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Absence of the Autophagy Adaptor SQSTM1/p62 Causes Childhood-Onset Neurodegeneration with Ataxia, Dystonia, and Gaze Palsy

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SQSTM1 (sequestosome 1; also known as *p62*) encodes a multidomain scaffolding protein involved in various key cellular processes, including the removal of damaged mitochondria by its function as a selective autophagy receptor. Heterozygous variants in *SQSTM1* have been associated with Paget disease of the bone and might contribute to neurodegeneration in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Using exome sequencing, we identified three different biallelic loss-of-function variants in *SQSTM1* in nine affected individuals from four families with a childhood- or adolescence-onset neurodegenerative disorder characterized by gait abnormalities, ataxia, dysarthria, dystonia, vertical gaze palsy, and cognitive decline. We confirmed absence of the SQSTM1/p62 protein in affected individuals' fibroblasts and found evidence of a defect in the early response to mitochondrial depolarization and autophagosome formation. Our findings expand the *SQSTM1*-associated phenotypic spectrum and lend further support to the concept of disturbed selective autophagy pathways in neurodegenerative diseases.

Neurodegenerative disease causes severe disability or even early death and many families and affected individuals remain without a specific molecular diagnosis. The identification of underlying gene defects in both common and rare conditions provided fundamental new insights into pathophysiology and basic cellular processes. Examples are the discovery of gene defects underlying Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS). Proteins encoded by affected genes such as PARKIN (PARK2), PINK1, OPTN, SQSTM1/p62, and TBK1 offer a new perspective on the involvement of mitochondria. Specifically, these proteins are thought to be involved in the maintenance of a functioning pool of mitochondria by regulating their turnover by selective autophagocytic processes.[1,2] One recent model suggests that after loss of mitochondrial membrane potential or accumulation of misfolded proteins, PINK1 generates a phospho-ubiquitin signature on mitochondria to induce recruitment of the primary autophagy adaptors OPTN and NDP52, which then engage with the autophagy machinery (ULK1, DFCP1, WIPI1, and LC3).[3] Although TBK1-dependent phosphorylation of OPTN and NDP52 is thought to be crucial for robust mitophagy, the presence of additional/alternative autophagy adaptors such as TAX1BP1, NRB1, and SQSTM1/p62 is dispensable for mitophagy itself.[3]

In addition to its function in autophagy, the multidomain scaffold protein SQSTM1/p62 plays a role in diverse other key cellular pathways; heterozygous *SQSTM1* (MIM: 601530) variants have been suggested to contribute to the pathogenesis of such diverse presentations as Paget disease of the bone (PDB [MIM: 167250]), ALS, FTD (MIM: 616437),[4] and recently distal myopathy with

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Table 1. Genetic and Clinical Findings in Individuals with Biallelic *SQSTM1* Loss-of-Function Variants

		SQSTM1 Variants	Phenotypic Features									MRI Findings	
ID	Sex	cDNA (NM_003900.4), Protein (NP_003891.1)	AaO[a]	AaLE[a]	Gait Abn.	Ataxia	Dystonia	Dysarthria	Gaze Palsy	MND	Cognitive Decline	CA	Iron
F1:II.1	M	c.[2T>A];[2T>A], p.[?];[?]	10	45	+*	+*	+	+	+	+L	–	ND	ND
F1:II.3	F	c.[2T>A];[2T>A], p.[?];[?]	12	42	+*	+	+	+	+	+U/L	+*	–	+
F1:II.6	F	c.[2T>A];[2T>A], p.[?];[?]	15	33	+*	+*	+	+	–	–	+	–	+
F2:II.2	F	c.[311_312del];[311_312del], p.[Glu104Valfs48*];[Glu104Valfs48*]	10	31	+*	+*	+	+	+	–	+*	+	–
F2:II.3	F	c.[311_312del];[311_312del], p.[Glu104Valfs48*];[Glu104Valfs48*]	10	18	+*	+*	+	(+)	+	–	+*	+	–
F2:II.z	F	c.[311_312del];[311_312del], p.[Glu104Valfs48*];[Glu104Valfs48*]	10	12	+*	+*	+	(+)	+	–	+*	+	–
F3:II.1	F	c.[286C>T];[286C>T], p.[Arg96*];[Arg96*]	7	18	+*	+*	–	+*	–	–	+	+	–
F4:II.1	F	c.[286C>T];[286C>T], p.[Arg96*];[Arg96*]	8	33	+	+*	–	+	+	–	+	–	–
F4:II.4	M	c.[286C>T];[286C>T], p.[Arg96*];[Arg96*]	8	17	+	+*	+	+	+	–	+	–	–

Abbreviations are as follows: AaLE, age at last examination; AaO, age at onset; abn., abnormalities; CA, cerebellar atrophy; MND, motor neuron disorder (L, lower MND; U/L, upper and lower MND); ND, not done; *, among the presenting symptoms; +, affected; (+), mildly affected; –, not affected.
[a]Ages in years.

rimmed vacuoles.[5] Here, we report on the phenotypic spectrum associated with absence of SQSTM1/p62 as well as the results of our studies of mitophagy-related phenotypes in mutant fibroblast cell lines.

