**Identification of mutation in *GTPBP2* in patients of a family with neurodegeneration
accompanied by iron deposition in the brain**

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29 **Abstract**

30 We aimed to identify the genetic cause of a neurologic disorder accompanied with mental
31 deficiency in a consanguineous family with three affected siblings by linkage analysis and
32 exome sequencing. Iron accumulation in the brain of the patients was a notable phenotypic
33 feature. A full-field electroretinography revealed generalized dysfunction of photoreceptors,
34 bipolar cells and amacrine cells. A splice site mutation in *GTPBP2* that encodes GTP binding
35 protein 2 was identified in the patients and considered possible cause of their disease. The
36 mutation was empirically shown to cause deletion of exon 9 of the gene and result in production
37 of a truncated protein lacking conserved C-terminus domains. GTPBP2 is a member of the
38 GTPase superfamily of proteins. A recent report of identification of another splice site mutation
39 in *Gtpbp2* in mice that causes neurodegeneration and retinal damage provides supportive
40 evidence for our finding. The conditions in the affected individuals of the family studied may
41 define a novel form of neurodegeneration with brain iron accumulation (NBIA), and *GTPBP2*
42 may be a novel NBIA gene.

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1.1.Introduction

A definitive clinical diagnosis for neurodegenerative presentations is presently often challenging. This is largely due to notable variability in presentations of individuals reported to be affected with a specific disorder, and to overlaps in presentations in individuals reported to be affected with different disorders. An example of the former is variability of symptoms of patients diagnosed with pantothenate kinase-2-associated neurodegeneration (PKAN, NBIA-1) (Dezfouli et al., 2013; Schneider et al., 2012), and an example of the latter is overlap in symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and hereditary motor and sensory neuropathy with proximal predominance (HMSN-P) (Campellone, 2013; Lillo et al., 2014; Swinnen and Robberecht, 2014). ALS and FTD have only recently been distinguished as different diseases, and HMSN-P may be a variant form of ALS (Campellone, 2013; Ishiura et al., 2012; Lillo et al., 2014). With progress in identification of causative genes for various neurodegenerative diseases, a genetic based classification may be most meaningful (Richard A Armstrong, 2012; Mitsui and Tsuji, 2014). But even this approach has its shortcomings as mutations in the same gene and even identical mutations are sometimes associated with different presentations and different clinically based diagnoses. For example, mutations in *C9orf72* are associated with ALS, FTD, Alzheimer's disease, corticobasal syndrome, supranuclear palsy, Parkinson's disease, olivopontocerebellar degeneration, and sleeping disorder (Richard A. Armstrong, 2012; Lesage et al., 2013; Lindquist et al., 2013). All this reflects the biological complexity associated with neurologic disorders. It is hoped that identification of even more causative genes, knowledge of their functional properties, and accurate descriptions of clinical phenotypes will culminate in better understandings of etiologies of the diseases and features that distinguish various disorders at the molecular and cellular levels.

Here, we describe genetic analysis in a family whose affected individuals appear to be affected with a Mendelian neurologic disease not easily classified as one of the known disorders. The phenotypic presentations included mental retardation and ataxia and dystonia features. We identified a mutation in *GTPBP2* that may be cause of the disease.

1.2.Subjects and Methods

This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran. Participants or guardians consented to participate.

1.2.1. Subjects

A family (DS-100) with four siblings affected with a neurologic disorder was referred for genetic analysis (Fig. 1). Two brothers are unaffected. The siblings had consanguineous unaffected parents, suggesting autosomal recessive inheritance. Detailed clinical data on the eldest affected child is not available, but the mother reports that her features were very similar to those of her other affected children. She was kept at an institution for the mentally retarded from age of 6 years and died there due to unknown causes at age of 21 years. Phenotypic features of the three living affected siblings are summarized in Table 1. Videos showing these patient are available in supplementary video files Video 1- 4.

Many anomalies are shared by the affected individuals. The parents report that all had delays in developmental milestones. All were diagnosed with moderate mental retardation during childhood and assessed to be “trainable”; one (DS-100-3) could not complete first grade in an elementary school for mentally deficient children, and her two brothers never attended school.

