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# Identification of copy number variation in the gene for autosomal dominant optic atrophy, *OPA1*, in a Chinese pedigree

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**ABSTRACT.** Autosomal dominant optic atrophy (ADOA) is an optic neuropathy characterized by bilateral optic nerve pallor and decreased visual acuity. It has been reported to be associated with two genes, *OPA1*, *OPA3*, and the *OPA4*, *OPA5*, and *OPA8* loci. However, mutations

 

in *OPA1* constitute the most prevalent cause of ADOA. The purpose of this study was to identify the underlying genetic defect in a Chinese pedigree with ADOA. DNA from six members of a Chinese pedigree was collected for testing genomic and copy number variation (CNV) by targeted region capture and next generation sequencing (targeted NGS). A new developmental CNV detection method was applied to analyze the sequence data. Further verification of CNV was performed by real-time polymerase chain reaction (PCR). Three members of the pedigree with clinically diagnosed ADOA were screened for pathogenic genes related to ophthalmic genetic disease. No eligible pathogenic point mutations associated with ADOA disease-causing genes were found in pedigree members with ADOA. Upon further analysis for CNVs, we found a heterozygous deletion in exons 1-9 of *OPA1*, which was confirmed by real-time PCR. In this study we used a new developmental method to detect CNVs associated with ADOA in a Chinese pedigree. To our knowledge, this is the first case of ADOA caused by a CNV of the *OPA1* gene in Chinese patients. The findings suggest that CNVs might be an important mutation type in Chinese patients with ADOA, and that CNV screening should be performed when point mutation screens are negative in these patients.

**Key words:** Autosomal dominant optic atrophy; Mutation; Pedigree; Copy number variation; Optic atrophy protein 1

## INTRODUCTION

Autosomal dominant optic atrophy (ADOA MIM 165500), also called Kjer's Optic Atrophy, is an optic neuropathy characterized by a bilateral degeneration of the optic nerves, causing slowly progressive visual loss beginning during the first decade of life (Kjer, 1959; Lenaers et al., 2012). ADOA is the most common form of inherited optic neuropathy, with a prevalence of 1 in 35,000 in the north of England and 1 in 12,000 in Denmark (Yu-Wai-Man et al., 2010; Almind et al., 2012). The disease has approximately 70% penetrance and variable expression (Toomes et al., 2001; Lenaers et al., 2012), and there are at least two genes, *OPA1*, *OPA3*, and three loci, including *OPA4*, *-5*, and *-8* that have been implicated in ADOA (Lenaers et al., 2012). In the report of Reynier et al. (2004), it was indicated that *OPA3* was responsible for autosomal recessive optical atrophy as well as ADOA. Genetic analysis of ADOA families identified two loci on chromosome 18q12.2-q12.3 (*OPA4*) and chromosome 22q (*OPA5*) (Kerrison et al., 1999; Barbet et al., 2005). Recently, Carelli et al. (2011) identified a new locus (*OPA8*) on chromosome 16q21-q22 in an Italian pedigree with complicated ADOA. Additional loci and genes, such as *OPA2*, *-6*, and *-7*, are described in relation to X-linked or recessive optic atrophy (Katz et al., 2006; Desir et al., 2012). However, to date, *OPA1* is the most prevalent known cause of ADOA.

*OPA1* is located on chromosome 3q28-q29 and consists of 28 coding exons that encode a 960-amino acid multifunctional mitochondrial inner membrane protein, that functions as a dynamin-like mitochondrial GTPase (Alexander et al., 2000; Delettre et al., 2001; Thiselton et al., 2001). The protein plays key roles in the mitochondrial fusion process, cristae

remodeling, and control of the release of cytochrome C (Olichon et al., 2003; Amati-Bonneau et al., 2008). Recently, mutations in *OPA1* have been shown to underlie ADOA (Fuhrmann et al., 2009; Gallus et al., 2012). In a large-scale study of suspected hereditary optic neuropathy cases, Ferré et al. (2009) found that *OPA1* mutations were identified in at least 75% of patients with ADOA. In addition, in reports from a Danish population, Almind et al. (2012) have found *OPA1* mutations in 90% of families with ADOA, including 15 novel mutations, through the use of multiplex ligation dependent probe amplification analysis and DNA sequencing. In the Chinese population, Yen et al. (2010) found that *OPA1* gene mutations were causative in Chinese patients with ADOA as well (Chen et al., 2013), and Li et al. (2008) identified an additional two novel mutations of the *OPA1* gene in Chinese families with ADOA.

Copy number variation (CNV), a type of genomic structural variation ranging over 1-kb genomic distance, can cause human genetic variation that might impact gene dosage, cause gene disruption, gene fusion, or result in position effects (Zhang et al., 2009; Conrad et al., 2010). Increasing evidence has suggested that CNVs play an important role in human disease (Fanciulli et al., 2010; Girirajan et al., 2011). In the study of Almind et al. (2011), the researchers aimed to identify CNVs in the *OPA1* gene in Danish patients with ADOA, and they found that ten families (10% of all patients) had *OPA1* deletions. However, in contrast to this report, in an Italian pedigree with complicated ADOA, CNVs were not found (Carelli et al., 2011). The role of CNV in ADOA, therefore, is still unknown. Chinese patients with ADOA have been reported to be associated with mutations in *OPA1* (Li et al., 2008; Yen et al., 2010; Chen et al., 2013); however, CNVs of the *OPA1* gene in Chinese patients has not been evaluated. In this study, we investigated genetic findings including CNVs in a Chinese pedigree with ADOA.

