OPTN 691_692insAG is a founder mutation causing recessive ALS and increased risk in heterozygotes

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ABSTRACT

Objective: To detect genetic variants underlying familial and sporadic amyotrophic lateral sclerosis (ALS).

Methods: We analyzed 2 founder Jewish populations of Moroccan and Ashkenazi origins and ethnic matched controls. Exome sequencing of 2 sisters with ALS from Morocco was followed by genotyping the identified causative null mutation in 379 unrelated patients with ALS and 1,000 controls. The shared risk haplotype was characterized using whole-genome single nucleotide polymorphism array.

Results: We identified 5 unrelated patients with ALS homozygous for the null 691_692insAG mutation in the optineurin gene (*OPTN*), accounting for 5.8% of ALS of Moroccan origin and 0.3% of Ashkenazi. We also identified a high frequency of heterozygous carriers among patients with ALS, 8.7% and 2.9%, respectively, compared to 0.75% and 1.0% in controls. The risk of carriers for ALS was significantly increased, with odds ratio of 13.46 and 2.97 in Moroccan and Ashkenazi Jews, respectively. We determined that 691_692insAG is a founder mutation in the tested populations with a minimal risk haplotype of 58.5 Kb, encompassing the entire *OPTN* gene.

Conclusions: Our data show that *OPTN* 691_692insAG mutation is a founder mutation in Moroccan and Ashkenazi Jews. This mutation causes autosomal recessive ALS and significantly increases the risk to develop the disease in heterozygous carriers, suggesting both a recessive mode of inheritance and a dominant with incomplete penetrance. These data emphasize the important role of *OPTN* in ALS pathogenesis, and demonstrate the complex genetics of ALS, as the same mutation leads to different phenotypes and appears in 2 patterns of inheritance.

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GLOSSARY

AAO = age at onset; **AJ** = Ashkenazi Jewish; **ALS** = amyotrophic lateral sclerosis; **AR** = autosomal recessive; **CI** = confidence interval; **fALS** = familial amyotrophic lateral sclerosis; **FTD** = frontotemporal dementia; **IBD** = identical by descent; **MJ** = Moroccan Jewish; **OR** = odds ratio; **PCA** = principal component analysis; **sALS** = sporadic amyotrophic lateral sclerosis; **SNP** = single nucleotide polymorphism.

Amyotrophic lateral sclerosis (ALS) is a complex and heterogeneous neurodegenerative disease, characterized by loss of upper and lower motor neurons, affecting individuals worldwide, with a global incidence of 2–3 people per 100,000 per year.¹ The disease leads to respiratory failure and death within 1–5 years after symptom onset. Up to 50% of patients may also have symptoms of cognitive impairment, and about 13% develop symptoms of frontotemporal dementia (FTD).^{2,3}

In the last decade, there has been an exponential increase in the discovery of genes and mutations associated with familial ALS (fALS) and sporadic ALS (sALS), some with biological function evidence for causality. Mutations in *C9ORF72*, *SOD1*, *TDP-43*, and *FUS* account for the majority of fALS, and although the etiology of most sALS is unknown, about 10% are caused by mutations in genes associated with ALS.⁴

Jews of Ashkenazi and North Africa origins are considered to be genetically homogeneous subgroups due to religious and cultural customs that maintained them as isolated communities.^{5,6}

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Supplemental data
at Neurology.org

Figure The 691_692insAG mutation in exon 6 of *OPTN* in patients with amyotrophic lateral sclerosis (ALS)

(A) A family of Moroccan origin segregating the ALS disease. The father died at age 36 years, probably from ALS. Three sisters, II-1, II-2, and II-3, dark gray circles, had ALS. Sister II-4, light gray circle, developed rapidly progressive presenile dementia with late signs of motor neuron damage; sister II-5, white circle, is healthy. Line across symbols = deceased. (B)

As such, with reduced heterogeneity and large blocks of linkage disequilibrium, they are considered an important model for identifying disease-causing mutations of simple and complex traits and diseases.⁷ ALS is observed in these populations apparently with a similar incidence as in the worldwide population, and few cases can be explained at present by known genes.^{8–10}

We report a nonsense 691_692insAG mutation in optineurin (*OPTN*) detected by exome next-generation sequencing in a homozygous state in 2 sisters of Moroccan Jewish descent, followed by genotyping the mutation in hundreds of patients with ALS and ethnically matched controls.

