significant nucleotide change identified in all coding sequences of this region. In addition, the mutation alters the C-terminal domain of the protein, by removing and replacing with an alternative sequence the highly conserved DAG binding-like motif of this domain, presumably leading to loss of function. However, since the frame-shift mutation occurs towards the end of the protein, it is possible that loss of function is only partial, with some residual function maintained by the mutant protein, which may be protected against degradation by the presence of the longer alternative sequence. The partial loss of function hypothesis would be in agreement with the apparent very low frequency of the disease, since it would imply that the common complete loss of function mutations of the same gene, when homozygous, would either not be viable or result in a different, more severe disease. The fact that the parents and healthy carriers showed no evidence of related signs or symptoms excludes the possibility that the mutation could be dominant or dominant negative. We named the novel protein encoded by the *KIAA0226* gene rundataxin, as it contains an N-terminal RUN domain and a C-terminal DAG binding-like domain. RUN domains are protein–protein binding domains that usually interact with small GTPases and could confer a role in multiple Ras-like GTPase signalling pathways (Callebaut *et al*., 2001). DAG binding motifs are zinc finger-type motifs first identified in protein kinase C and DAG kinases (Colon-Gonzalez and Kazanietz, 2006). The function of rundataxin is still unknown but comparison with paralogues of known function may help to shed light on its function. Very little is known about the mammalian paralogues PKHM3, PKHM4, DEFI8 and C13ORF18, while PKHM1 has been found mutated in osteopetrosis in rats and humans (Van Wesenbeeck *et al*., 2007). Rat Plekhm1 is highly expressed in osteoclasts and colocalizes with Rab7, a small GTPase associated with late endosomes/lysosomes, suggesting that rundataxin may also be associated with vesicular trafficking and signalling pathways. Another protein containing both an N-terminal RUN domain and a C-terminal cystein-rich lipid-binding domain (a phospholipid-binding FYVE domain) is RUN and FYVE domain-containing protein 1 (RUFY1), also named Rabip4, an effector of Rab4. Mari and colleagues (2001) reported that RUFY1 is associated with early endosome membranes via its RUN domain. In Chinese hamster ovary cells, RUFY1 appears as an early endosomal protein, colocalizing with early endosome antigen1 (EEA1), an effector of Rab5. Based on protein homologies, it appears that the rundataxin pathway is distinct from the ataxia pathways involving deficiency in mitochondrial, nuclear or chaperone proteins and is more related to pathways disrupted in spastic paraplegias for which the defective proteins NIPA1, spastin, maspardin, atlastin, alsin and spartin are involved in vesicular trafficking (Zhu *et al*., 2003; Goytain *et al*., 2007; Rismanchi *et al*., 2008; Connell *et al*., 2009; Edwards *et al*., 2009; Hanna and Blackstone, 2009). For example alsin, which is mutated in infantile-onset ascending hereditary paralysis and amyotrophic lateral sclerosis 2, is a guanine nucleotide exchange factor for the small GTPase Rab5 and plays a role in intracellular endosomal trafficking. In addition, the ulceromutilating sensory neuropathy (HMSN2B, previously known as CMT2B) is caused by mutations in Rab7 (Verhoeven *et al*., 2003; Houlden *et al*., 2004; Meggouh *et al*., 2006) and may present a molecular pathway similar to rundataxin deficiency. However, none of the three patients with the rundataxin mutation presented with pyramidal signs or motor neuropathy, indicating

Figure 2 Continued
indicated on the left. Regions of homozygosity by descent are shaded in grey. Selected single-nucleotide polymorphisms (from the array analysis) that delineate recombination breakpoints in the patients are also indicated. The mother is also homozygous over the 11p15.1-q13.2 region, presumably because she is also born from consanguineous parents (**A**), and is therefore non-informative over this region. Analysis with flanking markers indicates that Patient 3 has received a different maternal haplotype than Patients 1 and 2, therefore excluding linkage to this interval. In addition, two healthy siblings share the same parental homozygous haplotypes as Patients 1 and 2, therefore also excluding linkage to this interval. (**D**) Microsatellite marker analysis at chromosome 3q27.3-qter in all family members, as in **C**. Haplotype segregation confirmed linkage of the 3q27.3-qter locus to the disease in Family AR. ND = not determined.