90 The affected sons underwent surgery for scoliosis and kyphoscoliosis during childhood. One of
91 the sons (DS-100-5) also had pectus carinatum. During the course of their lives, the siblings were
92 treated with several typical and atypical antipsychotics and mood stabilizer to control impulsivity
93 and abnormal behavior. The parents report that motor and mental performance gradually
94 improved as they grew. Three standard neurological examinations over the past six years showed
95 stability in their conditions and progression of neurodegeneration was not evidenced. The
96 patients, presently aged 34, 30, and 29 years, were cooperative and oriented during a recent
97 examination. They can perform common daily activities and participate in simple social
98 interactions. They present with variable dystonia and mild to moderate ataxia as described
99 below. Their gait and stance were wide based and finger to nose tests were mildly abnormal.
100 Sensory examinations were normal. There were indications of autonomic dysfunction in the
101 patients, including skin thickening, mottling and loss of hair on the legs. The head hair of all
102 three was thin, sparse, and brittle, but microscopic examination of their hairs was normal and
103 there was no shaft disorder. Electromyography and nerve conduction studies showed normal
104 sensory and motor action potentials but neurogenic pattern in distal upper and lower extremity
105 muscles, suggesting chronic motor neuronopathy. Brain magnetic resonance imaging (MRI)
106 showed cerebellar vermain atrophy, and susceptibility-weighted imaging (SWI) revealed
107 abnormal signal (hypointensity) in the globus pallidus and substantia nigra in favor of abnormal
108 iron deposition (Fig. 2). White matter loss was not evidenced. Results of biochemical
109 investigations were normal. However, iron related parameters (serum iron, transferrin, and
110 ferritin levels) were near the lower edge of the normal range in all the patients (Table S1).

111 Dystonia and ataxia related manifestations are further described below. DS-100-3 had abnormal
112 movements of the left hand since childhood. She now has facial twitching that is more prominent

in the lower face. Abnormal hand posturing was evident bilaterally in this patient; it was more prominent in the left side with myoclonus like dystonic jerks when starting action or taking posture. Both feet had abnormal postures with plantar flexion and inversion. Her wide based stance and gait and mild dysmetria in hands suggested mild ataxia. Muscle tone and muscle strength were normal. Deep tendon reflexes (DTR) were normal except that ankle jerks were absent. Plantar reflex was neutral in the right side and showed fanning pattern in the left side. In patients DS-100-4 and DS-100-5, muscle tone was reduced in upper extremity and in calf muscles; the latter resulted in foot plantar posturing. Forces were mildly reduced generally with mild intrinsic hand muscle wasting and mild hammer toe. All DTRs were reduced and plantar reflexes were downward bilaterally in DS-100-4. Tendon reflexes were normal in DS-100-5 except for absence of ankle jerks. The decreased reflexes in the patients and the skeletal deformities are consistent with involvement of anterior horn cells and cerebellum. Fundus examination did not show retinal abnormality in these three patients' eyes. However, full-field electroretinography (ERG) of both eyes of DS-100-3 and DS-100-4 revealed reduced amplitude and delayed implicit time of both a and b waves in the scotopic and photopic conditions (Fig. S1). These findings are compatible with abnormal function of cone and rod photoreceptors, horizontal cells and amacrine cells. In addition, DS-100-5 had severe myopia and had early onset cataract for which he underwent surgery at the age of 28.

1.2.2. Genome-wide linkage analysis

Genome-wide single nucleotide polymorphism (SNP) genotyping was performed on all seven living members of family DS-100 and an unaffected offspring of DS-100-7 using Human370CNV-Quad Infinium HD BeadChips and the iScan reader (Illumina; www.illumina.com) (Fig. 1). Homozygous regions with a minimum length of 1 Mb shared

among the affected individuals and not homozygous in the unaffecteds were sought using the Homozygosity Detector Tool (Illumina). The chip data output was subsequently exported to Microsoft Excel software and homozygous regions were confirmed within the EXCLUDEAR spreadsheet. Nucleotide positions are according to Build 37.3 of the human genome sequence (<http://www.ncbi.nlm.nih.gov/mapview/>).

1.2.3. Exome sequencing

Exome sequencing was done on patient DS-100-5 DNA. Exons were enriched using the TruSeq® Exome Enrichment kit and sequenced on an Illumina HiSeq® 2000 system (Illumina). Sequence alignment and variant calling were performed against human reference genome UCSC NCBI37/hg19 using CASAVA software (Illumina). Additionally, NextBio (<http://www.nextbio.com/b/nextbio.nb>), ENSEMBL (<http://asia.ensembl.org/info/docs/tools/index.html>), and Enlis Genomics (<http://www.enlis.com/>) softwares were used for variant detection. Preliminary filtering was done to identify homozygous variations positioned within the locus identified by linkage analysis. Subsequently, SNPs with a minimal allele frequency of >0.3% in the dbSNP database (<http://www.ncbi.nlm.nih.gov/>), the 1000 Genomes databases (www.1000genomes.org), the NHLBI Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), or the Exome Aggregation Consortium database (<http://exac.broadinstitute.org/>), or observed in the exomes of 22 unrelated healthy Iranians or Iranians affected with non-neurologic diseases were removed. Finally, variations that did not affect amino acid change or splicing were filtered out. The same protocol was used for copy number variations.

