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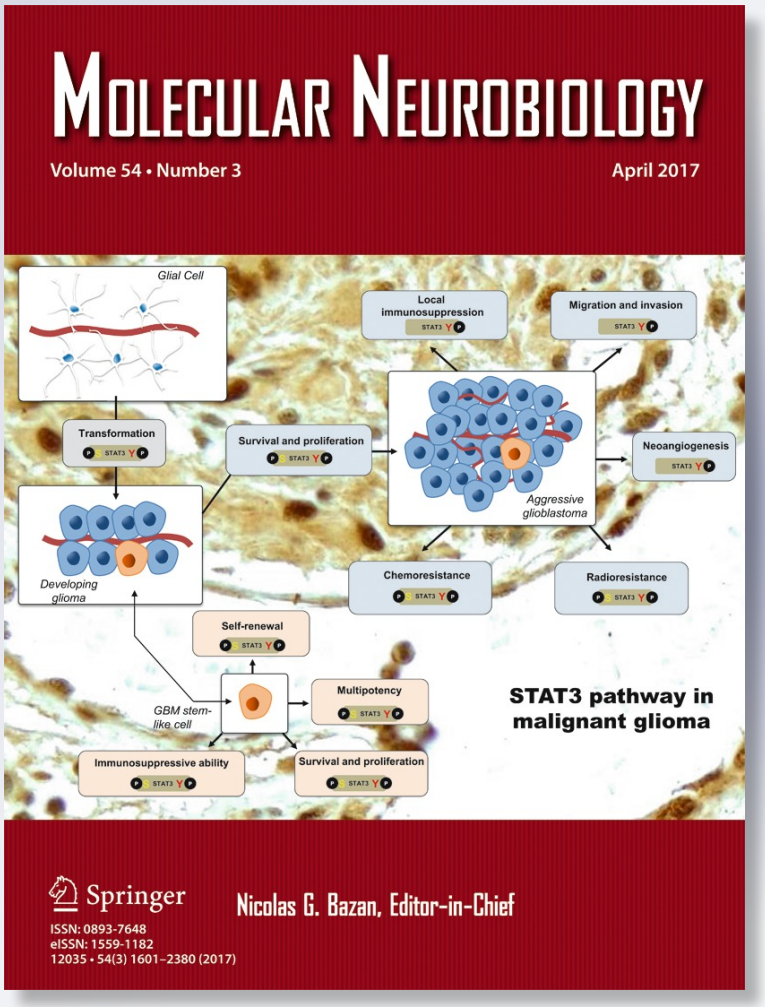

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The *OPA1* Gene Mutations Are Frequent in Han Chinese Patients with Suspected Optic Neuropathy

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Abstract While many patients with hereditary optic neuropathies are caused by mitochondrial DNA (mtDNA) mutations of Leber's hereditary optic neuropathy (LHON), a significant proportion of them does not have mtDNA mutation and is caused by mutations in genes of the nuclear genome. In this study, we investigated whether the *OPA1* gene, which is a pathogenic gene for autosomal dominant optic atrophy (ADOA), is frequently mutated in these patients. We sequenced all 29 exons of the *OPA1* gene in 105 Han Chinese patients with suspected LHON. mtDNA copy number was quantified in blood samples from patients with and without *OPA1* mutation and compared to healthy controls. In silico program-affiliated prediction, evolutionary conservation analysis, and in vitro cellular assays were performed to show the potential pathogenicity of the mutations. We identified nine *OPA1* mutations in eight patients; six of them are located in exons and three are located in splicing sites. Mutation c.1172T>G has not been reported before. When we combined our data with 193 reported Han Chinese patients with optic neuropathy and compared to the available data of 4327 East Asians by the Exome Aggregation Consortium (ExAC), we found a significant enrichment of potentially pathogenic *OPA1* mutations in Chinese patients. Cellular assays for OPA1 mutants c.869G>A and c.2708_2711del showed abnormalities in OPA1 isoforms, mitochondrial morphology, and cellular reactive oxygen species (ROS) level. Our results indicated that screening *OPA1* mutation is needed for clinical diagnosis of patients with suspected optic neuropathy.

Keywords *OPA1* · Suspected optic neuropathy · Pathogenicity · Mitochondrial dysfunction · Han Chinese

Introduction

Hereditary optic neuropathy is one of the most common genetic diseases, with typical symptom of progressive or acute loss of vision [1]. Primary mitochondrial DNA (mtDNA) mutations (for Leber hereditary optic neuropathy (LHON), OMIM535000) and the *OPA* gene mutations (for autosomal dominant optic atrophy (ADOA), OMIM165500) are two main causes for this disease. There are many patients suspected with optic neuropathy but without any known LHON primary mtDNA mutations. To characterize the pathogenic mutation and optimize a molecular screening method is very important for proper diagnosis of these patients.

The *OPA1* gene is located in chromosome 3q28-29 and encodes a dynamin-related GTPase protein, which localizes to mitochondrial inner membrane and inter-membrane space [2]. The OPA1 is critical for mitochondrial function and morphology maintenance. First, OPA1 is a fusion protein that balances the fusion/fission process of mitochondrial inner

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membrane. OPA1 mutations could induce an overflow of cytochrome c from mitochondria and stimulate cellular apoptosis [3, 4]. Second, OPA1 is an essential component of mitochondrial cristae and inner membrane. Abnormal OPA1 protein impairs cellular function and causes mitochondrial respiratory chain dysfunction [4]. Finally, OPA1 protein regulates mitochondrial calcium (Ca^{2+}) homeostasis and signaling, and mitochondrial Ca^{2+} retention capacity decreases in OPA1 knockdown cells [5].

The *OPA1* gene was expressed in all tissues and had a high level in human retina and optic nerve [6]. Pathogenic OPA1 mutations could impair mitochondrial function in these tissues and caused a variety of mitochondrial disorders. The *OPA1* gene mutations occurred in more than 60 % of ADOA patients [1], and mutations of the *OPA3* gene could be also found in ADOA patients [7]. In this study, we screened all 29 exons of the *OPA1* gene for mutations in 105 Han Chinese patients with suspected optic neuropathy. We observed an enrichment of *OPA1* pathogenic mutations in these patients. Further functional assays showed that mutations c.869G > A and c.2708_2711del affected mitochondrial function.

Materials and Methods

Subjects

The patients were diagnosed as suspected optic neuropathy at the Pediatric and Genetic Clinic of the Eye Hospital, Zhongshan Ophthalmic Center, and/or local medical centers. All patients ($n = 105$) were sporadic cases without a family history of disease and were genetically unrelated. These patients had been analyzed for mtDNA sequence variations in our previous study [8] and were confirmed to have no LHON primary mtDNA mutations (m.11778G > A, m.14484T > C, m.3460G > A, and m.3635G > A).

Mutation Screening for the *OPA1* Gene

Genomic DNA was isolated from whole blood by using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instruction. A total of 31 pairs of primers were designed to amplify and sequence all exons (including the 3′-UTR region) and the exon and intron boundary regions of the *OPA1* gene (Table S1). In brief, PCR was performed in a total of 50-μL reaction volume containing 5-μL 10× PCR buffer (Mg^{2+} plus), 2.5 units of TaKaRa rTaq (TaKaRa Bio Inc., Dalian, China), 400 μM of each deoxynucleotide triphosphate (dNTP), 0.2 μM of each primer, and 50 ng DNA. We followed the standard nomenclature recommendations of the HGVS (http://www.HGVS.org/mutnomen/) to name the mutation(s), and mRNA sequence of OPA1 isoform 1 (NM_015560) was used as the reference sequence.

Quantification of mtDNA Copy Number

We quantified the mtDNA copy number in individuals of three groups (group no. 1: patient 1, suspected LHON patients with *OPA1* mutations, $n = 8$; group no. 2: patient 2, suspected LHON patients without *OPA1* mutations, $n = 12$; and group no. 3: control, healthy controls from the general populations reported in our previous study, $n = 17$ [9]) using the same method described in our previous study [10]. Real-time quantitative PCR with SYBR® Premix ExTaq TM II Kit (TaKaRa Biotechnology Co. Ltd., Dalian, China) was performed on the BioRad iQ5 Real-Time PCR System (Bio-Rad Laboratories Inc., USA). The single-copy nuclear β-globin gene was used as the reference gene. Primers and procedure were same to our previous study [10].