We performed exome sequencing on genomic DNA from affected individuals from four families with a so far etiologically unresolved childhood- or adolescence-onset neurodegenerative syndrome manifesting with gait abnormalities and ataxia. Clinical and genetic findings are summarized in Table 1, pedigrees are shown in Figure 1, and neuroimaging findings are in Figure 2. A summary of the phenotypes is provided below, and the detailed individual case reports are provided in the Supplemental Data. Informed consent was obtained from all affected individuals or their guardians. The study was approved by the local ethics committees.

Family 1 consists of three affected siblings, one male (F1:II.1) and two females (F1:II.3, F1:II.6), and three healthy siblings born to healthy unrelated parents of German origin. All affected individuals presented with gait problems in their early teen years, progressing to wheelchair dependence at ages 16–32 years. Other presenting symptoms included urinary incontinence, upper limb ataxia, and cognitive decline. Last clinical examination at ages 45, 42, and 33 years, respectively, revealed generalized dystonia, dysarthria, and a cerebellar syndrome in all three affected siblings, as well as vertical gaze palsy and a pyramidal syndrome in two. None had a history of psychiatric symptoms, seizures, tremor, or visual impairment. Brain MRI showed accumulation of iron in basal ganglia in individuals F1:II.3 and F1:II.6 (Figure 2). A muscle biopsy in F1:II.6 was unremarkable regarding morphology, histochemistry, ultrastructure, and mitochondrial enzyme analysis.

Family 2 consists of three affected sisters (F2:II.2, F2:II.3, F2:II.4) and nine reportedly healthy siblings, born to healthy parents from the United Arab Emirates who were unaware of consanguinity. All affected siblings had a remarkably similar course with unsteadiness of gait, coordination problems, and cognitive decline from the age of 10 years. At last clinical examination at ages 31, 18, and 12 years, respectively, all were still able to walk independently but showed short and thin stature, a cerebellar syndrome, marked vertical gaze palsy, hearing loss, and a dystonic or athetotic movement disorder. Brain MRI showed mild cerebellar atrophy in all affected siblings (Figure 2).

Family 3 consists of one affected sister (F3:II.1) and one healthy brother, born to healthy unrelated parents from Finland. The affected sister presented at the age of 7 years with tremor, oculomotor apraxia, and dysarthria. Last clinical examination at age 18 years showed a cerebellar syndrome and cognitive impairment. Brain MRI revealed mild to moderate cerebellar atrophy (Figure 2). Muscle biopsy was unremarkable regarding morphology, histochemistry, and ultrastructure but respiratory chain enzyme analysis showed a slight decrease in complex IV activity.