METHODS Massive parallel sequencing and data analysis. Exome libraries were prepared for DNA samples extracted from peripheral blood of 2 sisters with ALS (figure, A, II-1 and II-2) using Illumina Nextera Rapid Capture Exome kit following manufacture protocol, and sequenced on the Illumina NextSeq500 sequencing platform (Illumina Inc., San Diego, CA), at The Center for Genomic Technologies, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel. For the quantification and validation of the genomic library, the Qubit 2.0 Fluorometer system (Life Technologies, Carlsbad, CA) and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) were used.

The NextSeq500 system generated .bcl files, followed by performance of demultiplexing of indexed reads and generation of FASTQ files. The sequences were aligned to the hg19 reference genome with BWA version 0.7.10,¹¹ and applied Picard tools version 1.126 (<http://picard.sourceforge.net>) for SAM/BAM file conversion, indexing, and marking duplicates. We used GATK version 3.2¹² base quality score recalibration and indel realignment, and performed single nucleotide polymorphism (SNP) and indel discovery and genotyping across the 2 samples simultaneously using standard hard filtering parameters and variant quality score recalibration according to GATK Best Practices recommendations.^{13,14} Variants analysis and filtering was done using SNP and Variation Suite v8.2 (Golden Helix, Inc.,

Sanger sequencing chromatograms: II-1, II-2, and II-4 are homozygous to the AG insertion, II-5 is heterozygous, as presented in overlapping chromatogram peaks. The AG insertion is marked in the box. Cntrl = control. (C) Fragment analysis of the 4 sisters. II-1, II-2, and II-4 have a single homozygous peak, the mutant allele at 210 bp. II-5 is heterozygous with 2 peaks presented at 208 and 210 bp. Cntrl with a single homozygous peak at 208 bp. (D) Schematic presentation of *OPTN* protein, its domains, and regions of interactions. CC = coiled-coil domains; Htt = huntingtin; LZ = Leucine zipper domain; UBAN = ubiquitin binding in ABIN and NEMO domain; Z = zinc finger motif. At the bottom, the effect of the AG insertional mutation on the mRNA open reading frame and protein translation is presented. The AG insertion at codon 127 changes 21 amino acids before introducing a premature stop codon.

Bozeman, MT, www.goldenhelix.com). We filtered out all variants with quality score less than 30, variants with read depth less than 10, and heterozygous variant calls with alternate to wild-type allele ratio less than 15%. We used 5 different functional prediction algorithms that determine if exonic variant is deleterious (SIFT, PolyPhen2, Mutation tester, Mutation Assessor, FATHMM). Variants that had less than 4 algorithms with a deleterious score, according to dbNSFP NS functional prediction 2.9, were filtered out. We then filtered out variants with frequency higher than 1% (based on 1000 Genomes, phase 3). Finally, we removed synonymous variants, as well as variants located in intergenic regions or introns.

Population. The study included 379 unrelated Jewish patients with ALS followed at the ALS Clinic at Tel Aviv Medical Center, Tel Aviv, Israel (table 1). All patients had a diagnosis of clinically definite or probable ALS according to the revised El Escorial criteria.¹⁵ The recruitment interval spanned 10.5 years, from July 2004 to December 2014. We excluded from this study patients with ALS who were identified in the past as carrying either *C9orf72* or *SOD1* mutation (6 unrelated Ashkenazi patients with ALS with *C9orf72* mutation and 2 unrelated Jews of Moroccan descent, one with *SOD1* mutation and one with *C9orf72* mutation).

All patients underwent an interview to disclose ancestry; family history of ALS, dementia, or other neurodegenerative diseases; age at onset (AAO), defined as the age when the first symptoms appeared; and affected site at disease onset, defined as the site where the first symptoms appeared (limb or bulbar). Disease duration, defined as time from first symptoms to death or tracheostomy, was recorded for all patients.