Plasmid Construction

Total RNA was extracted from whole blood of a healthy donor according to the manufacturer's instructions using the TRIzol Reagent (Invitrogen, USA). Around 1 μg RNA was used to prepare complementary DNA (cDNA) by using the Transcriptor First-Strand cDNA Synthesis Kit (Roche, USA). The coding region of the wild-type *OPA1* gene was amplified by primer pair OPA1-CDS-F (5′-CCGCTCGAGATGTGGCGACTACGTCGGG-3′)/OPA1-CDS-R (5′-CGCGGATCCTTTCTCCTGATGAAGAGCTT-3′), with cDNA as the template. The restriction endonuclease sites were designed in the 5′-end of each primer (*Xho* I site in OPA1-CDS-F and *Bam* HI site in OPA1-CDS-R are underlined). The amplicon was digested, purified, and cloned into plasmid pcDNA3.1. The flag tag was added to the C-terminal of the OPA1 protein for further detection. The OPA1 mutants identified in patients were generated by the site-directed mutagenesis PCR method. In brief, PCR was performed in a total of 50-μL reaction volume containing 5-μL 10× PCR buffer, 1 unit of KOD-Plus-Neo (TOYOBO, Japan), 1.5 mM Mg^{2+}, 200 μM of each dNTP, 0.3 μM of each primer (Table S2), and 50-ng plasmid DNA. The following PCR condition was used: 1 cycle of 94 °C for 1 min, 30 cycles of 94 °C for 30 s and 65 °C for 7 min, and one extension cycle of 72 °C for 10 min.

Cell Culture and Transfection

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 100 u/mL penicillin, 100 μg/mL streptomycin, and 10 % FBS in a 12-well plate at 37 °C with 5 % CO_2. A mixture of 2-μg plasmid DNA and 3 μL of FuGENE HD transfection reagent (Roche, USA)

was transfected when cells were grown to 80 % confluence. Cells without any transfection or cells transfected with empty pcDNA3.1 vector were used as the controls. Cells were harvested at 48 h after the transfection.

Western Blot and Confocal Fluorescence Microscopy

Western blot was performed to show the expression of the OPA1 mutants following a similar procedure as described in our recent study [11]. In brief, HeLa cells were collected and lysed in lysis buffer (Beyotime, China) to extract total protein. About 20 μg protein was electrophoresed in 12 % SDS polyacrylamide gel and was transferred to a polyvinylidene difluoride membrane (Bio-Rad, USA). The membrane was blocked in 5 % (*w*/*v*) nonfat milk for 2 h at room temperature, then incubated with monoclonal mouse anti-FLAG antibody (Abmart, China) (1:5000) overnight at 4 °C. After three washes with TBST (each wash 5 min), the membrane was incubated with anti-mouse IgG peroxidase-conjugated secondary antibody (KPL, Gaithersburg, MD, USA) (1:10,000) for 1 h at room temperature. Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA) was used to visualize the epitope.

Confocal fluorescence microscopy was used to investigate the localization of the OPA1 mutants and mitochondrial morphology. HeLa cells were co-transfected with pcDNA3.1-OPA1 fused with flag tag and DsRed-Mito. After transfection for 48 h, cells were fixed with paraformaldehyde (PFA; 4 % (*w*/*v*) in phosphate-buffered saline (PBS)) for 10 min and were permeabilized using 0.2 % (*v*/*v*) Triton X-100, followed by a block in 5 % (*w*/*v*) bovine serum albumin for 1 h at room temperature. Cells were incubated with primary antibody (anti-FLAG, Abmart, 1:5000) overnight at 4 °C, then with a FITC-conjugated secondary antibody (KPL, 1:50) for 1 h at room temperature after three washes with PBS. 4′,6-Diamidino-2-phenylindole (DAPI) (Roche) was used to color nucleus. Cells were imaged using the Olympus FluoView™ 1000 confocal microscope (Olympus, Melville, NY, USA) at 488 and 563 nm, respectively.

Measurement of Reactive Oxygen Species

Cellular reactive oxygen species (ROS) level was measured following the method described in our previous study [12]. In brief, cells were incubated in PBS containing 2-μM DCFH-DA probe (Sigma-Aldrich, USA, D6883) at 37 °C for 20 min. After three washes with PBS, cells were detected by flow cytometry (BD, Vantage SE, USA) at 535 nm.

Data Analysis

We searched the OPA1 mutations identified in patients in the available datasets, including dbSNP Short Genetic Variants (http://www.ncbi.nlm.nih.gov/projects/SNP); Exome Aggregation Consortium (ExAC), Cambridge, MA (http://exac.broadinstitute.org; accessed on December 16, 2015); and eOPA1 (http://mitodyn.org/home.php) [13, 14]. Evolutionary conservation of the OPA1 mutation was performed in the following eight species: *Caenorhabditis elegans* (CAA87771), *Drosophila melanogaster* (NP_610941), *Danio rerio* (NP_001007299), *Gallus gallus* (NP_001034398), *Mus musculus* (NP_001186106), *Bos taurus* (NP_001179890), *Pan troglodytes* (XP_003310226), and *Homo sapiens* (NP_056375). Five prediction algorithms (PolyPhen2 HumDiv and HumVar [15, 16], LRT [17], MutationTaster [18], and SIFT [19, 20]) were used to evaluate the potential pathogenicity of each mutation. We combined our data with 193 Chinese patients with optic neuropathy [21] and compared to 4327 East Asians by the ExAC. Fisher's exact test was used to show the frequency difference of the *OPA1* mutation between Chinese patients and the ExAC East Asians. Statistical difference of mtDNA copy number and cellular ROS levels between two groups was tested by two-tailed Student's *t* test. A P value < 0.05 was considered as statistically significant.

Results

High Frequency of *OPA1* Mutations in Han Chinese Patients with Optic Neuropathy

A total of nine mutations were identified in 8 out of 105 patients with suspected optic neuropathy. All mutations were heterozygous (Fig. 1). Among them, five (c.565G > A, p.E189K; c.575C > T, p.A192V; c.869G > A, p.R290L; c.1172T > G, p.L391R; and c.1465A > G, p.K489E) were missense mutations, three mutations (c.556 + 2T > G, c.2819 − 2A > G, and c.2614 − 2A > G) were located in the splice site of the intron and exon, and one mutation (c.2708_2711del) caused a truncated protein (Table 1). Mutations c.575C > T and c.1465A > G co-occurred in one patient. The web-based searches showed that mutation c.1172T > G (p.L391R) had not been reported previously. Mutations c.869G > A, c.1172T > G, and c.1465A > G were located in highly conserved domain of the GTPase and changed a highly conserved amino acid. Mutations c.565G > A and c.575C > T were not conserved in the eight species analyzed in this study (Fig. 2a). When we applied the five reported algorithms (PolyPhen2 HumDiv and HumVar [15, 16], LRT [17], MutationTaster [18], and SIFT [19, 20]) to predict the potential pathogenicity of these missense mutations, we found that mutation c.565G > A was predicted to be benign by all five algorithms; mutation c.575C > T was predicted to be possibly damaging by three of the five algorithms; and mutations c.869G > A, c.1172T > G, and c.1465A > G