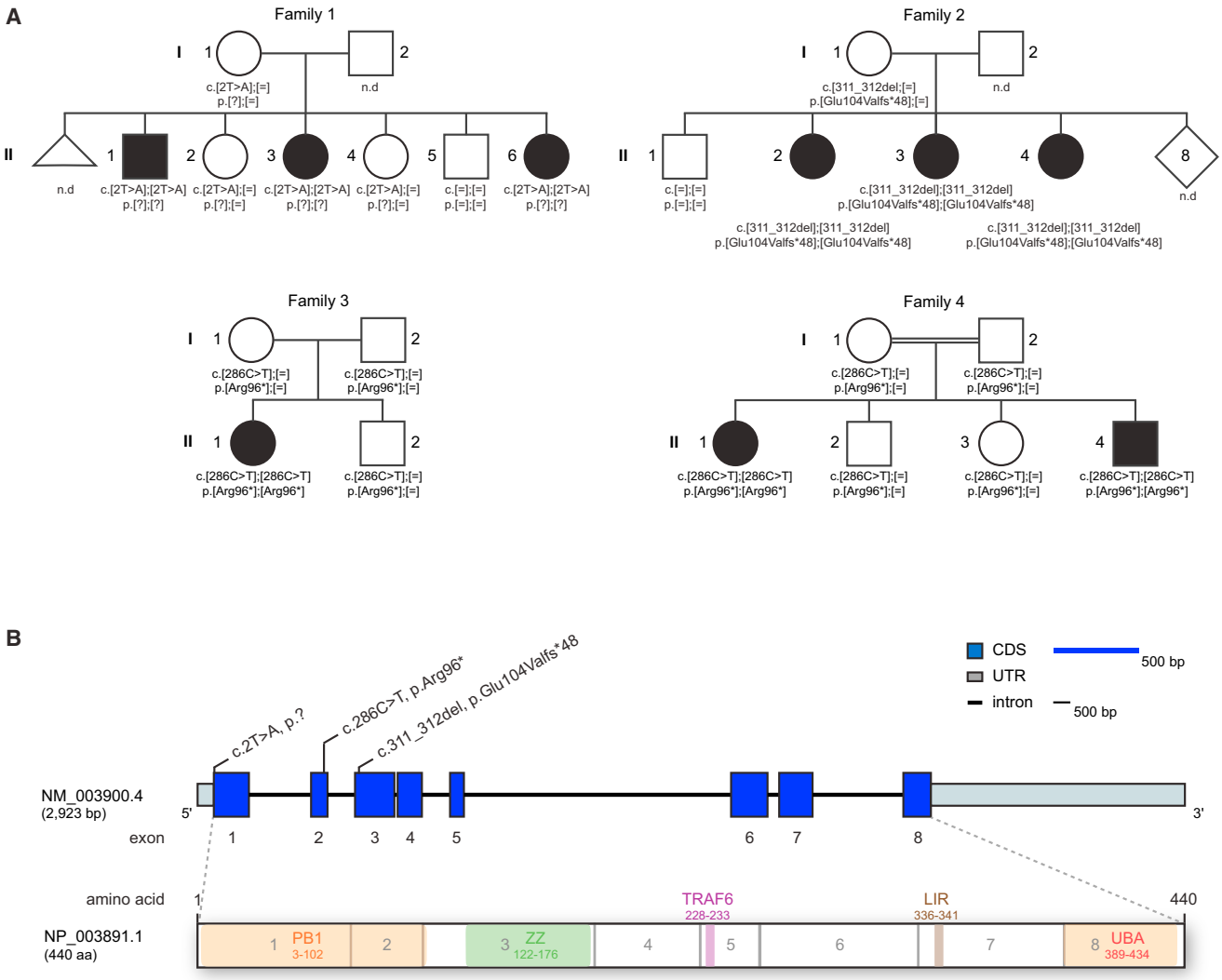


Figure 1. Pedigrees of Investigated Families and Structure of *SQSTM1*

(A) Pedigrees of four families with mutations in *SQSTM1*. Mutation status of affected (closed symbols) and healthy (open symbols) family members. n.d., not determined.

(B) Gene structure of *SQSTM1* with known protein domains and motifs of the gene product and localization of the identified mutations. Intronic regions are not drawn to scale. Abbreviations are as follows: PB1, Phox 1 and Bem1p; ZZ, zinc finger; TRAF6, tumor necrosis factor receptor-associated factor 6; LIR, LC3-interaction region; KIR, Keap1-interacting; UBA, ubiquitin-associated.

Family 4 consists of two affected individuals, one female (F4:II.1) and one male (F4:II.4), and two healthy siblings, born to healthy consanguineous parents of Kurdish descent. Both affected siblings presented at the age of 8 years with a syndrome of ataxia, dysarthria, and vertical gaze palsy. Last clinical examination at ages 33 and 17 years, respectively, revealed a cerebellar syndrome and cognitive impairment. Although F4:II.1 is still walking with a broad-based gait at age 33 years, her brother (F4:II.4) is wheelchair bound at age 17 years. Brain MRI and EEG showed normal findings in both. In F4:II:4, mitochondrial ATP production rate and respiratory chain enzyme activities were toward the lower end of normal, with complex IV activity being mostly affected. In agreement with this, fibroblasts grown on galactose as carbon source showed a mild complex IV defect (Figure S1).

We performed exome sequencing at three centers (Munich [families 1 and 2], Helsinki [family 3], and Stockholm [family 4]) on genomic DNA from three affected individuals of family 1, individuals F2:II.2 and F3:II.1, as well as on both affected individuals and their parents of family 4, essentially as described previously.[6]

In family 1, individuals F1:II.1 and F1:II.3 have been investigated in a prior experiment with a SureSelect Human All Exon 38 Mb enrichment kit (Illumina). A search for recessive-type non-synonymous variants with a minor allele frequency (MAF) < 0.1% in an in-house database containing 7,000 control exomes and the Exome Aggregation Consortium (ExAC) Server (09/2015) failed to prioritize likely pathogenic variants common to both individuals. We speculated that we had missed the responsible variant due to insufficient coverage of target sequences and