1.2.4. Screening of c.1237-1G>T in GTPBP2

Exon 9 and adjacent intronic sequences of *GTPBP2* of all DS-100 family members were PCR amplified and Sanger sequenced. The amplicon contains nucleotide c.1237-1G>T. Sequences were analyzed with Sequencher 4.8 software (Gene Codes Corporation, Ann Arbor, MI). *GTPBP2* reference sequences were NT_007592.15, NM_019096.3, and NP_061969.3. Variation c.1237-1G>T was also screened in 800 unrelated healthy Iranians by an allele-specific-PCR-protocol.

1.2.5. GTPBP2 cDNA synthesis and amplification

RNA was isolated from leukocytes of DS-100 patients and a control individual, and c-DNA was synthesized by standard protocols (Maniatis and Sambrook, 1982). Regions of *GTPBP2* cDNA were sequenced as described below. All primer sequences are available upon request.

1.2.6. Expression of wild type and mutant GTPBP2 encoding proteins

Human wild type *GTPBP2* cDNA cloned in pCMV6-XL6 (pCMV6-XL6-wtGTPBP2) was purchased (ORIGENE, MD). The mutant sequence cloned in pUC57 was also purchased and sub-cloned into pCMV6-XL6 to create pCMV6-XL6-mutGTPBP2 (GenScript, NJ). HEK293 cells were transfected separately with pCMV6-XL6-wtGTPBP2 and pCMV6-XL6-mutGTPBP2 using Lipofectamine 2000 (Life Technologies, NY). Proteins were extracted 48 hours after transfection. Western blotting was performed using rabbit antibody generated against the amino terminus of human GTPBP2 (ABIN463313, antibodies-online Inc., GA) and the ECL Advance Luminol based chemiluminescence detection kit according to the manufacturer's instructions (GE Healthcare Life Sciences, UT). A horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:80,000; Sigma-Aldrich, NY) was used as secondary antibody.

1.3.Results

Only a single region that was homozygous in the three affected members of family DS-100 and not homozygous in the remaining five unaffected individuals was identified (Fig. S2). The 13.5 Mb locus was on 6p21.1-6p13, contained 24733 marker SNPs, and was bound by proximal and distal markers, respectively, rs206765 (32996919 bp) and rs4711853 (46469484 bp). The region contained 234 annotated protein encoding genes and 18 open reading frames (ORFs) (<http://www.ncbi.nlm.nih.gov/mapview/>). Review of the literature and information available at Online Mendelian Inheritance in Man (OMIM; <http://www.ncbi.nlm.nih.gov/omim>) with regards to expression profile, function, and association with neurodegenerative diseases, identified 17 reasonable candidate genes including *SYNGAP1* (OMIM 612621), *KCNK5* (OMIM 603493), *MOCS1* (OMIM 252150), and *GLO1* (OMIM 138750). Direct sequencing of the exons of these genes in DS-100-5 did not identify a potential disease causing variation. Given the multitude of remaining genes in the locus, exome sequencing was performed on the DNA of DS-100-5. The filtering criteria used identified a single novel candidate variation, c.1237-1G>T in *GTPBP2* that encodes GTP binding protein-2. The variation affects the highly conserved -1 position of the acceptor site in intron 8. Direct sequencing of the variation in all DS-100 family members evidenced complete segregation with disease status (Fig. 1, Fig. S3). It was not observed in 800 controls. These results suggested that the c.1237-1G>T in *GTPBP2* may be cause of the neurologic disease in the family.