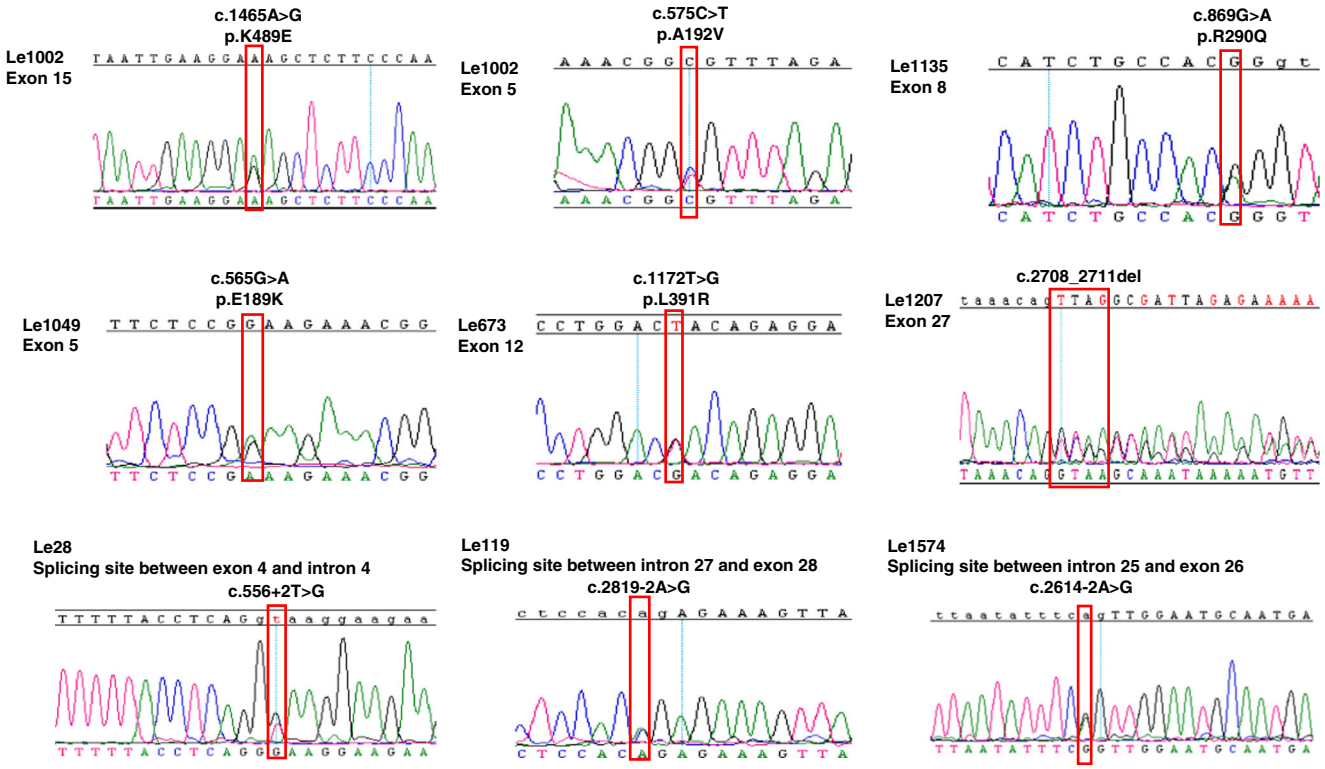

Fig. 1 Sequencing electropherograms showing the *OPA1* mutations in Han Chinese patients with optic neuropathy. Mutations c.565G > A, c.575C > T, c.869G > A, c.1172T > G, and c.1465A > G are missense mutations; mutations c.556 + 2T > G, c.2819 − 2A > G, and c.2614 − 2A > G are located in the splice site of the intron and exon; and mutation c.2708_2711del is a frame shift mutation. Mutations c.575C > T and c.1465A > G co-exist in patient Le1002

were predicted to be damaging by at least four algorithms, thus were highly possible to be pathogenic.

We further combined our data with the reported *OPA1* data in 193 Chinese patients [21], and compared to the East Asian

Table 1 OPA1 mutations in 105 Han Chinese patients with optic neuropathy

Sample	Mutation	ID in dbSNP	Function	Protein mutation	SIFT score[a]	Polyphen2 HDIV score[b]	Polyphen2 HVAR score[c]	LRT score[d]	MutationTaster[e]	Prediction[f]
Le1049	c.565G > A	rs569675223	Missense	p.E189K	0.92 (*T*)	0.039 (*B*)	0.025 (*B*)	0.079 (*N*)	0.927 (*N*)	*B*
Le1002	c.575C > T	rs34307082	Missense	p.A192V	0.16 (*T*)	0.994 (*P*)	0.591 (*P*)	0.729 (*N*)	1 (*D*)	*P*
	c.1465A > G	rs201301622	Missense	p.K489E	0.1 (*T*)	0.997 (*P*)	0.886 (*P*)	0 (*D*)	1 (*D*)	*D*
Le1135	c.869G > A	rs121908375	Missense	p.R290L	0.4 (*T*)	1 (*P*)	0.997 (*D*)	0 (*D*)	1 (*A*)	*D*
Le673	c.1172T > G	–	Missense	p.L391R	0.0 (*D*)	1 (*P*)	0.995 (*D*)	0 (*D*)	1 (*D*)	*D*
Le1207	c.2708_2711del	–	Frame shift	–	–	–	–	–	–	–
Le28	c.556 + 2T > G	–	Splicing	–	–	–	–	–	–	–
Le1574	c.2614 − 2A > G	–	Splicing	–	–	–	–	–	–	–
Le119	c.2819 − 2A > G	–	Splicing	–	–	–	–	–	–	–

[a] A SIFT score ≤ 0.05 was regarded as deleterious (*D*), and a score value >0.05 was regarded as tolerated (*T*) [19, 20]

[b] Polyphen2 HDIV score ≥ 0.957, probably damaging (*D*); 0.453 < Polyphen2 HDIV score <0.956, possibly damaging (*P*); and Polyphen2 HDIV score ≤ 0.452, benign (*B*) [15, 16]

[c] Polyphen2 HVAR score ≥ 0.909, probably damaging (*D*); 0.447 < Polyphen2 HVAR score <0.909, possibly damaging (*P*); and Polyphen2 HVAR score ≤ 0.446, benign (*B*) [15, 16]

[d] *D* deleterious, *N* neutral, and *U* unknown [17]

[e] *A* disease causing automatic, *D* disease causing, *N* polymorphism, and *P* polymorphism automatic [18]

[f] *B* benign, predicted to be benign by more than three of the five algorithms; *P* possibly damaging, predicted to be (possibly) damaging by three of the five algorithms; and *D* damaging, predicted to be (possibly) damaging by at least four of the five algorithms

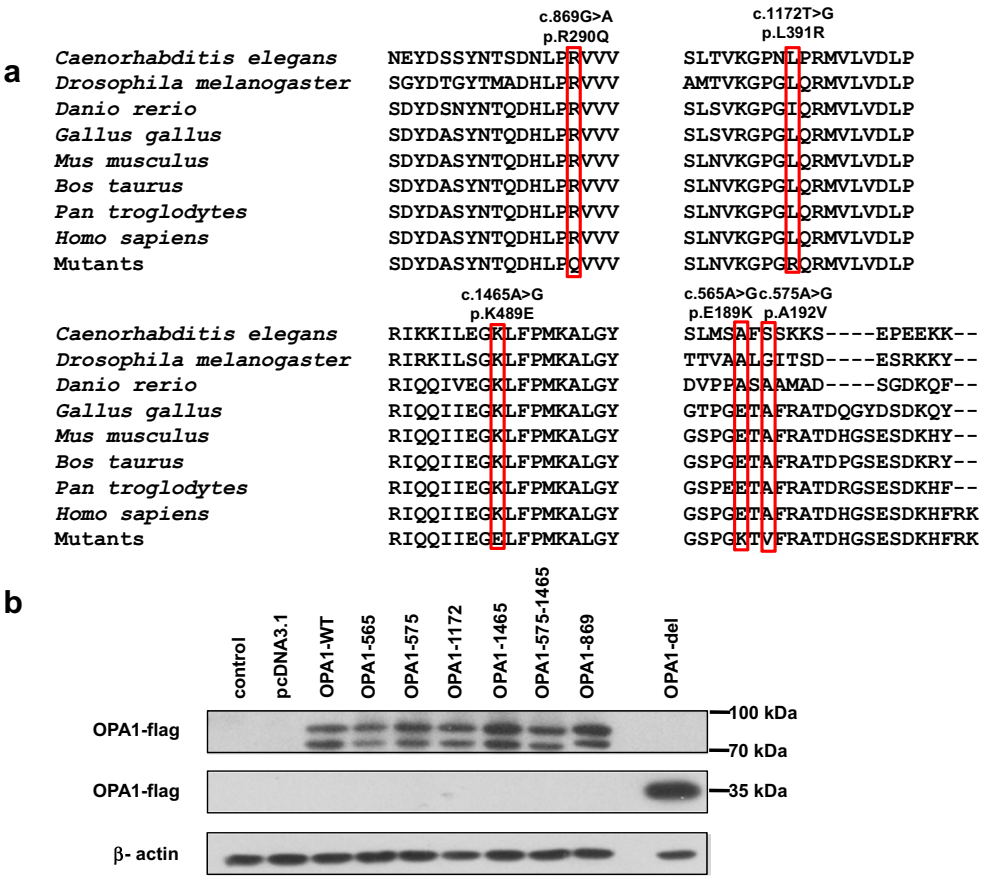

Fig. 2 Evolutionary conservation analysis and isoform expression of the OPA1 mutants. **a** Eight species were used to analyze the evolutionary conservation of the *OPA1* gene mutations. Sequences of *Caenorhabditis elegans* (CAA87771), *Drosophila melanogaster* (NP_610941), *Danio rerio* (NP_001007299), *Gallus gallus* (NP_001034398), *Mus musculus* (NP_001186106), *Bos taurus* (NP_001179890), *Pan troglodytes* (XP_003310226), and *Homo sapiens* (NP_056375) were retrieved from GenBank. **b** Western blot of the OPA1 isoforms in HeLa cells overexpressing different mutants. The two OPA1 isoforms were tagged by flag. A truncated protein (about 35 kDa) was observed in HeLa cells overexpressing mutant c.2708_2711del. The untransfected cells were as the control

population retrieved from the ExAC database (http://exac.broadinstitute.org), which contains the exome sequencing data of 4327 individuals. Mutation c.2708_2711del had a significantly higher frequency in Chinese patients with optic neuropathy than in the ExAC East Asians ($P = 0.00002$; Table 2). We retrieved all potentially pathogenic OPA1 mutations with a minor allele frequency (MAF) less than 0.01 in the East Asian population from the ExAC dataset (Supplementary Table 3) and grouped them into four categories (missense mutations, frame shift mutations, nonsense mutations, and splicing mutations; Table 3). Comparison of the four mutation categories between Han Chinese patients with optic neuropathy and the ExAC East Asian population revealed that these potentially pathogenic mutations are significantly enriched in Chinese patients (Table 3).