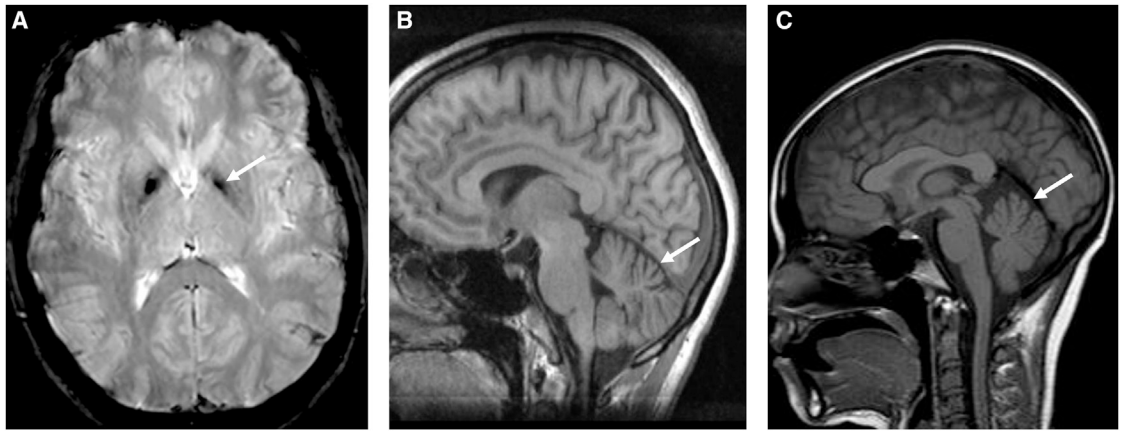


Figure 2. Neuroimaging Findings in SQSTM1/p62 Variant Individuals
(A) Brain MRI (T2-weighted image, axial view) of individual F1:II.6 at the age of 33 years, demonstrating iron accumulation in the globus pallidus internus.
(B) Brain MRI (T1-weighted image, sagittal view) of individual F2:II.2 at the age of 31 years, demonstrating mild cerebellar atrophy.
(C) Brain MRI (T1-weighted image, sagittal view) of individual F3:II.1 at the age of 18 years showing mild to moderate upper vermian atrophy.

processed DNA of the third affected individual (F1:II.6) with an updated version of the enrichment kit, SureSelect Human All Exon 50 Mb V3, potentially providing an improved coverage of the coding regions. This analysis identified seven genes carrying potential compound heterozygous or homozygous variants, with *SQSTM1* being the only gene carrying two predicted loss-of-function alleles (Table S1). Manual inspection of the sequencing data confirmed that the *SQSTM1* (GenBank: NM_003900.4) variant c.2T>A (p.?) in exon 1 was missed as an alternative call in the exome datasets of the two other siblings due to insufficient coverage of this genomic position, which reflects a general problem of GC-rich first exons. Subsequent Sanger sequencing confirmed the c.2T>A variant in a homozygous state in all affected individuals, with the mother and healthy siblings being heterozygotes or wild-types. These results were in line with the observation that the *SQSTM1* locus is located within one of six regions of interest of a previously performed SNP-array linkage study performed on this family. Because no paternal samples were available for carrier testing, we cannot exclude a large deletion on the paternal chromosome. However, analysis of the gene dosage in individual F1:II.6's exome dataset using exome depth as described previously did not indicate any copy-number variation.[7,8]

In individual F2:II.2, sequencing was performed using a SureSelect Human All Exon 38 Mb enrichment kit. A search for genes carrying putative compound heterozygous or homozygous rare variants identified 30 candidate genes, 7 of which were affected by variants rated likely detrimental in silico (see Table S1). Segregation analysis by Sanger sequencing on DNAs of further family members excluded a likely clinical relevance of variants in five putative candidate genes, leaving a homozygous frameshift variant c.311_312del (p.Glu104Valfs*48) in *SQSTM1* as the likely candidate. No material was available to confirm a heterozygous carrier status of the father. However, we did not detect any evidence of a large heterozygous deletion in the exome data of individual F2:II.2.[8]

In individual F3:II.1, the exome targets of the affected individual's DNA were captured with the Agilent SureSelect Human All Exon V5 whole exome kit followed by sequencing with the Illumina HiSeq 2000 platform. The variant calling pipeline of the Finnish Institute of Molecular Medicine (FIMM) was used for the reference genome alignment and variant calling.[9] Variants with a Combined Annotation Dependent Depletion (CADD) C-score less than 10 were excluded.[10] A search for recessive-type nonsynonymous variants with a MAF $< 0.1\%$ on the ExAC Server (09/2015) prioritized three candidate genes: *LLGL1* (MIM: 600966), *RYR1* (MIM: 180901), and *SQSTM1*. Variants in *RYR1* have been associated with unrelated autosomal-recessive inherited phenotypes (MIM: 255320 and 117000). Variants in *LLGL1* have so far not been associated with human disease and although an effect of the predictively synonymous variant on splicing cannot be excluded, we considered it unlikely to be associated with the disease of individual F3:II.1. A homozygous *SQSTM1* loss-of-function variant with a CADD score of 45, c.286C>T (p.Arg96*), remained as the top scoring candidate.

Family 4 was sequenced on the Hiseq 2500 platform (Illumina) using the Agilent SureSelect Human All Exon V4 whole exome kit. The resulting sequences were analyzed using an in-house Mutation Identification Pipeline (MIP) as previously described.[11] All called variants are scored and ranked using the Mutation Identification Pipeline weighted sum model, which uses multiple parameters but emphasizes Mendelian inheritance patterns, conserved, rare, and protein-damaging variants. This left a homozygous c.286C>T (p.Arg96*) *SQSTM1* loss-of-function variant as the top-scoring variant. Sanger sequencing of parents and affected siblings confirmed that the