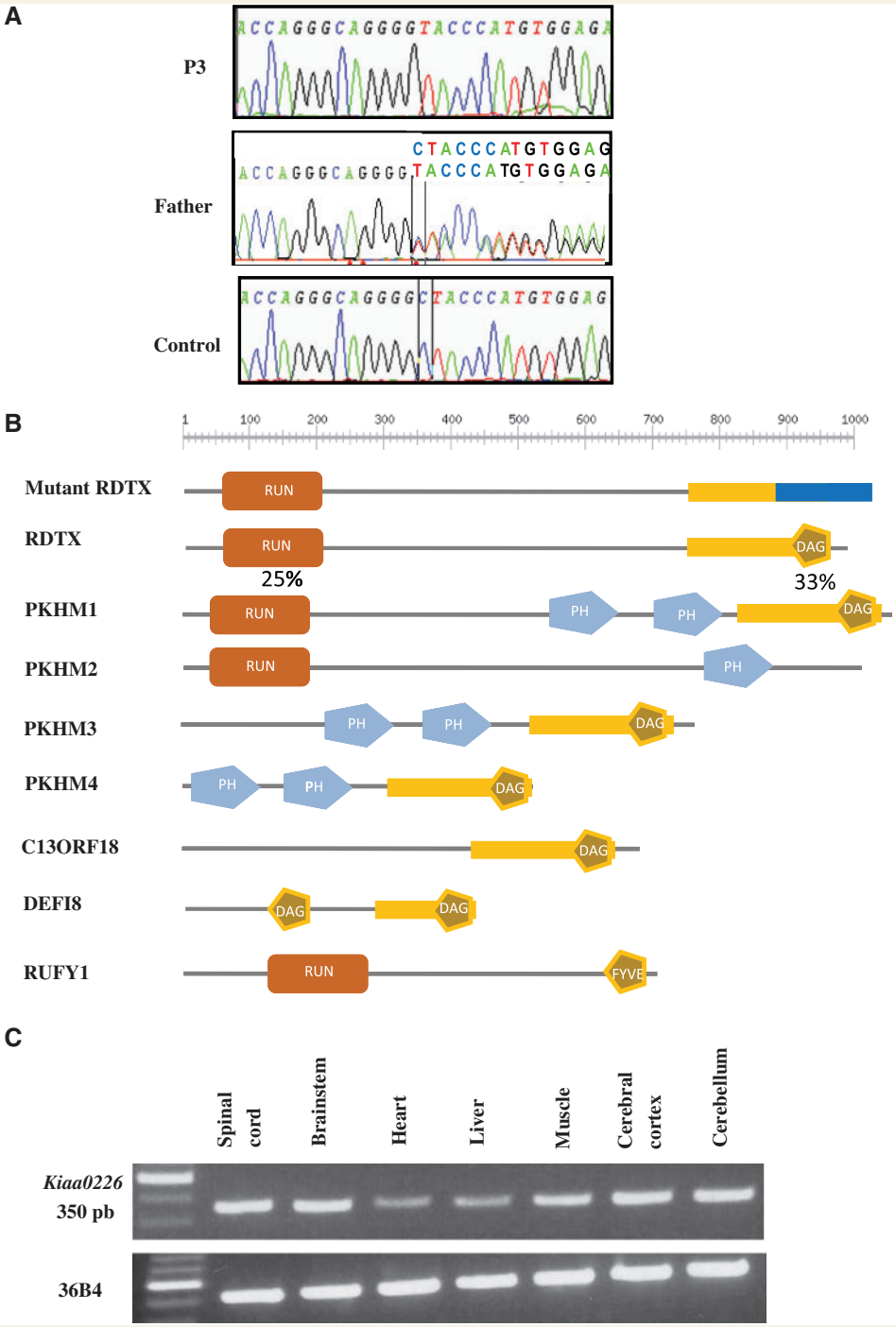


Figure 3 Analysis of the rundataxin gene and protein. (**A**) Sequence of rundataxin exon 19 in Patient 3 (P3), her father and a control individual. Patient 3 is homozygous for the deletion of the cytidine at position 2927, while her carrier father is heterozygous for this mutation. (**B**) Domains and human paralogues of rundataxin (RDTX). The predicted mutant rundataxin is shown on top of normal rundataxin. The novel 145-amino-acid sequence resulting from the shifted reading frame is depicted in blue. The percentage below the RUN and DAG binding-like domains of rundataxin indicates amino-acid identity with PKHM1 over these domains. RUN and FYVE domain-containing protein 1 (RUFY1) contains at its C-terminus a FYVE domain that is another type of lipid-binding zinc finger. (**C**) Expression analysis of *rundataxin* in adult mouse tissues. Mice *rundataxin* is ubiquitously expressed, though more prominently in the nervous system. 36B4, encoding the acidic ribosomal phosphoprotein P0 (RPLP0), was used as internal control. Similar results were obtained using *GAPDH* as an internal control (data not shown).

that *RDTX* was not *a priori* an obvious candidate gene for this disease.

Rab proteins constitute the largest family of monomeric small GTPases, which function in the tethering/docking of vesicles to their target compartment, leading to membrane fusion. However, Rab proteins have also been implicated in vesicle budding and, more recently, in the interaction of vesicles with cytoskeletal elements. Rab5 is localized at early endosomes, Rab6 is localized at the Golgi network, Rab7 and Rab9 are markers of late endosomes. The finding that Rab proteins have several functions suggests that all steps of vesicle transport could be coordinated by the same regulatory machinery (Zerial and McBride, 2001). Testing the hypothesis that rundataxin is also associated with intracellular vesicles through its RUN and DAG-binding-like domains should shed light on the mechanism that leads to ataxia in the case of rundataxin deficiency and broaden the range of mechanisms leading to recessive ataxias.