Altered Isoform Expression of OPA1 Mutants

Two OPA1 isoforms, long-OPA1 (l-OPA1) and short-OPA1 (s-OPA1), were recognized by Western blot in HeLa cells transfected with different OPA1 mutants (Fig. 2b). The s-OPA1 isoform in cells overexpressing mutant c.869G > A was somewhat larger than that of cells overexpressing with wild-type or other mutants. Cells that overexpressed OPA1 mutant with mutation c.2708_2711del showed no bands for l-OPA1 and s-OPA1, but a truncated protein with a size around 35 kDa was observed, which might be caused by the premature stop codon (Fig. 2b). These results suggested that the *OPA1* gene mutations identified in Chinese patients affected the expression level of different isoforms, which might lead to further change of cellular function.

OPA1 Mutants Impair Mitochondrial Function

Cellular localization of the ectopically expressed OPA1 mutant (marked by green fluorescence) was overlapped with mitochondria (red fluorescence), which was consistent with the notion that OPA1 is a mitochondrial protein and suggested that these mutants did not affect cellular localization of the OPA1 (Fig. 3). However, we found that mitochondrial morphology had a change from a network distribution to a dot distribution in the cells overexpressing mutant c.2708_2711del, which indicated the impairment of mitochondrial fusion (Fig. 3). No apparent change of mitochondrial morphology was observed in cells overexpressing the other mutants. We further quantified the cellular ROS level in all transfected cells and observed mild increase of ROS levels in cells overexpressing all mutants, albeit the difference was not significant (Fig. 4a).

Table 2 Frequency of the *OPA1* gene mutation in Han Chinese patients with optic neuropathy and in East Asian population from the ExAC dataset

Mutation	Sample	ID in dbSNP	Function	Protein mutation	Chinese patients[a]	East Asian[b]	*P* value[c]
c.565G > A	Le1049	rs569675223	Missense	p.E189K	1/596	6/8652	0.37
c.575C > T	Le1002	rs34307082	Missense	p.A192V	10/596	220/8654	0.22
c.1465A > G	Le1002	rs201301622	Missense	p.K489E	1/596	1/8372	0.13
c.869G > A	Le1135	rs121908375	Missense	p.R290L	1/596	0/8654	0.06
c.1172T > G	Le673	–	Missense	p.L391R	1/596	0/8654	0.06
c.2708_2711del	Le1207	–	Frame shift	–	4/596	0/8654	0.00002
c.556 + 2T > G	Le28	–	Splicing	–	1/596	1/8372	0.13
c.2614 − 2A > G	Le1574	–	Splicing	–	1/596	0/8654	0.06
c.2819 − 2A > G	Le119	–	Splicing	–	1/596	0/8654	0.06
c.49_50insGG	Le1608	–	Frame shift	–	1/596	0/8654	0.06
c.190_194del	Le2028	–	Frame shift	–	1/596	0/8654	0.06
c.985 − 1G > A	Le2146	–	Splicing	–	1/596	0/8654	0.06
c.989C > G	Le1524	–	Missense	p.T330S	1/596	0/8654	0.06
c.991 − 992_del	Le1656	–	Frame shift	–	1/596	0/8654	0.06
c.1129G > A	Le2028	–	Missense	p.V337I	1/596	1/8436	0.13
c.2119G > T	Le1599	–	Nonsense	–	1/596	0/8654	0.06
c.2389A > T	Le1432	–	Nonsense	–	1/596	0/8654	0.06
c.2470C > T	Le1601	–	Nonsense	–	1/596	0/8654	0.06
c.2164 − 2A > G	Le1574	–	Splicing	–	1/596	0/8654	0.06

[a] Allele count was based on 298 Chinese patients with optic neuropathy from this study and our previous study [21]

[b] Allele counts in East Asians retrieved from Exome Aggregation Consortium (ExAC), Cambridge, MA (http://exac.broadinstitute.org; accessed on December 16, 2015)

[c] Fisher's exact test

Decrease of mtDNA Copy Number in Patients with OPA1 Mutations

OPA1 plays an important role in mtDNA maintenance and distribution [22]. We measured the mtDNA copy number in patients with and without OPA1 mutations and healthy individuals. We found that the average mtDNA copy number was relatively decreased in the eight patients with OPA1 mutations in comparison to that of patients without mutation or normal controls, whereas the average mtDNA copy number was similar in the patients without *OPA1* mutations and normal controls (Fig. 4b). Note that this pattern was based on limited number of individuals analyzed in this study and there was a large variance within each group.

Table 3 Distribution of four categories of possibly pathogenic *OPA1* mutations in Han Chinese patients with optic neuropathy and in East Asian population from the ExAC dataset

Mutation category	Chinese patients with optic neuropathy[b]	East Asian[c]	*P* value[d]
Pathogenic missense[a]	5/596	9/8654	1.34×10^{-3}
Frame shift	7/596	0/8654	4.46×10^{-9}
Nonsense	3/596	2/8654	2.41×10^{-3}
Splice donor/acceptor	5/596	1/8654	6.21×10^{-6}

[a] Missense mutations with MAF < 0.01 in the East Asian population from the ExAC dataset were predicted to be (possibly) damaging by at least four of the five prediction algorithms

[b] 298 Han Chinese patients with optic neuropathy from this study and our previous study [21]

[c] 4327 East Asians retrieved from Exome Aggregation Consortium (ExAC), Cambridge, MA (http://exac.broadinstitute.org; accessed on December 16, 2015)

[d] Fisher's exact test

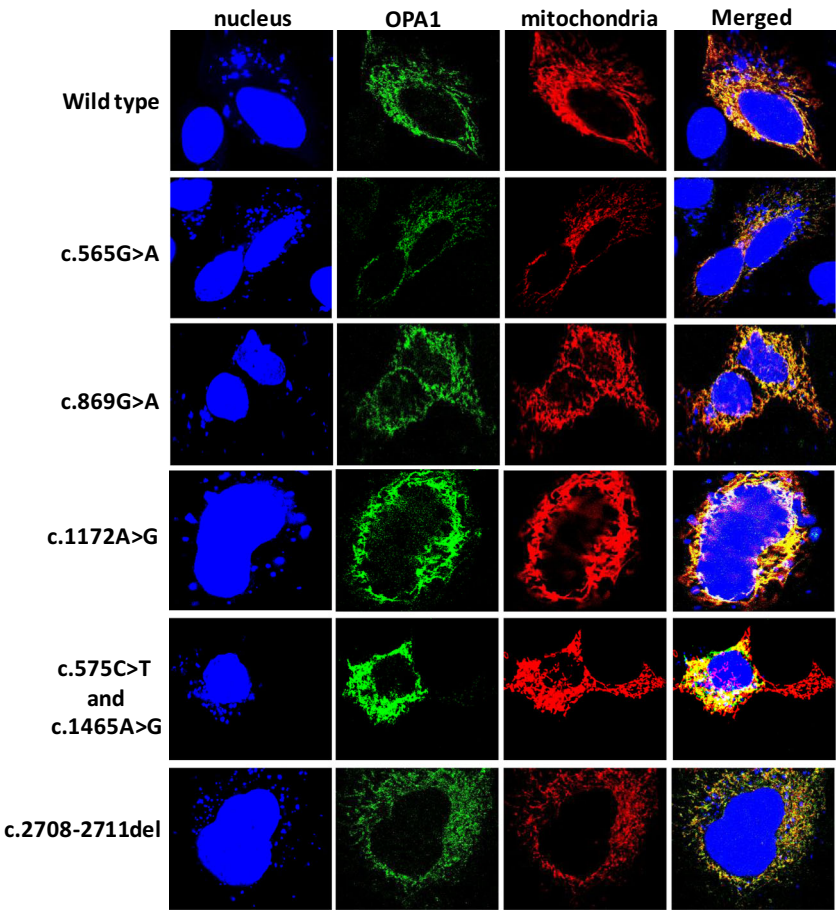

Fig. 3 Mitochondrial morphology in HeLa cells overexpressing OPA1 mutants. Cell nucleus was stained by DAPI. The OPA1 protein was demonstrated by green fluorescence. Mitochondria were marked by red fluorescence

Discussion

ADOA and LHON are two common ophthalmological diseases caused by gene mutations and can be diagnosed according to clinical symptoms and family history [23, 24]. There are many sporadic cases with symptoms similar to LHON but without any known LHON primary mtDNA mutations [7, 8, 25], and the exact etiological factor was unclear.