## MATERIAL AND METHODS

### Patients and control subjects

Four members of a Chinese pedigree (total six members) were clinically diagnosed with ADOA at the Ophthalmic Center of West China Hospital. The diagnosis was based on routine clinical procedures, including the standard of refraction and determination of best corrected visual acuity, visual evoked potential (VEP) recording, and electroretinogram (ERG) recording. We utilized unaffected relatives as control subjects. The study was approved by Ethics Committee of BGI-Shenzhen (No. 13066), and followed the tenets of the Declaration of Helsinki. Patients and control subjects were informed of the nature of the study and gave their written informed consent.

### Targeted region capture and next generation sequencing (targeted NGS)

Genomic DNA was extracted from peripheral blood collected from patients and control subjects using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. High quality genomic DNA (1 μg) was sheared to 150-250-bp fragments which were subjected to Illumina DNA library preparation (Illumina, San Diego, CA, USA). Libraries from an estimated 20 samples (4 ADOA family samples and 16 control samples for CNV identification) were pooled together, and enriched for target sequences using a NimbleGen EZ choice Probe Library (Roche Nimblegen, Madison, WI, USA) to cap-

ture the target region, which covered 283 protein-coding genes associated with ophthalmic genetic disease, and contained 1.5 Mb of capture target region. These 283 genes included the five known optic atrophy disease-causing genes, *OPA1*, *OPA3*, *TME*M126A (*OPA7*), *SOX2*, and *PAX6*. Enriched libraries were sequenced using the HiSeq2000 platform (Illumina, San Diego, CA, USA) at a paired-end read length of 90 bp according to the manufacturer protocol, to generate an output of >0.65 GB data covering the target region to depth of 200X.

## Sequence alignment and variation detection

The obtained HiSeq2000 short sequence reads were aligned to the human genome (hg19) using the BWA software (Li and Durbin, 2009). The SOAP SNP software (Li et al., 2009) was used for the detection of single nucleotide variations (SNVs). A quality control valuation (Q value) for the SNVs detection was performed by SOAP SNP, and the cut-off value was 20. Indels were identified using Samtools (Tools for alignments in the SAM format) Version: 0.1.18, http://samtools.sourceforge.net/. The variants were annotated based on the database integrated by BGI-Shenzhen.

## Mutation screening

To identify mutations, the following three-step procedure was performed: the first stage was pedigree analysis, to identify the mutations that were shared by all patients but not present in unaffected members. Next, we ascertained the allele frequency in three databases: the 1000 human genome dataset, single nucleotide polymorphism database (dbSNP), and HGMD. In addition, we sequenced 200 healthy human subjects to build our internal control database. An allele frequency of 0.01 was used as a cut off value to filter out the variants. Third, synonymous mutations were filtered out unless reflecting the pathogenic mutation, potential splice site mutations (10 bp near coding region), or coding mutations.

## CNV analysis

CNV analysis was performed based on the module described previously (Wei et al., 2014). The depth of each captured exon was adopted for z-score calculation (also named the standard score). Specifically, we used the absolute z-score (|z|) >2.58 as the cut-off value, since it represented 99% normal sample bilateral tailed regions. Regions with an absolute z-score (>2.58) were defined as deletion (<-2.58) or duplication (>2.58) regions.

$$z = \frac{\overline{X} - \mu}{\sigma}$$

In this formula:

$$\overline{X} = \mathrm{Nom}_{exon} = \frac{\text{mean depth of certain exon}}{\text{mean depth of all target region in the same sample}}$$

$$\mu = \frac{\sum \mathrm{Nom}_{exon}}{\mathrm{N}}$$

which is the average mean depth of a specific exon in all samples; and $\sigma$ is the standard deviation of a specific exon in all samples from the same batch.

The CNV identification pipeline required a large number of samples to obtain a reasonable average value of exon depth for z-score calculation in the Gaussian distribution. Four samples in this pedigree and 16 control samples in the same batch were pooled (as mentioned in materials and methods) to establish a standard for z-score calculation to analyze CNVs. We focused on CNVs for the candidate pathogenic genes *OPA1*, *OPA3*, *TME*M126A, *SOX2*, and *PAX6*.

## Real-time polymerase chain reaction (PCR)

DNA quantification PCR for *OPA1* was carried out in triplicate using an ABI 7500 real-time PCR system (Applied Biosystems Inc., Foster City, CA, USA). Amplification of complementary DNA was performed using SYBR Green PCR Master Mix (ABI) and initiated by the polymerase activation step for 15 sec at 95°C. Amplification was obtained by 40 cycles of 30 s at 60°C with a 1-min annealing and extension step at 72°C. Relative gene expression was determined using a previously described method (Livak and Schmittgen, 2001). Primer sequences are summarized in Table 1.