Sixty-nine patients (18.2%) were of North African/Moroccan origin (Moroccan Jewish [MJ]) on both parental sides and 310 (81.8%) were of Ashkenazi Jewish ancestry (AJ) on both parental sides (table 1). A total of 1,000 control samples were analyzed, including 750 anonymous DNA samples from young healthy individuals, aged 20–45 years, mostly women (400 MJ and 350 AJ), who underwent routine genetic screening tests and were randomly selected, and 250 healthy elderly AJ (age range 44–89 years; average age 67.9 ± 10.0 years; 151 men and 99 women).

Standard protocol approvals, registrations, and patient consents. All participants provided informed consent before entering the study. All DNA samples were coded and tested in an anonymous manner. The Institutional and National Supreme Helsinki Committees for Genetics Studies approved the study protocols and the informed consents.

Mutation screening. Genomic DNA was isolated from peripheral blood using standard protocols. To detect the 2 base insertion mutation, FAM-labeled primer pairs were used for fragment analysis on the 3130XL genetics analyzer to observe a 208-bp product for the wild-type allele and a 210-bp product for the mutated allele (forward primer labeled with FAM-5'-TGGTGCCAGCCTTAGTTTGAT-3' and reverse primer 5'-TGAGGAGCCGCTGGAGTTCA-3'). The same primers unlabeled were used for PCR amplification and Sanger sequencing to validate the next generation sequencing mutation results, as well as all samples found carrying the mutation by fragment analysis, using standard protocols (BigDye Terminator v3.1 Cycle Sequencing kit; Applied Biosystems, Foster City, CA).

Statistical analysis. Differences among groups in continuous variables were tested using analysis of variance; χ^2 or Fisher exact test were used for comparison of categorical variables. Goodness-of-fit test with one degree of freedom was applied to look for any deviation from the Hardy-Weinberg equilibrium in the 2 groups of controls (600 AJ and 400 MJ). Significance of allele frequencies was calculated with an online calculator (<http://graphpad.com/quickcalcs/contingency2/>) and http://www.medcalc.org/calc/odds_ratio.php to determine odds ratios (ORs). SPSS software V.15 (SPSS Inc., Chicago, IL) was used for all data analyses unless otherwise mentioned.

Genome-wide SNP genotyping and analysis. Fifty-seven unrelated individuals were genotyped on the HumanOmniExpress v1.1 BeadChip (>713,000 SNPs, Illumina) at Gene By Gene, Ltd., Houston, TX. These samples included 20 heterozygous for the *OPTN* 691_692insAG mutation (6 MJ-ALS, 8 AJ-ALS, 3 MJ controls, and 3 AJ controls), 5 with homozygous mutation (1 AJ-ALS and 4 MJ-ALS), and 32 individuals with homozygous wild-type allele (8 MJ-ALS, 8 AJ-ALS, 8 MJ

Table 1 Patients with amyotrophic lateral sclerosis (ALS) of Moroccan (MJ-ALS) and Ashkenazi (AJ-ALS) Jewish origin screened for the *OPTN* nonsense 691_692insAG mutation

	MJ-ALS	AJ-ALS	Total	p Value ^a
No. of patients (% of total)	69 (18.2)	310 (81.8)	379 (100)	
Age at onset, y, mean $\pm$ SD	54.2 $\pm$ 13.3	63.1 $\pm$ 11.4	59.5 $\pm$ 12.2	2.84 $\times$ 10 ^{-8b}
Male, n (%)	40 (58.0)	186 (60.0)	226 (59.6)	
Familial ALS, n (%)	5 (7.9) ^c	4 (1.3) ^d	9 (2.5)	0.01 ^b
Disease duration, mo, ^e mean $\pm$ SD, no. of patients	38.9 $\pm$ 28.2, 49	35.2 $\pm$ 25.7, 243	35.8 $\pm$ 26.1, 292	0.401
Site of onset, n (%)				
Bulbar	19 (27.5)	83 (26.8)	102 (26.9)	0.897
Limb	50 (72.5)	227 (73.2)	277 (73.1)	

^a Difference between MJ-ALS and AJ-ALS.