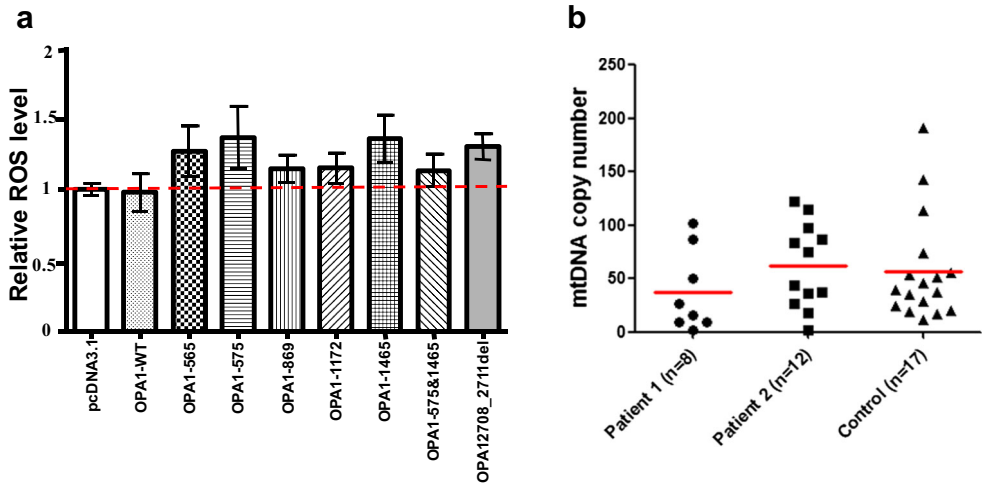

Fig. 4 Cellular ROS level in HeLa cells overexpressing OPA1 mutants and mtDNA copy number in patients with and without OPA1 mutations. **a** HeLa cells overexpressing OPA1 mutants (OPA1-mutation) had a relatively higher cellular ROS level than those cells overexpressing wild-type OPA1 (OPA1-WT) or empty expression vector pcDNA3.1. **b** mtDNA copy number in Han Chinese patients with optic neuropathy and normal controls. Patient 1—patients with optic neuropathy and OPA1 mutations, patient 2—patients with optic neuropathy but no OPA1 mutations, and control—healthy individuals reported in our previous study [9]

The *OPA1* gene was one of the most possible causal genes for this disease [7, 25]. Hitherto, many *OPA1* gene mutations had been reported (cf. eOPA1 [http://mitodyn.org/home.php]) and the mutation spectra were somewhat different among populations [7, 21, 26, 27]. The Chinese patients with optic neuropathy in this study expressed acute vision loss but had no known LHON primary mtDNA mutations [8]. Genetic analysis of the *OPA1* gene mutations in these patients will undoubtedly help to make a proper diagnosis.

In this study, we identified an enrichment of potentially pathogenic OPA1 mutations in Chinese patients with optic neuropathy. The in silico program-affiliated prediction of the pathogenicity by using five algorithms showed that three mutations (c.869G > A, c.1172T > G, and c.1465A > G) might be damaging by changing the GTPase activity. Our current finding, together with the mutations reported in Han Chinese patients [21, 25], suggested that the mutation spectrum of the *OPA1* gene in Chinese patients was different from that of populations of European ancestry. Mutation c.575C > T was previously reported in a glaucoma patient [28] and Chinese patients with suspected hereditary optic neuropathic [21] and had a relatively high frequency (2.5 %) in the ExAC East Asians (Table 2). Moreover, it caused no apparent mitochondrial morphology change in HeLa cells (Fig. 3). Therefore, variant c.575C > T may be regarded as a benign polymorphism. Mutation c.869G > A was previously reported in a French ADOA family and caused mitochondrial dysfunction [29]. Similar to mutations c.868C > T, c.808G > A, and c.869G > T [30], c.869G > A was located in highly conservative region of the GTPase and was predicted to be damaging (Table 1), which might influence the function of OPA1 by changing the expression of isoforms and its structure (Fig. 2). Mutant c.869G > A led to a larger s-OPA1 than that of wild type (Fig. 2); this result was consistent with a previous study that identified the isoform variation and function impairment in fibroblast cell of patients with c.869G > A [29]. Mutation c.2708_2711del was a hot spot mutation [21, 31, 32] and produced a truncated OPA1 protein of 35 KDa, which heavily affected the mitochondrial morphology (Fig. 3). However, mutation c.2708_2711del might not lead to seriously clinical symptoms according to previous study in ADOA patients [32]. Mutations c.556 + 2T > G, c.2819 − 2A > G, and c.2614 − 2A > G were located in splicing sites of exons and introns.

Cellular ROS level could affect many disorders, such as tumors and liver inflammation [33, 34]. It has been identified that the *OPA1* gene mutations could increase ROS level, accelerate lattice cell apoptosis, and shorten the life span in Drosophila model [35, 36]. Chen et al. [37] identified that the heterozygous OPA1 mutation led to decreased mtDNA copy number, increased ROS level, and fragmented mitochondria in mouse model. Similar to these studies, we identified an increasing ROS level in HeLa cells overexpressing OPA1 mutants and a decreasing mtDNA copy number in patients with the OPA1 mutations, although the difference was not statistically significant (perhaps due to the relatively small number of patients under consideration). The increasing ROS could induce release of *OPA1* and cytochrome c from mitochondria to cytosol, which would lead to mitochondrial fragmentation and cell death [38]. Our current results, together with others [36, 37], suggested that increasing ROS and mitochondrial dysfunction might be one of the causes for optic neuropathy.

In sum, the *OPA1* gene mutations are frequent in Han Chinese patients with optic neuropathy. In silico prediction and functional assays indicated that some of these mutations are pathogenic and affect cellular ROS level, expression of OPA1 isoforms, and mitochondrial morphology. The significant enrichment of potentially pathogenic mutations in Han Chinese patients with suspected optic neuropathy indicated that screening the *OPA1* gene mutations should be encouraged for precise diagnosis of patients.

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Compliance with Ethical Standards Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study. The Institutional Review Boards of the Zhongshan Ophthalmic Center and the Kunming Institute of Zoology approved this study.

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Supplementary Table 1. Primer pairs for PCR and sequencing the entire exons of the *OPA1* gene