The most likely effect of the variation was predicted to be skipping of exon 9, although other mis-splicing patterns were also conceivable. To ascertain skipping of exon 9 and other potential mis-splicings that would affect splicing of adjacent exons, five primer sets were designed (Fig. 3A). RT-PCRs were performed on leukocyte RNAs of affected individuals and a control

individual. The forward (4F) and reverse (4R) PCR primers designed to detect skipping of exon 9 spanned, respectively, exons 7 and 8 and exons 10 and 11. Assuming exon 9 skipping, these primers were expected to produce an amplicon that was 59 bp shorter on the mutant (337 bp) as compared to the wild type (396 bp) template. The expected size difference was observed, and no other splicing anomaly was detected (Fig.3B). Direct sequencing evidenced exon 9 deletion and splicing of exon 8 to exon 10 in the mutated cDNA (Fig. 3C). Again, no other anomaly was detected by sequencing. The comparable intensity of the electrophoresis RT-PCR DNA bands produced on the wild type and mutated templates and results of quantitative analysis by real time PCR suggest that the mutated mRNA does not undergo extensive nonsense-mediated mRNA decay in the leukocytes (Brognia and Wen, 2009). The mis-spliced *GTPBP2* mRNA is expected to cause deletion of 20 amino acids encoded by exon 9 and a subsequent frameshift that creates a stop codon within exon 10. It would encode a 416 amino acid truncated protein that is 186 amino acids shorter than the wild type protein. We were unable by Western blot experiments to detect *GTPBP2* protein leukocytes of patients and controls. However, blotting of proteins from HEK293 cells transfected with wild type and mutated *GTPBP2* coding sequences showed that the mutated sequence produced a truncated protein of expected size (Fig. 3D).

1.4.Discussion

We identified a mutation in *GTPBP2* as possible cause of disease in patients of a family whose symptoms include early onset mental deficiency, movement anomalies, and indications of altered iron metabolism including iron deposition in the brain. Retinal abnormalities were also evidenced. Although strong confirmation for the role of this gene in disease etiology would be identification of a mutation in a second family, our findings and studies in mice described below

226 are highly supportive. *GTPBP2* and its encoded protein were first identified in mice by
227 searching for sequences similar to *GTPBP1* and by yeast two-hybrid screening in a brain cDNA
228 library using BH-protocadherin-c as bait (Kudo et al., 2000; Senju et al., 2000; Watanabe et al.,
229 2000). *GTPBP1* and *GTPBP2* are members of the GTPase superfamily whose members are
230 involved in cell proliferation and differentiation, intracellular transport, regulation of
231 cytoskeleton, and protein synthesis (Bourne et al., 1990; Kaziro et al., 1991). The proteins are
232 characterized by conserved GTP-binding motifs. Molecular phylogenetic analysis suggests that
233 *GTPBP1* and *GTPBP2* and a few other proteins form a distinct cluster (GP-1 family) within the
234 superfamily (Kudo et al., 2000; Watanabe et al., 2000). The family closest to the GP-1 family in
235 the superfamily includes human translation factor EF-1 α and orthologous proteins from other
236 species (Kudo et al., 2000; Watanabe et al., 2000). These two families include proteins from
237 mammals, nematodes, and archaebacteria. Alignment of the amino acid sequences of these
238 proteins evidences that in addition to the GTP binding sites in the amino-termini, several motifs
239 within the carboxyl-termini are also highly conserved (Watanabe et al., 2000). The evolutionary
240 conservation of these motifs in distally related organisms signals a functional significance. The
241 conserved C-terminus blocks of eEF1 α bind GTP/GDP and aminoacyl-tRNAs and facilitate
242 delivery of charged tRNAs to ribosomes during translation (Sasikumar et al., n.d.). The truncated
243 *GTPBP2* protein in family DS-100 retains the conserved GTP-binding sequences, but lacks the
244 conserved C-terminal motifs.

245 The function of *GTPBP2* is not well known. Publications relating to this protein and its gene are
246 not numerous. Although *GTPBP1* and *GTPBP2* are homologous (~45% amino acid sequence
247 identity), the two proteins appear to have non-overlapping functions (Kirmizitas et al., 2014;
248 Woo et al., 2011). In mice, *GTPBP1* enhances exosome-mediated mRNA turnover and perhaps

also 3'-UTR mediated mRNA degradation. While GTPBP2 does not have these roles, it was reported that this protein can stabilize reporter mRNA (Woo et al., 2011). More recently, empirical evidence in support of essential functions for the C-terminus of GTPBP2 that is deleted in the truncated protein of DS-100 patients was reported (Kirmizitas et al., 2014). It was shown that GTPBP2 has roles not shared with GTPBP1 in BMP signaling in *Xenopus* embryos and that these roles are mediated by direct interaction between the C-terminus of GTPBP2 with Smad1. Smads in vertebrates and non-vertebrates, after recruiting other effectors, bind promoters of target genes and affect transcription and chromatin remodeling (Itoh and ten Dijke, 2007; Ross and Hill, 2008).