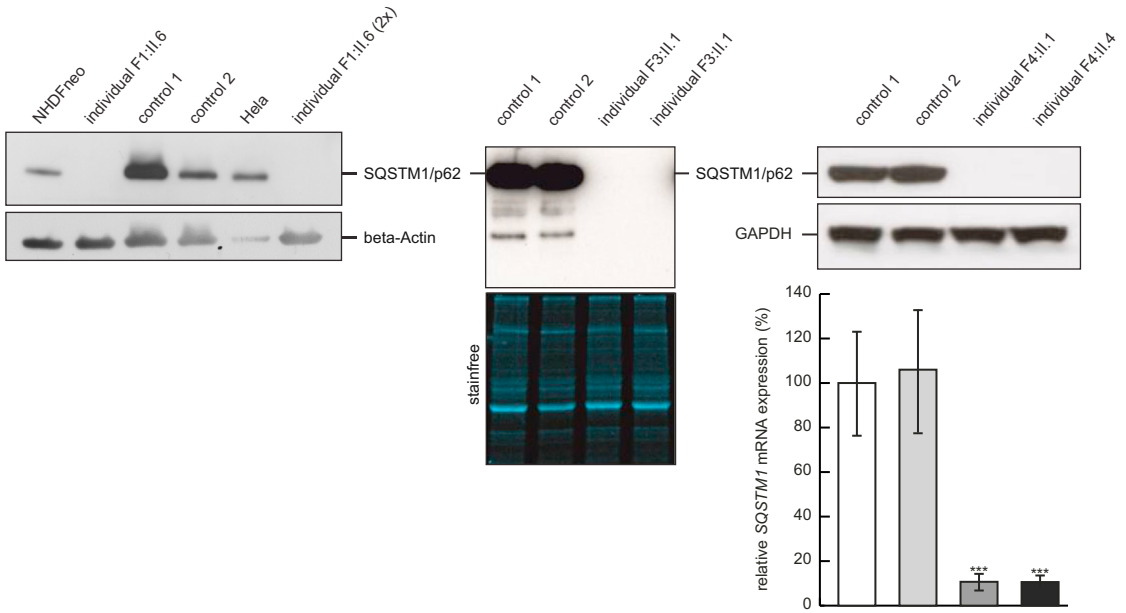


Figure 3. Investigation of SQSTM1/p62 Protein and RNA Levels
Western blot studies in SQSTM1/p62 variant fibroblast cell lines indicating that the homozygous variants c.2T>A (p.?) and c.286C>T (p.Arg96*) result in a loss of SQSTM1/p62 protein. Immunoblotting was done with anti-SQSTM1/p62 antibody (F1:II.6 with Progen cat# GP62-C; F3:II.1 and F4:II.1,4 with Cell Signaling cat# 5114).
In SQSTM1/p62 variant fibroblasts cell lines of F4:II.1,4, total RNA was isolated for qPCR analysis using Trizol. Reverse transcription for qRT-PCR analysis was performed using High Capacity cDNA Reverse Transcription Kit (Life Technologies). qRT-PCR was performed on a QuantStudio 6 (Life Technologies) with Platinum SYBR Green qPCR supermix-UDG (Life Technologies) and gene-specific primers. Error bars indicate ± SEM; ***p ≤ 0.001; two-tailed unpaired t test. The first two columns refer to controls 1 and 2, and the last two columns refer to affected individuals F4:II.1 and F4:II.4.

SQSTM1 variant co-segregated with the phenotype in line with autosomal-recessive inheritance.

Biallelic loss-of-function *SQSTM1* variants were absent from 7,000 in-house exomes (Munich) of individuals with unrelated phenotypes and no such homozygous variants were observed in ~120,000 alleles of the ExAC Server (09/2015). Western blot studies in primary fibroblasts available from individuals F1:II.6, F3.II.1, F4:II.1, and F4:II.4 showed absence of the SQSTM1/p62 protein accompanied by a severe reduction in *SQSTM1* mRNA steady-state levels in fibroblasts from individuals F4:II.1 and F4:II.4 (Figure 3). In summary, the identification of three different biallelic *SQSTM1* loss-of-function variants in four unrelated families with a similar clinical phenotype establishes *SQSTM1* as a gene confidently implicated in this neurodegenerative disease. The identified variants c.286C>T (p.Arg96*) and c.311_312del (p.Glu104Valfs*48) affect all three predicted *SQSTM1* isoforms. In contrast, the variant c.2T>A (p.?) affects only the start codon of one out of three predicted isoforms, and while arguing for the importance of this isoform in the pathogenesis of the disease, it raises the possibility of residual mRNA/protein amount. RNA sequencing in fibroblasts and whole blood suggested partial expression of the two other isoforms (Figure S2). However, the antibody directed against the C terminus of the SQSTM1/p62 protein common to all three isoforms failed to provide evidence of any translated *SQSTM1* gene products, at least in fibroblasts.

The mutation c.286C>T has been detected in families F3 and F4. In order to test for the possibility of a shared ancestral change, we compared the variation observed in the ~2 Mb region surrounding the variant. Identified rare (MAF < 1% in public databases) variants were not shared between subjects of families 3 and 4 carrying the c.286C>T variant, indicating that this is unlikely a single ancestral variant but arose independently in the two families, in line with the reportedly Finnish and Kurdish descent of families 3 and 4, respectively.