Primer	Sequence (5'-3')	Amplicon
OPAl-1F	GATTGCTCCAGTCCGTTCC	Exon 1
OPAl-1R	AGATAGAGATAAGACAGCCCC	
OPA1-2F	TGGGGCTGTGTTTCCTTTAG	Exon 2
OPA1-2R	GCAAAACCAGGAGGAATGTC	
OPA1-3F	TATTTGGCATGCAGAGCATC	Exon 3
OPA1-3R	TCTCTTTCCTCGAGATGACCA	
OPA1-4F	GGGTTGTCATGAGGATTAAACAA	Exon 4
OPA1-4R	TCTTTAAATTAGATCAAACAGCTTC	
OPA1-4bF	TGCCCTATCGTAATATGAAATCTG	Exon 4b
OPA1-4bR	GGCATGTTAAGGTCAGCAAAA	
OPA1-5F	CATTCACCTTTTCATTGACTCG	Exon 5
OPA1-5R	TCTTTCAAGACTACCTACATGAACAA	
OPA1-5bF	TCTCCTCCCCAATTTCCTCT	Exon 5b
OPA1-5bR	ATGATCTTGGCAGAAATGGC	
OPA1-6F	AAAAATTTAACTTGCTGTACATTCTG	Exon 6
OPA1-6R	CACCTTCCAAATTTTGCTCTG	
OPA1-7F	TTTGGAAGATTTTAATTTAGACT	Exon 7
OPA1-7R	CTTAGAAACTGGTACTGATG	
OPA1-8F	GACACATCTGTTATATTTTTTTCTT	Exon 8
OPA1-8R	GGCAAGTTCACTATCAATTTTT	
OPA1-9F	TGAATAAGTGTTCTTTGTTTTGTGG	Exon 9
OPA1-9R	TGAACAGGTCTCACTGAAGCA	
OPA1-10F	GCAATGCAGTAGCCCTGTCT	Exon 10
OPA1-10R	GCTCATCCAGGTTATAAAATTACACA	
OPA1-11F	TTTTCTTAGGATTTTGTGTTTTCCA	Exon 11
OPA1-11R	GCAGCAATCTAAACATCAATACC	
OPA1-12F	CCCCCAAACTTTAGCATTGT	Exon 12
OPA1-12R	AAATGTCATTATAAATGCTTCACACA	
OPA1-13F	TTGTGTGAAGCATTTATAATGACA	Exon 13
OPA1-13R	AATGCAAAGCTTGGATTGCT	
OPA1-14F	TACATCTTAAAATTCCACAGTGTCAT	Exon 14
OPA1-14R	CTCGCAACAAAGAATTTGACC	
OPA1-15F	ATCTCACATTAATTTTTCCCACTTTT	Exon 15
OPA1-15R	TGATGGTCAAATTTTCATCAAG	
OPA1-16F	CTGTTGTTTTCAAATAATAAAGAGT	Exon 16
OPA1-16R	CTAGAAATCCGATGTTAAACTAATA	
OPA1-17F	TGTCCTTTTTGTCATTTTAATATAC	Exon 17
OPA1-17R	ATGTCTTAATTTGCTTGCTTC	
OPA1-18F	TCTGGAAAGAAGGAGGGTCA	Exon 18
OPA1-18R	GCTTATCAGATTTTTCTCTCAACAT	
OPA1-19F	CCTCCCTTTGGTTATCTCTGAA	Exon 19

OPA1-19R	AAGTTTTAGCATTTCATTATAACCTGT	
OPA1-20F	TGATACTTCAGTCAAGCTGTTTTT	Exon 20
OPA1-20R	TCACAGCTCCTACTCCCTTCA	
OPA1-21F	GTTTGGCTTGAGCTCGTGTT	Exon 21
OPA1-21R	ATATGAGGCTGATACCCCAGT	
OPA1-22F	CAAGTTGGCTTTTTCTCTTCTTG	Exon 22
OPA1-22R	TTGGATGACTCCTTCACCACT	
OPA1-23F	GTTAAGAAAGCAAGACCATTATTAGTT	Exon 23
OPA1-23R	TGCCTGAATTAAAATGAACAAAA	
OPA1-24F	TCAAGCACCAAATTATGAACCA	Exon 24
OPA1-24R	GCAGATTCCTGCTTCTCAGC	
OPA1-25F	AATAACCTTGCTTTTGCTTTT	Exon 25
OPA1-25R	ATACTTCTCTTTGTTTTTGGA	
OPA1-26F	TTTTCATTTTAACTTTGCATCTGG	Exon 26
OPA1-26R	TGGGAAGTATTTTGGCATCC	
OPA1-27F	ATGAAACTCTGTTGGGAATG	Exon 27
OPA1-27R	GGTATTGAGCCTGGAACATC	
OPA1-28F	CCTCCTGATTTGTGATACCTTTG	Exon 28
OPA1-28R	CAAGCAGGATGTAAATGAAGCA	
OPA1-29F	TAAAACTAGCAAAGGTAAGCG	Exon 29
OPA1-29R1	TACGTTACAATACTGATCTCC	
OPA1-29F4	CACTTGGTATGCTCGGTTGA	Exon 29
OPA1-29R2	TAATGAAAATGACAGCCTTGG	
OPA1-29F1	GATGTATGTTACGGGCGCTT	Sequencing exon 29
OPA1-29F2	CGAAGAAATTTGCCTTGGAA	Sequencing exon 29
OPA1-29F3	TCCCCCTCTCAGCTGAATAA	Sequencing exon 29
OPA1-29F5	TGGAGGGAGATCTTCATTTCTT	Sequencing exon 29
OPA1-29F6	CAAAGCTCTGTATGGAAGACTGAA	Sequencing exon 29
OPA1-29F7	GCCTCACAATGTGGGAATTT	Sequencing exon 29

Supplementary Table 2. Primers for constructing the expression vector of the OPA1 mutants.

Mutation	Primer	Sequence (5'-3')
c.565G>A	c.565A-F	CCTCAGGTTCTCCGAAAGAAACGGCGTTTAG
(p.E189K)	c.565A-R	CTAAACGCCGTTTCTTTCGGAGAACCTGAGG
c.575C>T	c.575T-F	CCGGAAGAAACGGTGTTTAGAGCAACAGATC
(p.A192V)	c.575T-R	GATCTGTTGCTCTAAACACCGTTTCTTCCGG
c.869G>A	c.869A-F	AAGATCATCTGCCACAGGTTGTTGTGGTTGG
(p.R290L)	c.869A-R	CCAACCACAACAACCTGTGGCAGATGATCTT
c.1172T>G	c.1172G-F	AAAGGCCCTGGACGACAGAGGATGGTGCTTG
(p.L391R)	c.1172G-R	CAAGCACCATCCTCTGTGGTCCAGGGCCTTT
c.1465A>G	c.1465G-F	GCAGATAATTGAAGGAGAGCTCTTCCCAATG
(p.K489E)	c.1465G-R	CATTGGGAAGAGCTCTCCTTCAATTATCTGC
c.2708-2711del	c.2708-2711del-F	ATACTGAAGGATCCGATTACAAGGATGAC
	c.2708-2711del-R	GTCATCCTTGTAATCGGATCCTTCAGTAT

Supplementary Table 3. Potentially pathogenic OPA1 mutations retrieved from the ExAC database.