A study most relevant to the findings reported here was published very recently (Ishimura et al., 2014). It was shown that a homozygous splice site mutation in *Gtpbp2* caused neurodegeneration accompanied with truncal ataxia symptoms in mice. Apoptosis of brain neurons and degeneration of retina neurons were evidenced during development. These effects manifested only in a mouse strain that also harbored a mutation in a gene that encodes tRNA^{Arg}_{UCU}; neither mutation alone caused the disease phenotype. The tRNA gene encodes an isoacceptor specifically expressed in brain tissue. Contrary to wild type GTPBP2, the mutated GTPBP2 protein was unable to rescue ribosome stalling induced by the mutated tRNA. GTPBP2 domains, including C-terminal domains, have homology with domains of Hbs1 which is a protein that interacts with the ribosome recycling protein Pelota; GTPBP2 can also interact directly with Pelota (Doma and Parker, 2006; Pisareva et al., 2011). Interestingly, Dom34, the non-vertebrate homolog of Pelota, is involved in degradation processes of non-functional mRNAs (Doma and Parker, 2006). Disruptions in various components of the translation machinery including tRNAs and aminoacyl-tRNA-synthetases can lead to intracellular accumulation of misfolded proteins,

and it is well known that neurons are particularly sensitive to protein misfolding (Antonellis and Green, 2008; Lee et al., 2006; Zhao et al., 2005). In fact, accumulation of misfolded proteins is a hallmark of neurodegeneration (Dobson, 2003; Drummond and Wilke, 2008; Ross and Poirier, 2004). *GARS* is an example of a gene that encodes a protein, glycyl-tRNA synthetase, which functions in the translation process and whose dysfunction results in neurologic disorders. Mutations in *GARS* cause Charcot-Marie-Tooth Disease, Axonal, Type 2D (CMT2D; OMIM: 601472) and Neuronopathy, Distal Hereditary Motor, Type VA (HMN5A; OMIM: 600794). The consequences of the *GTPBP2* mutation of family DS-100 may also be disruptions in translation and/or disruptions in mRNA metabolism. The DS-100-5 exome sequence data did not reveal candidate mutations in genes known to be directly involved in translation, including genes that encode tRNAs, ribosomal RNAs, ribosomal proteins, and translation factors (Table S2).

Iron accumulation in the brain of all three DS-100 affected individuals suggests that their clinical presentations may represent a novel form of neurodegeneration with brain iron accumulation (NBIA), and that *GTPBP2* may be an NBIA causing gene (Kurian et al., 2011; Schipper, 2012; Schneider et al., 2013; Schneider and Bhatia, 2013). NBIA constitutes a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by iron deposition in the basal ganglia, usually in the first decades of life. Known causative genes for the NBIA disorders include *PANK2*, which encodes pantothenate kinase 2; *PLA2G6*, which encodes phospholipase A2; *CP*, which encodes ceruloplasmin; *FTL*, which encodes ferritin light chain; and *C19orf12*, which encodes an orphan mitochondrial protein (Devos et al., 2009; Hartig et al., 2011; Hayflick et al., 2003; McNeill et al., 2008; Morgan et al., 2006; Zhou et al., 2001). Among these genes, mutations are most common in *PANK2*, *PLA2G6*, and *C19orf12* that cause, respectively, NBIA subtypes PKAN, PLAN (phospholipase A2G6-associated

neurodegeneration), and MPAN (mitochondrial membrane protein-associated neurodegeneration). Notably, the major clinical features of our patients, dystonia, ataxia, motor neuronopathy, and cognitive dysfunction, also manifest in the major subtypes of NBIA (Hartig et al., 2013; Kurian and Hayflick, 2013). As with most known NBIA genes, *GTPBP2* is not known to directly participate in iron homeostasis (Schipper, 2012). Systemic anomalies in iron metabolism are generally not found in NBIA patients. The observed low levels of iron, transferrin, and/or ferritin in the blood of DS-100 patients may be irrelevant to accumulation of iron in the brain evidenced by MRI.

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Figure legends

Figure 1- DS-100 pedigree. Filled shapes: affected individuals; *: individuals included in the linkage analysis; arrow: exome sequenced individual. Genotypes of individuals ascertained by direct sequencing are presented: N, normal *GTPBP2* allele; M, mutated *GTPBP2* allele.