The scaffold protein SQSTM1/p62 is highly conserved among metazoans and was originally identified as a signal adaptor for the atypical protein kinases C sub-family (aPKCs).[12,13] SQSTM1/p62 serves as a signaling hub in a variety of key cellular processes such as cell differentiation, cell growth, osteoclastogenesis, tumorigenesis, amino acid sensing, and oxidative stress response.[14,15] Its interactions with binding partners are mediated by multiple domains (Figure 1). SQSTM1/p62 is found in different compartments of the cell including the cytoplasm, nucleus, autophagosomes, and lysosomes. Upon stress induction, SQSTM1/p62 is activated to enable selective autophagy, e.g., of cells infected by bacteria, protein aggregates, and damaged mitochondria.[16–20] Earlier studies had suggested that SQSTM1/p62 is responsible for the PARKIN-mediated direction of ubiquitinated mitochondria to the autophagosome.[17] However, growing evidence indicates that SQSTM1/p62 is not essential for mitophagy itself[3,18] but

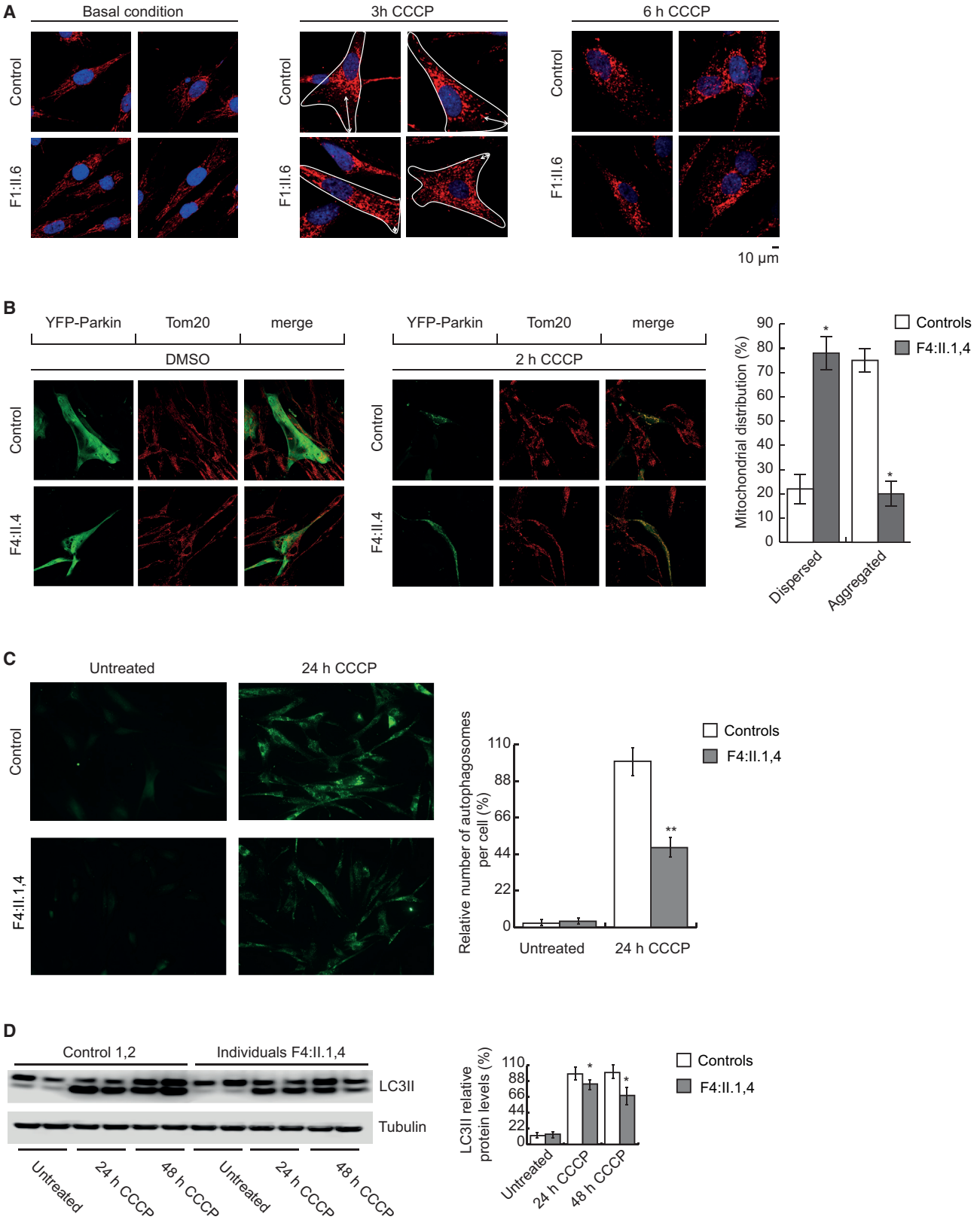


Figure 4. Investigation of Aggregation of Depolarized Mitochondria and Autophagosomal Formation
(A) Naive (no exogenous PARKIN) control and SQSTM1/p62 variant fibroblasts were treated with 20 μM CCCP for 3 and 6 hr and fixed with 4% PFA. Mitochondria were immunostained with the mitochondrial SSBP and detected with a fluorescent-labeled secondary antibody (AF568, red), nuclei were stained with DAPI (blue), and images were acquired by confocal microscopy. Depolarization of mitochondria using the protonophore CCCP led to the collapse of the mitochondrial network in control and in SQSTM1/p62 variant cells.