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Supplementary material

Supplementary material is available at *Brain* online.

References

Allikmets R, Raskind WH, Hutchinson A, Schueck ND, Dean M, Koeller DM. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). Hum Mol Genet 1999; 8: 743–9.

Anttonen AK, Mahjneh I, Hamalainen RH, Lagier-Tourenne C, Kopra O, Waris L, et al. The gene disrupted in Marinesco–Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 2005; 37: 1309–11.

Callebaut I, de Gunzburg J, Goud B, Mornon JP. RUN domains: a new family of domains involved in Ras-like GTPase signaling. Trends Biochem Sci 2001; 26: 79–83.

Campuzano V, Montermini L, Lutz Y, Cova L, Hindelang C, Jiralerspong S, et al. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum Mol Genet 1997; 6: 1771–80.

Chen G, Lee E. The product of the ATM gene is a 370-kDa nuclear phosphoprotein. J Biol Chem 1996; 271: 33693–7.

Colon-Gonzalez F, Kazanietz MG. C1 domains exposed: from diacylglycerol binding to protein-protein interactions. Biochim Biophys Acta 2006; 1761: 827–37.

Connell JW, Lindon C, Luzio J P, Reid E. Spastin couples microtubule severing to membrane traffic in completion of cytokinesis and secretion. Traffic 2009; 10: 42–56.

Edwards TL, Clowes VE, Tsang HT, Connell JW, Sanderson CM, Luzio JP, et al. Endogenous spartin (SPG20) is recruited to endosomes and lipid droplets and interacts with the ubiquitin E3 ligases AIP4 and AIP5. Biochem J 2009; 423: 31–9.

Engert JC, Berube P, Mercier J, Dore C, Lepage P, Ge B, et al. ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Genet 2000; 24: 120–5.

Gabsi S, Gouider-Khouja N, Belal S, Fki M, Kefi M, Turki I, et al. Effect of vitamin E supplementation in patients with ataxia with vitamin E deficiency. Eur J Neurol 2001; 8: 477–81.

Goytain A, Hines RM, El-Husseini A, Quamme GA. NIPA1(SPG6), the basis for autosomal dominant form of hereditary spastic paraplegia, encodes a functional Mg2+ transporter. J Biol Chem 2007; 282: 8060–8.