Figure 2- Brain magnetic resonance images of DS-100 patients. A. Axial susceptibility weighted imaging (SWI) showing globus pallidus (hollow arrows). Images from left to right on top row are from patient DS-100-3, patient DS-100-4, and patient DS-100-5. The images on bottom row are from a 30 years old normal individual (left) and a patient genetically diagnosed with PKAN (right). The images show mild hypointensity in the globus pallidus of the DS-100 patients and strong hypointensity in the PKAN patient. **B.** Axial susceptibility weighted imaging (SWI) showing substantia nigra (filled arrows) of DS-100-4 (left), a normal individual (middle), and a PKAN patient (right). The figure shows abnormal iron deposition in substantia nigra of patient DS-100-4 and severe iron deposition in substantia nigra of PKAN patient. **C.** Sagittal T2 weighted MRI of DS-100-5 (left) and a normal individual (right). Cerebellar vermian atrophy (arrow) is evident in the image of DS-100-5. **D.** Axial T2 weighted MRIs of DS-100-3 (left), DS-100-4 (middle), and DS-100-5 (right) that show vermian atrophy and increased diameter of 4th ventricle. All images were obtained within the past year.

Figure 3- Effect of c.1237-1G>T in *GTPBP2* on exon skipping and protein truncation. A. Exons of *GTPBP2* are numbered. Position of c.1237-1G>T mutation in acceptor site of intron 8 is show with open arrow. Filled arrows indicate positions of primers on cDNA used for RT-PCR. The primers expanded adjacent exons to prevent amplification of potentially contaminating

genomic DNA. **B.** Electrophoresis of RT-PCR products of DS-100 affected individuals and a control individual with PCR primers 4F and 4R which are expected to produce a 396 bp amplicon on control DNA; skipping of exon 9 during splicing in affected individuals (DS-100-3, DS-100-4, and DS-100-5) is expected to cause synthesis of an amplicon 59 bp shorter. **C.** Sequencing chromatograms of cDNA of control individual and DS-100 patients. Comparison of the chromatograms shows region in cDNA of control (bracketed by lines) corresponding to exon 9 of *GTPBP2* that is deleted in the cDNA of the patients. **D.** Western blot analysis of wild-type and mutated GTPBP2 expressed in HEK293 cells. The mutation is expected to cause premature translational termination and production of a 45.6 kDa protein. Lamin B was used as a loading control.

Table 1- Phenotypic features of DS-100 patients[#]

	DS-100-3	DS-100-4	DS-100-5
Present age (yrs.)	34	30	29
Sex	Female	Male	Male
Delayed developmental milestones (age)			
Independent sitting (1 yr.)	+	+	+
Independent walking (2 yrs.)	+	+	+
First speech (3 yrs.)	+	+	+
Intelligence quotient α	43-50	47-50	40-50
Scoliosis or kyphoscoliosis	-	+	+
Pectus carinatum	-	-	+
Generalized tonic clonic seizures	-	-	+*
Wide based gait & stance	+	+	+
Abnormal finger to nose test	+	+	+
Thin sparse brittle hair	+	+	+
Electromyography & nerve conduction studies			
Abnormal sensory action potentials (SNAPs)	-	Not done	-
Abnormal compound motor action potentials (CMAPs)	-	Not done	-
Neurogenic pattern on needle examination	+	Not done	+
MRI: cerebellar vermain atrophy	+	+	+
SWI: hypointensity in globus pallidus & substantia nigra	+	+	+
Tremor of hands and jaws	+	-	-
Abnormal hand posturing	+	-	-
Reduced muscle tone	-	+	+
Reduced muscle strength	-	+	+
Reduced deep tendon reflexes	+ β	+	+ β
Abnormal plantar reflexes	+	+	+
Myopia	-	-	+
Early onset cataract	-	-	+
Anomalies of the retina	+	+	Not done
Serum biochemistry			
Abnormal muscle enzyme levels	-	-	-
Abnormal thyroid hormone levels	-	-	-
Abnormal electrolyte levels	-	-	-
Abnormal liver &/or kidney function tests γ , δ	-	+	-

[#]All features listed below except intelligence quotient were from examinations and measurements within the past year. α , The Wechsler Adult Intelligence Scale (WAIS) test; *Four seizures between age of 15 yrs. and present age of 29 yrs.; β , evidenced only by absence of ankle jerks; γ , abnormal in at least one of three factors measured in serum: iron, transferrin, ferritin; δ , all measurements were close to lower edge of normal range: see Table S1. MRI, magnetic resonance imaging; SWI, susceptibility-weighted imaging. +, present; -, absent.

Figure 1

Figure 2

Figure 3