(legend continued on next page)

seems to be indispensable for perinuclear clustering of depolarized mitochondria in a process of self-oligomerization.[3,18,19] Furthermore, TBK1-dependent phosphorylation of SQSTM1/p62 at Ser403 has been shown to promote the efficacy of autophagosomal engulfment of ubiquitinated mitochondria at an early phase of mitochondrial depolarization.[21] We therefore studied perinuclear clustering and clearance of mitochondria in naive as well as GFP-PARKIN- and YFP-PARKIN-overexpressing control and SQSTM1/p62 variant fibroblasts after depolarization of mitochondria with the protonophore carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP), the established tool to study PARKIN-dependent mitophagy in cultured cells. In naive SQSTM1/p62 variant cell lines, reduced perinuclear clustering of mitochondria was detected after 3 hr CCCP treatment compared to control cells (Figure 4A). In PARKIN-overexpressing cells (transduced with GFP-PARKIN or transfected with YFP-PARKIN), depolarization of mitochondria and subsequent collapse of the mitochondrial network led to a translocation of PARKIN to the mitochondria as indicated by co-localization with mtSSBP (Figure S3) and TOM20 (Figure 4B), replicating results of a wide literature in the field.[3,18] After 2 hr of CCCP treatment, we found a clear mitochondrial clustering in control YFP-PARKIN-overexpressing cells but not in SQSTM1/p62 variant cells (Figure 4B). No perinuclear clustering of mitochondria was observed after 2 hr treatment of oligomycin and antimycin (Figure S4). Differences in perinuclear clustering were less obvious between stably overexpressing GFP-PARKIN control and SQSTM1/p62 variant fibroblasts after 3 hr of CCCP treatment (Figure S3). We observed no difference in the overall clearance of mitochondria after 24 hr CCCP treatment in all tested conditions, in line with SQSTM1/p62 being dispensable for the PARKIN-dependent disposal of depolarized mitochondria in fibroblasts (Figures S3 and S4). In agreement, we observed no difference in mitochondrial clearance in PARKIN-overexpressing cells, treated with the respiratory chain inhibitors oligomycin and antimycin after 24 hr (Figure S4) in these cells. We next investigated the autophagic flux in SQSTM1/p62-deficient and control cell lines by monitoring changes in the levels of LC3I/II upon starvation with and without addition of the lysosomal inhibitors bafilomycin or NH_4Cl. Concordant with a functional redundancy of involved autophagy adaptors, we observed no obvious differences between control and SQSTM1/p62 variant cell lines in these assays (Figures S5 and S6). Colocalization of the autophagosomal marker LC3II and YFP-PARKIN was not affected upon CCCP treatment (Figure S7). However, depolarization of mitochondria with CCCP did result in a reduced autophagosome formation in naive SQSTM1/p62-deficient cells (Figures 4C and 4D), whereas after treatment with oligomycin-antimycin, no differences were observed between SQSTM1/p62 variant and control cell lines (Figure S8). These observations suggest that in dividing cells, SQSTM1/p62 contributes to the early regulation of mitophagy including perinuclear clustering of mitochondria and autophagosome formation upon depolarization, but that it is redundant for mitochondrial removal and autophagic flux.

In mice, the knock out of *Sqstm1* leads to mature-onset obesity, leptine resistance, and impaired glucose tolerance and insulin resistance potentially mediated by disturbed regulation of adipocyte differentiation due to enhanced basal ERK activity.[22] Furthermore, the chronic absence of Sqstm1/p62 promotes mature-onset neurodegeneration with accumulation of hyperphosphorylated tau and neurofibrillary tangles in hippocampal and cortical neurons manifesting with increased anxiety, depression, and loss of short-term memory.[23] In agreement, individuals deficient of SQSTM1/p62 did present with severely increased tau and phospho-tau levels in CSF (see F4:II.1,4 clinical description in the Supplemental Data).

To our knowledge, biallelic *SQSTM1* loss-of-function mutations have not yet been reported in humans. The present study establishes absence of SQSTM1/p62 as a molecular

After 3 hr of CCCP treatment, perinuclear clustering of mitochondria can be observed in control cells, which is less evident in SQSTM1/p62 variant fibroblasts (arrows indicate distance from outer cell membrane to clustered mitochondria). No obvious differences between cell lines were observed after 6 hr of treatment.

(B) Control and SQSTM1/p62 variant fibroblasts were transfected with YFP-Parkin (Addgene #23955),[28] treated with CCCP (20 μM) for 2 hr, followed by 4% PFA fixation. Mitochondria were immunostained using antibodies against the mitochondrial protein TOM20 (Santa Cruz cat# sc-11414, RRID: AB_793274) and detected with a fluorescent-labeled secondary antibody (AF568, red). Images were acquired by confocal microscopy. Depolarization of mitochondria using the protonophore CCCP led to the collapse of the mitochondrial network in control and in SQSTM1/p62 variant cells. After 2 hr, mitochondrial clustering can be observed in control cells, with reduced clustering in SQSTM1/p62-deficient cells. Right panel: quantification of percent of cells showing a dispersed or aggregated mitochondrial distribution after 2 hr of CCCP treatment. n = 3, 25 cells per experiment. Morphology classification was done according to previous reports studying the function of p62/SQSTM1 in mitophagy.