^b Significant.

^c Six patients had a questionable positive family history and were not included in the percentage calculation and analysis for association with familial ALS.

^d Eleven patients had a questionable positive family history and were not included in the percentage calculation and analysis for association with familial ALS.

^e Survival was calculated only for patients deceased.

controls, and 8 AJ controls). The analysis was done using SNP & Variation Suite v8.2 (Golden Helix, Inc.). Identical by descent (IBD) estimations were computed with SNP & Variation Suite v8.2 and presented as π , the overall fraction of alleles over the genome that are shared IBD between 2 individuals.

RESULTS Patients with ALS. The mean AAO was 59.5 years (SD 12.2 years, range 21–88 years), and was younger in MJ-ALS compared to AJ-ALS (54.2 and 63.1 years, respectively, t test, $p = 2.84 \times 10^{-8}$, table 1), confirming our previous data.¹⁶

fALS, defined as having at least one first- or second-degree relative with a diagnosis of ALS by accepted criteria,¹⁷ was noted in 2.5% of patients ($n = 9$), with more cases of fALS in MJ compared to AJ (7.9% and 1.3%, respectively, $p = 0.01$, table 1).

The mean disease duration was calculated for the deceased patients only and was 35.8 months (SD 26.1 months, range 5–251 months). The difference between the 2 ethnic groups (38.9 and 35.2 months in MJ and AJ, respectively) was not significant ($p = 0.401$, table 1). In most patients (73.1%, $n = 277$), the disease started in a limb, and the same distribution was observed in MJ and AJ ($p = 0.897$).

The Jewish family of Moroccan origin, which initiated this study, included 5 sisters, among whom 3 (figure, A, II-1, II-2, II-3) had classical definite ALS with predominant and early upper motor neuron involvement in the lower limbs that spread later to involve all body areas and the lower motor neurons, without cognitive involvement. The fourth sister (figure, A, II-4) developed rapidly progressive presenile dementia with late signs of motor neuron damage. Their AAO was 46, 43, 42, and 52 years (mean 45.7 years) and survival was 13, 47, 36, and 54 months (mean 37.5 months). The remaining fifth sister is healthy at age 70 years. The patients' father died at age 36 years and had an undiagnosed neurologic disease that might have been ALS.

Exome data analysis. Exome sequencing was performed in 2 sisters (figure, II-1 and II-2). Average error rate was 3.65. Variant calls from both were compared to each other, and genes that shared variants were crossed with the large public database of genes reported to be associated with ALS (Amyotrophic Lateral Sclerosis Online Genetics Database [6.0]; <http://alsod.iop.kcl.ac.uk/>). The best candidate as a causative mutation was the AG insertion in exon 6 of the optineurin gene (NM_001008211, *OPTN*691_692insAG, Chr10:13154465, reference genome UCSC hg19) in a homozygous state in both sisters (figure, B and C). DNA was not available for the third sister (figure, A II-3), but Sanger sequencing of the fourth affected sister and the unaffected sister identified this *OPTN* mutation in homozygous and heterozygous states, respectively (figure, A, II-4 and II-5, B, and C). This mutation changes the open reading frame of the protein at amino acid 127 and changes 21 amino acids before introducing a premature stop codon at codon 148 (figure, D). Aligning the variant calls of the 2 sisters along chromosome 10 identified a shared homozygous block of 4.14 Mb (Chr10:11,505,175–15,649,698).