Position	RSID	Reference	Alternate	Consequence	Transcript Consequence	Annotation	Allele count in the ExAC database	Allele number in the ExAC database	Number of homozygotes in the ExAC database	Allele count in East Asians	Allele number in East Asians	Homozygote count in East Asians	SIFT score [a]	SIFT pred [a]	Polyphen2 HDIV score [b]	Polyphen2 HDIV pred [b]	Polyphen2 HVAR score [c]	Polyphen2 HVAR pred [c]	LRT score	LRT pred [d]	MutationTaster score	MutationTaster pred [e]	Pathogenic score [f]
193332520	.	G	A	p.Cys14Tyr	c.41G>A	missense	1	121292	0	0	8654	0	0	D	0.998	D	0.991	D	0	D	1	D	5
193332579	.	C	T	p.His34Tyr	c.100C>T	missense	1	121374	0	0	8654	0	0.01	D	0.993	D	0.968	D	0	D	1	D	5
193332599	.	T	G	p.Ile40Met	c.120T>G	missense	4	121204	1	0	8654	0	0.01	D	0.996	D	0.964	D	0	D	0.933	D	5
193332634	.	G	A	p.Arg52Gln	c.155G>A	missense	1	121160	0	0	8592	0	0.07	T	0.999	D	0.945	D	0	D	1	D	4
193332735	.	A	G	p.Arg86Gly	c.256A>G	missense	1	120720	0	0	8570	0	0.04	D	0.985	D	0.916	D	0	D	1	D	5
193332750	.	G	A	p.Ala91Thr	c.271G>A	missense	1	121028	0	0	8634	0	0.16	T	0.993	D	0.971	D	0	D	1	D	4
193332750	.	G	C	p.Ala91Pro	c.271G>C	missense	1	121028	0	1	8634	0	0.02	D	0.998	D	0.987	D	0	D	1	D	5
193332757	.	T	C	p.Leu93Ser	c.278T>C	missense	1	121108	0	0	8644	0	0	D	0.999	D	0.994	D	0	D	1	D	5
193332760	.	C	T	p.Ala94Val	c.281C>T	missense	1	121104	0	0	8644	0	0.5	T	0.997	D	0.985	D	0	D	1	D	4
193332780	.	C	T	p.Arg101Cys	c.301C>T	missense	2	121126	0	0	8646	0	0	D	1	D	1	D	0	D	1	D	5
193332781	.	G	A	p.Arg101His	c.302G>A	missense	3	121118	0	0	8646	0	0	D	1	D	0.999	D	0	D	1	D	5
193332784	.	A	G	p.Tyr102Cys	c.305A>G	missense	4	121098	0	0	8648	0	0	D	1	D	0.999	D	0	D	1	D	5
193332795	.	G	C	p.Gly106Arg	c.316G>C	missense	1	120990	0	0	8648	0	0	D	1	D	0.999	D	0	D	1	D	5
193332799	.	C	T	p.Ser107Leu	c.320C>T	missense	3	120948	0	0	8648	0	0	D	0.99	D	0.674	P	0	D	1	D	4
193332808	.	G	C	p.Gly110Ala	c.329G>C	missense	2	120794	0	0	8648	0	0.06	T	0.999	D	0.996	D	0	D	1	D	4
193332829	.	A	T	p.Lys117Met	c.350A>T	missense	1	120498	0	0	8636	0	0.03	D	0.998	D	0.924	D	0	D	1	D	5
193333491	.	C	T	p.Pro127Leu	c.380C>T	missense	1	121398	0	0	8654	0	0	D	1	D	0.999	D	0	D	1	D	5
193335018	.	C	G	p.Pro167Arg	c.500C>G	missense	1	121152	0	0	8646	0	0.08	T	1	D	0.999	D	0	D	1	D	4
193335023	.	TTTG	T	p.Phe169_Asp170delin...	c.506_508delTTG	inframe deletion	15	121124	0	0	8640	0	-	-	-	-	-	-	-	-	-	-	-
193335058	.	G	T	p.Lys180Asn	c.540G>T	missense	1	120478	0	0	8578	0	0.04	D	0.986	D	0.655	P	0	D	1	D	4
193335076	.	T	G	c.556+2T>G	c.556+2T>G	splice donor	1	118182	0	1	8372	0	-	-	-	-	-	-	-	-	-	-	-
193349409	.	CAA	C	p.Lys212ArgfsTer4	c.634_635delAA	frameshift	1	120600	0	0	8638	0	-	-	-	-	-	-	-	-	-	-	-
193349441	.	T	C	p.Leu222Pro	c.665T>C	missense	4	120844	0	0	8648	0	0.03	D	0.999	D	0.909	D	0	D	1	D	5
193349444	.	T	G	p.Leu223Arg	c.668T>G	missense	1	120850	0	0	8648	0	0	D	0.998	D	0.926	D	0	D	1	D	5
193353214	.	A	T	p.Tyr229Phe	c.686A>T	missense	1	120702	0	1	8636	0	0.1	T	0.997	D	0.985	D	0	D	1	D	4
193353214	.	A	G	p.Tyr229Cys	c.686A>G	missense	1	120702	0	0	8636	0	0	D	1	D	0.997	D	0	D	1	D	5
193353218	.	G	T	p.Gln230His	c.690G>T	missense	5	120736	0	0	8640	0	0.03	D	0.998	D	0.993	D	0	N	1	D	4
193353231	.	C	T	p.Arg235Ter	c.703C>T	stop gained	1	120840	0	0	8644	0	-	-	-	-	-	-	-	-	-	-	-
193353248	.	C	G	p.Asn240Lys	c.720C>G	missense	1	121010	0	0	8650	0	0	D	1	D	1	D	0	D	1	D	5
193355012	.	T	C	p.Val271Ala	c.812T>C	missense	1	120562	0	0	8606	0	0.06	T	1	D	0.996	D	0	D	1	D	4
193355068	.	C	T	p.Arg290Trp	c.868C>T	missense	2	121090	0	0	8628	0	0	D	1	D	0.999	D	0	D	1	D	5
193355788	.	A	T	p.Glu306Asp	c.918A>T	missense	1	121022	0	0	8650	0	0	D	1	D	1	D	0	D	1	D	5
193355798	.	C	G	p.Gln310Glu	c.928C>G	missense	1	120996	0	1	8650	0	0.06	T	0.992	D	0.987	D	0	D	1	D	4
193355799	.	A	G	p.Gln310Arg	c.929A>G	missense	2	120978	0	0	8648	0	0.36	T	0.997	D	0.992	D	0	D	1	D	4
193355805	.	G	A	p.Arg312Gln	c.935G>A	missense	1	120938	0	0	8642	0	0.01	D	1	D	1	D	0	D	1	D	5
193355809	.	A	G	p.Ile313Met	c.939A>G	missense	1	120892	0	0	8642	0	0	D	1	D	1	D	0	D	1	D	5
193355840	.	C	T	p.Arg324Cys	c.970C>T	missense	5	119616	0	0	8610	0	0	D	1	D	1	D	0	D	1	D	5
193355853	.	A	C	p.Lys328Thr	c.983A>C	missense	1	117926	0	0	8476	0	0.16	T	1	D	0.998	D	0	D	1	D	4
193360569	.	G	A	p.Gly334Ser	c.1000G>A	missense	2	121334	0	0	8654	0	0.14	T	1	D	1	D	0	D	1	D	4
193360579	.	A	C	p.His337Pro	c.1010A>C	missense	1	121342	0	0	8654	0	0	D	1	D	0.999	D	0	D	1	D	5
193360597	.	A	C	p.Asp343Ala	c.1028A>C	missense	5	121328	0	0	8654	0	0	D	1	D	0.999	D	0	D	1	D	5
193360605	.	C	T	p.Arg346Trp	c.1036C>T	missense	1	121310	0	0	8654	0	0	D	1	D	1	D	0	D	1	D	5
193360617	.	C	G	p.Leu350Val	c.1048C>G	missense	1	121278	0	0	8652	0	0	D	1	D	1	D	0	D	1	D	5
193360623	.	AAAG	A	p.Glu354del	c.1055_1057delAAG	inframe deletion	2	121250	0	0	8652	0	-	-	-	-	-	-	-	-	-	-	-
193360777	.	G	C	p.Arg360Thr	c.1079G>C	missense	1	119644	0	0	8488	0	0	D	1	D	1	D	0	D	1	D	5
193360785	.	A	C	p.Ile363Leu	c.1087A>C	missense	1	119726	0	0	8506	0	0	D	0.967	D	0.897	P	0	D	1	D	4
193360809	.	G	A	p.Val371Met	c.1111G>A	missense	1	119426	0	0	8502	0	0	D	1	D	0.997	D	0	D	1	D	5
193360818	.	G	C	p.Gly374Arg	c.1120G>C	missense	1	119024	0	0	8484	0	0	D	1	D	1	D	0	D	1	D	5
193360819	rs146003075	G	A	p.Gly374Asp	c.1121G>A	missense	5	118972	0	0	8482	0	0.01	D	1	D	1	D	0	D	1	D	5
193360819	rs146003075	G	C	p.Gly374Ala	c.1121G>C	missense	1	118972	0	0	8482	0	0	D	1	D	1	D	0	D	1	D	5
193360827	.	G	A	p.Val377Ile	c.1129G>A	missense	3	118176	0	1	8436	0	0.43	T	0.999	D	0.927	D	0	D	1	D	4
193360839	.	G	C	c.1140+1G>C	c.1140+1G>C	splice donor	1	116620	0	0	8306	0	-	-	-	-	-	-	-	-	-	-	-
193361167	rs143319805	A	G	p.Ile382Met	c.1146A>G	missense	73	120454	0	0	8628	0	0	D	1	D	0.999	D	0	D	1	D	5
193361196	.	A	G	p.Gln392Arg	c.1175A>G	missense	1	120762	0	0	8634	0	0.03	D	0.993	D	0.931	D	0	D	1	D	5
193361201	.	A	G	p.Met394Val	c.1180A>G	missense	1	120764	0	0	8636	0	0	D	1	D	0.999	D	0	D	1	D	5
193361330	.	G	A	p.Gly409Asp	c.1226G>A	missense	1	120928	0	0	8608	0	1	T	1	D	1	D	0	D	1	D	4
193361336	.	C	G	p.Ala411Gly	c.1232C>G	missense	1	120960	0	1	8612	0	0.03	D	1	D	0.998	D	0	D	1	D	5
193361380	.	A	T	p.Met426Leu	c.1276A>T	missense	8	120838	0	0	8622	0	0.06	T	0.985	D	0.987	D	0	D	1	D	4
193361799	.	G	C	p.Asp450His	c.1348G>C	missense	1	121250	0	0	8630	0	0	D	1	D	0.994	D	0	D	1	D	5
193361800	.	A	G	p.Asp450Gly	c.1349A>G	missense	1	121260	0	0	8632	0	0	D	0.998	D	0.976	D	0	D	1	D	5
193361804	.	G	C	p.Leu451Phe	c.1353G>C	missense	1	121268	0	0	8632	0	0	D	0.999	D	0.987	D	0	D	1	D	5
193361836	.	C	T	p.Thr462Ile	c.1385C>T	missense	1	121336	0	0	8640	0	0	D	1	D	0.998	D	0	D	1	D	5
193361840	.	A	G	p.Ile463Met	c.1389A>G	missense	2	121346	0	0	8642	0	0.02	D	1	D	0.997	D	0	D	1	D	5
193361841	.	T	C	p.Phe464Leu	c.1390T>C	missense	1	121342	0	0	8640	0	0.73	T	0.999	D	0.998	D	0	D	1	D	4
193363532	.	G	A	p.Ser511Asn	c.1532G>A	missense	1	119968	0	1	8612	0	0.05	D	0.999	D	0.986	D	0	N	1	D	4
193363535	rs148834015	T	C	p.Ile512Thr	c.1535T>C	missense	8	119948	0	0	8612	0	0	D	1	D	0.999	D	0	D	1	D	5
193363543	.	A	C	p.Ile515Leu	c.1543A>C	missense	3	119802	0	0	8604	0	0	D	1	D	0.997	D	0	D	1	D	5
193364886	.	C	T	p.Thr541Ile	c.1622C>T	missense	1	116208	0	0	8330	0	0	D	1	D	0.998	D	0	D	1	D	5
193364933	.	C	T	p.Arg557Ter	c.1669C>T	stop gained	1	114298	0	0	8198	0	-	-	-	-	-	-	-	-	-	-	-
193365864	.	C	T	p.Arg571Cys	c.1711C>T	missense	1	120680	0	0	8592	0	0	D	1	D	0.998	D	0	D	1	D	5
193365865	rs140606054	G	A	p.Arg571His	c.1712G>A	missense	5	120708	0	0	8590	0	0.04	D	1	D	0.998	D	0	D	1	D	5
193365876	.	G	A	p.Glu575Lys	c.1723G>A	missense	1	120938	0	0	8608	0	0	D	1	D	0.999	D	0	D	1	D	5
193365903	.	C	T	p.Arg584Cys	c.1750C>T	missense	2	120994	0	0	8610	0	0	D	1	D	0.943	D	0	D	1	D	5
193365904	.	G	A	p.Arg584His	c.1751G>A	missense	1	120994	0	1	8610	0	0.04	D	0.999	D	0.832	P	0	D	1	D	4