(C) SQSTM1/p62 variant and control cell lines were treated for 24 hr with the protonophore CCCP (20 μM) and fixed with 4% PFA. Autophagosomes were immunostained, using antibodies against the autophagosomal protein LC3II (MBL International cat# M152-3, RRID: AB_1279144) and detected with a fluorescent-labeled secondary antibody (AF488, green). Images were acquired by fluorescence microscopy and autophagosomes were counted manually. Right panel: quantification of the relative number of autophagosomes in the cell. Treatment with CCCP resulted in reduced autophagosome formation in SQSTM1-deficient cells. n = 3, 150 cells per experiment.

(D) SQSTM1/p62 variant and control cell lines were treated for 24 and 48 hr with the protonophore CCCP (20 μM). Treatment with CCCP resulted in reduced LC3II levels formation in SQSTM1/p62-deficient cells. Right panel: quantification of the relative LC3II protein levels using ImageJ software.

In (B)–(D), *$p \leq 0.05$; **$p \leq 0.01$; two-tailed unpaired t test. Error bars indicate ± SEM from the mean of three replicates.

defect underlying a childhood- or adolescence-onset neurodegenerative disorder. The pivotal features of the affected individuals' phenotypes were gait abnormalities (9/9), ataxia mostly of the upper limbs (9/9), dysarthria (9/9), dystonia (7/9), vertical gaze palsy (7/9), and mild cognitive decline (7/9). The course was remarkably similar in all nine affected individuals with onset between age 7 to 15 years and relatively slow progression. Brain MRI showed cerebellar atrophy in four out of eight individuals and signal abnormalities in basal ganglia with iron accumulation in two out of eight individuals. Of note, extensive brain iron deposition in the basal ganglia and substantia nigra is a characteristic phenotypic feature of a clinically and genetically heterogeneous group of neurodegenerative disorders subsumed under the umbrella term NBIA (neurodegeneration with brain iron accumulation).[24] Although the exact pathomechanisms leading to neurodegeneration is unknown in most of the major NBIA forms, the identification of X chromosome *WDR45* (MIM: 300526) mutations in individuals with BPAN (NBIA 5 [MIM: 300894]) suggested defective autophagy as a key pathomechanism.[25] Screening of a large cohort of 250 idiopathic NBIA individuals for *SQSTM1* variants failed to detect clinically relevant biallelic variants, indicating that brain iron accumulation is probably an inconsistent feature in SQSTM1-associated neurodegeneration. Of note, also in the PLA2G6 (MIM: 603604)-associated NBIA subtype PLAN (MIM: 610217), brain iron accumulation may be absent or subtle early in the disease course.[24]

Given the role of SQSTM1/p62 as a key player in a variety of vital cellular processes, it was unexpected that its absence is compatible with survival above age 40 years and that affected individuals display such a circumscribed neurological phenotype. This observation together with the findings in mice argues for a redundancy of involved factors and pathways or effective compensatory mechanisms to maintain cellular homeostasis. Our data indicate that SQSTM1/p62 functions are of particular importance in the brain, where the postmitotic nature of neurons poses especial challenges to the removing of damaged organelles and misfolded proteins.

In humans, heterozygous missense and truncating *SQSTM1* variants have been associated with the progressive skeletal disorder Paget disease of the bone (PDB), the neurodegenerative disorders ALS and FTD,[4] and recently distal myopathy with rimmed vacuoles.[5] Furthermore, 5q copy number gains comprising *SQSTM1* have been associated with kidney cancer.[26] Although several disease alleles are common to both PDB and ALS/FTD, others have been proposed to be ALS/FTD specific.[4] Surprisingly, heterozygous carriers of *SQSTM1* variants in our families showed no skeletal defects or neurological disease. In keeping with this, a recent large exome-sequencing study of 2,869 ALS-affected case subjects and 6,405 control subjects, which confidently implicated *TBK1* (MIM: 604834) and *OPTN* (MIM: 602432) in ALS, failed to establish a significant enrichment of rare *SQSTM1* variants relative to control subjects.[27] Therefore, despite *SQSTM1* being a promising ALS candidate gene from a cell biological perspective, additional statistical and mechanistic evidence is needed for the implication of hypomorphic alleles, putatively dominant-acting variants, or *SQSTM1* haploinsufficiency in neurodegeneration. The puzzling variety of diseases and inheritance patterns associated with *SQSTM1* variants suggests complex genotype/phenotype relationships and points to the possibility of currently unidentified modifying genes and gene-environment interactions.

Supplemental Data

Supplemental Data include clinical descriptions, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.06.026.

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Web Resources

CADD, http://cadd.gs.washington.edu/
ExAC Browser, http://exac.broadinstitute.org/

OMIM, http://www.omim.org/
RRID, https://scicrunch.org/resources

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