The *OPTN* 691_692insAG mutation is strongly associated with ALS in the MJ population. All our unrelated MJ-ALS patients (69) were genotyped for this mutation. We detected 3 additional homozygous patients and 6 heterozygotes (5.8% and 8.7%, respectively, table 2). Among the control MJ samples, 3 were heterozygous (3/400, 0.75%) and none was homozygous. The association among the 3 genotypes and the disease was significant (Pearson $\chi^2 = 43.69$, $df = 2$, $p = 3.25 \times 10^{-10}$, table 2). There was also a significant association between *OPTN* 691_692insAG heterozygosity and ALS ($p = 3.6 \times 10^{-4}$), with ORs of 13.46 (95% confidence interval [CI] 3.28–55.27, $p = 0.0003$,

Table 2 Association between *OPTN* 691_692insAG genotypes and amyotrophic lateral sclerosis (ALS)

Study group/genotype	No. tested	% Homozygous MUT/MUT (n)	% Heterozygous WT/MUT (n)	% Homozygous WT/WT (n)	p Value (χ^2 , $df = 2$)
Patients with ALS					
Morocco	69	5.8 (4)	8.7 (6)	85.5 (59)	3.25×10^{-10} (43.69)
Ashkenazi	310	0.3 (1)	2.9 (9)	96.8 (300)	0.038 (6.53)
Total	379	1.3 (5)	4.0 (15)	94.7 (359)	6.490×10^{-7} (28.50)
Controls^a					
Morocco	400	0	0.75 (3)	99.2 (397)	
Ashkenazi	600	0	1.0 (6)	99.0 (594)	
Total	1,000	0	0.9 (9)	99.1 (991)	

Abbreviations: MUT = mutant *OPTN* 691_692insAG allele; WT = wild-type allele.

^aControl groups were in Hardy-Weinberg equilibrium.

Table 3 Effect of heterozygous *OPTN* 691_692insAG mutation

Patients with ALS	p Value of association ^a	OR	95% CI of OR	p Value of OR
Morocco	3.6×10^{-4}	13.46	3.28–55.27	0.0003
Ashkenazi	0.032	2.97	1.05–8.42	0.0407
Total	8.9×10^{-5}	4.60	2.00–10.61	0.0003

Abbreviations: ALS = amyotrophic lateral sclerosis; CI = confidence interval; OR = odds ratio.

^a Fisher exact test for Morocco and Pearson χ^2 test for Ashkenazi and total.

table 3), suggesting a significantly increased risk for ALS in heterozygous MJ. At the population level, the frequency of the mutant allele was 0.00375, significantly associated with disease risk (allelic OR 30.0, 95% CI 8.5–105.9, $p < 0.0001$).

***OPTN* 691_692insAG is associated with ALS also in the AJ population.** We further studied the possibility that this mutation is associated with ALS not only in the MJ discovery cohort, but also in an additional confirmatory cohort of patients. For this purpose, we genotyped 310 unrelated AJ-ALS patients and 600 AJ controls. One homozygous *OPTN* 691_692insAG patient with ALS was identified (0.3%) and 9 heterozygous (2.9%), all non-fALS (table 2). Six of the 600 AJ controls were heterozygous (1%) and none was homozygous to the mutation. A significant association was observed among the 3 genotypes and ALS (Pearson $\chi^2 = 6.53$, $df = 2$, $p = 0.038$, table 2).

As in the MJ population, there was a significant association between heterozygosity for *OPTN* 691_692insAG and ALS in AJ ($p = 0.032$), with OR of 2.97 (95% CI 1.05–8.42, $p = 0.0407$, table 3), suggesting a significantly increased risk for ALS in heterozygous AJ as well. At the population level, the frequency of the mutant allele was 0.005, significantly associated with disease risk (allelic OR 3.6, 95% CI 1.3–9.8, $p = 0.0121$).

Genotype/phenotype correlations: *OPTN* 691_692insAG is associated only with fALS. We did not find any effect of the 3 genotypes on AAO in the MJ or the AJ groups ($p = 0.175$ and $p = 0.373$, respectively), on disease duration ($p = 0.561$ and $p = 0.857$, respectively), or on the site of onset ($p = 0.537$ and $p = 0.145$, respectively). The *OPTN* mutation was significantly associated with fALS in the MJ-ALS population (Pearson $\chi^2 = 16.112$, $df = 2$, $p = 3.17 \times 10^{-4}$), but not in AJ-ALS.