Gros-Louis F, Dupre N, Dion P, Fox MA, Laurent S, Verreault S, et al. Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia. Nat Genet 2007; 39: 80–5.

Hanna MC, Blackstone C. Interaction of the SPG21 protein ACP33/maspardin with the aldehyde dehydrogenase ALDH16A1. Neurogenetics 2009; 10: 217–28.

Houlden H, King RH, Muddle JR, Warner TT, Reilly MM, Orrell RW, et al. A novel RAB7 mutation associated with ulcero-mutilating neuropathy. Ann Neurol 2004; 56: 586–90.

Koutnikova H, Campuzano V, Foury F, Dolle P, Cazzalini O, Koenig M. Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. Nat Genet 1997; 16: 345–51.

Lagier-Tourenne C, Tazir M, Lopez LC, Quinzii CM, Assoum M, Drouot N, et al. ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. Am J Hum Genet 2008; 82: 661–72.

Mari M, Macia E, Le Marchand-Brustel Y, Cormont M. Role of the FYVE finger and the RUN domain for the subcellular localization of Rabip4. J Biol Chem 2001; 276: 42501–8.

Meggouh F, Bienfait HM, Weterman MA, de Visser M, Baas F. Charcot-Marie-Tooth disease due to a de novo mutation of the RAB7 gene. Neurology 2006; 67: 1476–8.

Moreira MC, Barbot C, Tachi N, Kozuka N, Uchida E, Gibson T, et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat Genet 2001; 29: 189–93.

Moreira MC, Klur S, Watanabe M, Nemeth AH, Le Ber I, Moniz JC, et al. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat Genet 2004; 36: 225–7.

Muller DP. Vitamin E—its role in neurological function. Postgrad Med J 1986; 62: 107–12.

Narcisi TM, Shoulders CC, Chester SA, Read J, Brett DJ, Harrison GB, et al. Mutations of the microsomal triglyceride-transfer-protein gene in abetalipoproteinemia. Am J Hum Genet 1995; 57: 1298–310.

Nikali K, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lonnqvist T, et al. Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. Hum Mol Genet 2005; 14: 2981–90.

Ouahchi K, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, et al. Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. Nat Genet 1995; 9: 141–5.

Parfitt DA, Michael GJ, Vermeulen EG, Prodromou NV, Webb TR, Gallo JM, et al. The ataxia protein sacsin is a functional co-chaperone

that protects against polyglutamine-expanded ataxin-1. Hum Mol Genet 2009; 18: 1556–65.

Rismanchi N, Soderblom C, Stadler J, Zhu PP, Blackstone C. Atlastin GTPases are required for Golgi apparatus and ER morphogenesis. Hum Mol Genet 2008; 17: 1591–604.

Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, et al. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 1999; 99: 577–87.

Takashima H, Boerkoel CF, John J, Saifi GM, Salih MA, Armstrong D, et al. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. Nat Genet 2002; 32: 267–72.

Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. DNA Repair (Amst) 2004; 3: 1219–25.

Van Goethem G, Martin JJ, Dermaut B, Lofgren A, Wibail A, Ververken D, et al. Recessive POLG mutations presenting with sensory and ataxic neuropathy in compound heterozygote patients with progressive external ophthalmoplegia. Neuromuscul Disord 2003; 13: 133–42.

Van Wesenbeeck L, Odgren PR, Coxon FP, Frattini A, Moens P, Perdu B, et al. Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisors absent rats and humans. J Clin Invest 2007; 117: 919–30.

Verhoeven K, De Jonghe P, Coen K, Verpoorten N, Auer-Grumbach M, Kwon JM, et al. Mutations in the small GTP-ase late endosomal protein RAB7 cause Charcot-Marie-Tooth type 2B neuropathy. Am J Hum Genet 2003; 72: 722–7.

Zerial M, McBride H. Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2001; 2: 107–17.

Zhu PP, Patterson A, Lavoie B, Stadler J, Shoeb M, Patel R, et al. Cellular localization, oligomerization, and membrane association of the hereditary spastic paraplegia 3A (SPG3A) protein atlastin. J Biol Chem 2003; 278: 49063–71.