193365921	.	C	T	p.Arg590Trp	c.1768C>T	missense	3	120704	0	0	8594	0	0	D	1	D	1	D	0	D	1	D	5
193365922	rs147077380	G	A	p.Arg590Gln	c.1769G>A	missense	4	120670	0	0	8592	0	0.01	D	1	D	1	D	0	D	1	D	5
193366582	.	A	G	c.1771-2A>G	c.1771-2A>G	splice acceptor	1	120810	0	0	8642	0	-	-	-	-	-	-	-	-	-	-	-
193366654	rs146601330	A	T	p.Lys614Ile	c.1841A>T	missense	2	121106	0	0	8650	0	0.04	D	0.966	D	0.691	P	0	D	1	D	4
193372768	.	C	G	p.Ile655Met	c.1965C>G	missense	1	121046	0	0	8616	0	0.01	D	1	D	0.997	D	0	D	1	D	5
193374867	.	A	G	c.2014-2A>G	c.2014-2A>G	splice acceptor	1	120144	0	0	8600	0	-	-	-	-	-	-	-	-	-	-	-
193374953	.	C	T	p.Leu700Phe	c.2098C>T	missense	1	121290	0	0	8644	0	0.01	D	1	D	0.998	D	0	D	1	D	5
193376682	.	A	G	p.Ile725Val	c.2173A>G	missense	1	121292	0	0	8650	0	0.06	T	0.995	D	0.984	D	0	D	1	D	4
193376692	.	A	G	p.Asn728Ser	c.2183A>G	missense	1	121332	0	0	8652	0	0.06	T	0.999	D	0.985	D	0	D	1	D	4
193376773	.	G	A	p.Arg755His	c.2264G>A	missense	2	121030	0	0	8620	0	0.01	D	0.999	D	0.902	P	0	D	1	D	4
193376784	.	A	G	p.Thr759Ala	c.2275A>G	missense	1	120648	0	0	8564	0	0.11	T	0.997	D	0.985	D	0	D	1	D	4
193377300	.	C	G	p.Pro769Ala	c.2305C>G	missense	1	97952	0	0	7254	0	0	D	1	D	0.983	D	0	D	1	D	5
193377308	.	GAAA	G	p.Lys772del	c.2314_2316delAAA	inframe deletion	1	100400	0	0	7448	0	-	-	-	-	-	-	-	-	-	-	-
193377336	rs190235251	C	T	p.Arg781Trp	c.2341C>T	missense	19	97646	0	0	7206	0	0.01	D	1	D	0.96	D	0	D	1	D	5
193377346	.	A	G	p.Glu784Gly	c.2351A>G	missense	1	94440	0	0	6888	0	0	D	0.957	D	0.641	P	0	D	1	D	4
193380707	rs143252541	C	T	p.Arg818Trp	c.2452C>T	missense	4	121176	0	0	8640	0	0	D	1	D	0.997	D	0	D	1	D	5
193380720	.	A	T	p.Glu822Val	c.2465A>T	missense	1	121178	0	0	8640	0	0	D	1	D	0.991	D	0	D	1	D	5
193380737	.	G	A	p.Val828Ile	c.2482G>A	missense	1	121108	0	0	8642	0	0.04	D	0.999	D	0.991	D	0	D	1	D	5
193382679	.	C	T	p.Thr836Ile	c.2507C>T	missense	1	120082	0	0	8544	0	0.12	T	0.999	D	0.996	D	0	D	1	D	4
193382720	.	C	G	p.Leu850Val	c.2548C>G	missense	1	119800	0	1	8528	0	0.06	T	0.997	D	0.991	D	0	D	1	D	4
193382738	.	T	A	p.Cys856Ser	c.2566T>A	missense	1	119654	0	0	8524	0	0	D	1	D	0.994	D	0	D	1	D	5
193382754	rs112388167	A	G	p.Tyr861Cys	c.2582A>G	missense	1	119420	0	0	8522	0	0.02	D	1	D	0.997	D	0	D	1	D	5
193384115	.	C	T	p.Arg882Cys	c.2644C>T	missense	3	121388	0	0	8652	0	0	D	1	D	1	D	0	D	1	D	5
193384146	.	A	C	p.Asn892Thr	c.2675A>C	missense	1	121398	0	0	8654	0	0	D	1	D	0.989	D	0	D	1	D	5
193384956	rs80356530	CAGTT	C	c.2708-2_2709delAGTT	c.2708-2_2709delAGTT	splice acceptor	4	118722	0	0	8504	0	-	-	-	-	-	-	-	-	-	-	-
193384965	.	G	A	p.Arg905Gln	c.2714G>A	missense	1	120166	0	0	8598	0	0.01	D	1	D	0.994	D	0	D	1	D	5
193384998	.	A	T	p.Asp916Val	c.2747A>T	missense	2	121202	0	0	8640	0	0.03	D	0.997	D	0.843	P	0	D	1	D	4
193385015	.	GAGA	G	p.Lys924del	c.2764_2767delGAGAinsG	inframe deletion	2	121242	0	0	8648	0	-	-	-	-	-	-	-	-	-	-	-
193385045	rs145710079	C	T	p.Arg932Cys	c.2794C>T	missense	4	120922	0	1	8642	0	0	D	1	D	0.996	D	0	D	1	D	5
193385048	.	G	A	p.Val933Ile	c.2797G>A	missense	1	120834	0	0	8642	0	0	D	1	D	0.999	D	0	D	1	D	5
193385053	.	A	C	p.Gln934His	c.2802A>C	missense	1	120632	0	0	8636	0	0.05	D	0.985	D	0.827	P	0	D	1	D	4

[a] D: deleterious (score<=0.05); T: tolerated (score>0.05) (Kumar et al. 2009; Ng 2003) .□

[b] D: probably damaging (score>=0.957), P: possibly damaging (0.453<score<0.956); B: benign (<=0.452) (Adzhubei et al. 2013; Adzhubei et al. 2010).

[c] D: probably damaging (score>=0.909), P: possibly damaging (0.447<score<0.909); B: benign (score<=0.446) (Adzhubei et al. 2013; Adzhubei et al. 2010).

[d] D: deleterious; N: neutral; U: unknown (Chun and Fay 2009).

[e] A: disease causing automatic; D: disease causing; N: polymorphism; P: polymorphism automatic (Schwarz JM et al. 2014).

[f] The score means the number of algorithms that predicted the mutation as damaging mutation.

We retrieved all potentially pathogenic OPA1 mutations from the ExAC database (http://exac.broadinstitute.org).

The OPA1 mutations were regarded as potentially pathogenic when at least 4 algorithms showed a prediction to be damaging.