***OPTN* 691_692insAG is a founder mutation.** Whole-genome SNP genotyping ($>713,000$ SNPs) in 57 unrelated MJ and AJ individuals, followed by a principal component analysis (PCA), fully divided them into 2 distinct groups based on ancestry, Moroccan or Ashkenazi, confirming their separate

origins. No other separation or any stratification was observed within each group by the PCA. Since the proportion of heterozygous and homozygous patients with the *OPTN* mutation was much higher in the MJ-ALS group than in AJ-ALS, we tested the possibility that the MJ-ALS group is enriched by hidden relatedness. For this purpose, all pairwise IBD estimates (π) were determined using the genome-wide SNP genotype data (table e-1 on the *Neurology*[®] Web site at Neurology.org for MJ and table e-2 for AJ). The mean π of all individuals in the MJ and AJ populations was 0.24 ($SD \pm 3.79 \times 10^{-5}$) and 0.27 ($SD \pm 2.85 \times 10^{-5}$), respectively, with maximum estimation of IBD under 0.3 (0.257 in MJ and 0.282 in AJ), implying that there is no hidden relatedness in both populations.¹⁸ We also examined whether the estimated IBD was different between the groups of MJ: carriers of 1 or 2 copies of mutation and noncarriers. The mean pairwise estimations were 0.243 and 0.244, respectively (t test, $p = 0.392$, range 0.228–0.257 for both), further excluding the possibility of higher relatedness among MJ carriers of *OPTN*691_692insAG.

We then aligned the genotype calls of chromosome 10 for all individuals (table e-3). In the MJ group, out of all 13 unrelated individuals with 1 or 2 copies of the mutation, 12 shared a risk haplotype of 363 Kb (Chr10:12,861,945–13,225,119; rs1630635 to rs11258235). One control heterozygous MJ reduced the shared interval to 58.5 Kb, composed of 15 informative SNPs (Chr10:13,141,144–13,199,611; rs3829923 to rs7899305). This region encompasses the entire *OPTN* gene. In the AJ group, 9 out of all 12 unrelated individuals carrying the mutation (homozygous and heterozygous) shared a risk haplotype of 160Kb (Chr10:13,039,484–13,199,611; rs7074830 to rs7899305). This AJ haplotype was identical to the MJ haplotype. Three additional AJ-ALS heterozygotes reduced the risk interval to a 63.78-Kb segment (Chr10:13,135,831–13,199,661; rs1953314 to rs7899305). This minimal risk haplotype in AJ included the complete 58.5 Kb minimal risk haplotype of MJ, suggesting that this is a founder mutation.

DISCUSSION Important discoveries in recent years have broadened our understanding of the complexity of ALS. Although the main characteristic of the disease is motor neuron degeneration, it is now clear that patients with ALS may exhibit additional phenotypes, such as FTD, parkinsonism, ataxia, dystonia, and more.¹⁹ Moreover, genes that were identified as causal of ALS were found to also cause other diseases, a pleiotropy that adds to the intricacy of diagnosis and therapeutic interventions.

Studying complex diseases in isolated, relatively homogeneous populations simplifies the complexity,

as it reduces the genetic variations and increases linkage disequilibrium blocks. Rare variants with low effect become less rare, with an increased effect on the phenotype, and significance is achieved in a reduced study cohort size. In ALS, several isolated populations have shown high incidence of rare mutations, such as the mutation in the *TARDBP* gene in Sardinia,²⁰ and *C9orf72* mutation in European cohorts.²¹ We had previously shown the power of studying Jewish populations, such as AJ, in identifying variants with high effect in another neurodegenerative disease: Parkinson disease.²² Here we present a high frequency of *OPTN* 691_692insAG founder mutation in patients with ALS of MJ origin (14.5%), our discovery cohort, and in AJ (3.2%), our confirmatory group.

Mutations in *OPTN* in patients with ALS are rare in other populations and so far account for few reported cases. Initially, mutations in *OPTN* were described as the cause of autosomal recessive (AR) ALS in 3 Japanese families.²³ The 691_692insAG mutation was identified until now in only one patient with ALS in a heterozygous state, but its association with ALS was not established.²⁴ Since then, other heterozygous mutations were described^{25–32}; however, no defined association or family segregation was reported for these potential dominant mutations. A recent study suggested rare damaging variants in *OPTN* by using a dominant model, which were accounted for in only 0.62% of patients with ALS (and 0.23% in controls).³³ Another study suggested an additional more complex mode of inheritance for *OPTN* in neurodegenerative disease: a compound heterozygosity either within *OPTN* or together with the *TBK1* gene.³⁴ We showed for the first time that *OPTN* 691_692insAG mutation is associated with AR ALS. In addition, the remarkable differences in frequencies of mutation carriage between MJ-ALS and MJ controls, and the replication of these results in AJ, with significantly increased risk for ALS in heterozygous carriers, strongly suggest that this mutation also has a dominant effect on ALS in the same populations.

The data presented here, together with earlier reports, suggest an important role for *OPTN* in ALS pathogenesis and neurodegeneration. *OPTN* is involved in multiple cellular functions, including maintenance of the Golgi complex, protein trafficking, and exocytosis.³⁵ It recognizes various protein aggregates, and its depletion significantly increases protein aggregation.³⁶ Suppression of *OPTN* causes neuronal cell death and serves as an autophagy receptor for damaged mitochondria.^{37,38} Its presence in inclusions in sALS, as well as in other neurodegenerative diseases, suggests its involvement in a variety of neurodegenerative processes or a common shared pathway.

One sister in our index family exhibited a different course of disease development than her 2 sisters, with rapid progressive presenile dementia and late signs of

motor neuron damage. Of interest, a haploinsufficiency of *TBK1*, which is known to bind to and phosphorylate *OPTN*, was recently found to cause familial ALS and FTD,³⁹ suggesting *TBK1* as a candidate gene in similar cases.

Interestingly, the calculated OR in the MJ population (13.46) was much higher compared to AJ (2.97). Since we ruled out a potential influence of hidden relatedness on these results, it is reasonable to speculate that other variables, including genetic modifiers, can affect the magnitude of risk in heterozygous individuals. It is possible that MJ harbor additional genetic variants that increase the chance of heterozygous carriers to develop the disease. On the other hand, it is possible that AJ harbor protective alleles that decrease the risk of *OPTN* mutation carriers to develop ALS. Therefore, further genomic analysis is necessary to explore the mechanisms that affect penetrance in *OPTN*-ALS.

AUTHOR CONTRIBUTIONS

Dr. Orly Goldstein: design and conceptualization of the study, study coordination, acquisition of data, analysis and interpretation of the data, statistical analysis, drafting the manuscript, and revising the manuscript for intellectual content. Omri Nayshool: acquisition of the data and revising the manuscript for intellectual content. Dr. Beatrice Nefussy: study coordination, acquisition of the data, and revising the manuscript for intellectual content. Dr. Bryan J. Traynor: revising the manuscript for intellectual content. Dr. Alan E. Renton: revising the manuscript for intellectual content. Dr. Mali Gana-Weisz: study coordination, acquisition of the data, and revising the manuscript for intellectual content. Dr. Vivian E. Drory: design and conceptualization of the study, study coordination, acquisition of data, supervision of the study, obtaining funding, and revising the manuscript for intellectual content. Dr. Orr-Urtreger: design and conceptualization of the study, study coordination, analysis and interpretation of the data, drafting the manuscript, supervision of the study, obtaining funding, and revising the manuscript for intellectual content.

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This Week's *Neurology*[®] Podcast

Orthostatic tremor: Clinical, electrophysiologic, and treatment findings in 184 patients (see p. 458)

This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the February 2, 2016, issue of *Neurology*. In the second segment, Dr. Jeff Ratliff talks with Dr. Anhar Hassan about her paper on the clinical, electrophysiologic, and treatment findings in 184 patients with orthostatic tremor. Dr. Robert Gross interviews Dr. Gretchen Birbeck on being the section editor and curator of our new global neurology site, *Without Borders*, in our “What’s Trending” feature of the week. In the next part of the podcast, Dr. Ted Burns focuses his interview with Dr. James Grotta on a *Neurology Today*[®] story about the SPRINT trial and lowering blood pressure and stroke.

Disclosures can be found at Neurology.org.

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