**Table 1.** Sequences of the PCR primers.

| Primer | Sequence |
|---|---|
| exon1 | F: 5'-TCAGGCTCTTGCGGAAGTCCAT-3' |
| | R: 5'-GTCCAGGAATGACCCAGGAAGTG-3' |
| exon9 | F: 5'-TGTGGGAAGGTTGTTGTGGTTGG-3' |
| | R: 5'-AATATTCGAGCTTGGGCAATCATTTCC-3' |
| intron3 | F: 5'-AGGCTGAAGTGCGGTCTTGGA-3' |
| | R: 5'-ACTTGGGAGGCAGAGTCGGAAG-3' |
| ACTB | F: 5'-TGTCCACCTTCCAGCAGATGT-3' |
| | R: 5'-GCAACTAAGTCATAGTCCGCCTAG-3' |

PCR = polymerase chain reaction; ACTB = β-Actin.

# RESULTS

## Clinical characteristics in patients with ADOA

We have identified a Chinese pedigree consisting of four patients diagnosed with optic atrophy and two unaffected relatives (Figure 1). Clinical data are presented in Table 2. The proband (106-3) and two patients (106-2 and 106-5) had VEP recordings with prolonged latencies of the P100 waves and decreased amplitude. Only one patient (106-1) had VEP recordings solely with prolonged latency of the P100 wave. Two unaffected relatives exhibited normal ERG and VEP recordings.

## Mutation screening of candidate genes

Targeted NGS was performed on the DNA samples; the results are shown in Table 3. There were 50.69, 50.60, 50.39, and 51.74% reads of the target region with mean coverage depth of 271.16, 388.29, 242.76, and 390.4 in the samples 106-1, 106-3 106-5, and 106-6, respectively. The percentages of the target region covered by at least 20X for each sample were

 

98.32, 97.01, 96.96, and 97.47%, respectively, which suggested that sufficient coverage depth was achieved for variant calling (Table 3).

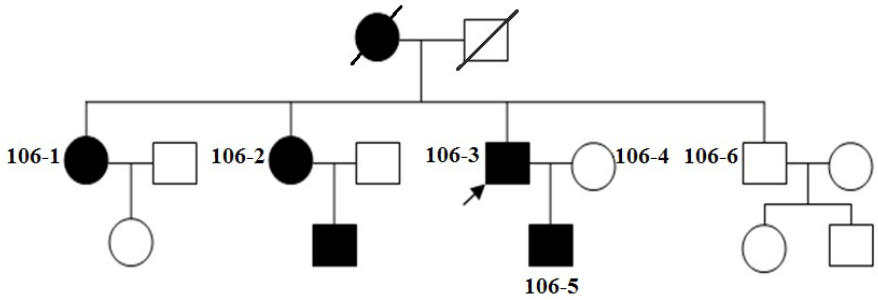


**Figure 1.** Pedigree 106 with ADOA. Index patient is indicated with an arrow. Slashed symbols are deceased members, filled symbols are individuals affected with ADOA, and open symbols are unaffected members. ADOA, autosomal dominant optic atrophy.

**Table 2.** Clinical characteristics of the pedigree members.

| Pedigree member | Gender | Age | Best corrected visual acuity | | ERG | VEP |
|---|---|---|---|---|---|---|
| | | | OD | OS | | |
| 106-1 | F | 58 | 0.7 | 0.7 | Normal | Prolonged latency |
| 106-2 | F | 56 | 0.2 | 0.1 | Normal | Prolonged latency and diminished amplitude |
| 106-3 | M | 54 | 0.1 | 0.1 | Normal | Prolonged latency and diminished amplitude |
| 106-4 | F | 50 | 1 | 1.2 | Normal | Normal |
| 106-5 | M | 27 | 0.06 | 0.1 | Normal | Prolonged latency and diminished amplitude |
| 106-6 | F | 46 | 1.2 | 1.2 | Normal | Normal |

F = female; M = male; OD = right eye; OS = left eye; ERG = electroretinogram; VEP = visual evoked potential.

The initial variant (SNVs + Indels) numbers identified were 3423, 3492, 3250, and 3865 in samples 106-1, 106-3, 106-5, and 106-6, respectively (Tables 4 and 5). After pedigree analysis and filtering against the dbSNP, 1000 Genome database, and HapMap, the numbers of rare variants were reduced to 22. Following exclusion of the variants found in the control samples, eight rare variants were found in all patient samples (106-1, 106-3, and 106-5) (Table 6). These eight variants were captured, and were found to be located within the G protein-coupled receptor 98 (*GPR98*), retinitis pigmentosa 1-like 1 (*RP1L1*), and fukutin related protein (*FKRP*) genes (Table 7). No other variants associated with pathogenic genes related to ADOA were found.

## CNV analysis

CNV was analyzed by a homemade pipeline of BGI-Shenzhen. The z-score of *OPA1* exons 1-2 in was less than -2.58 in all three affected members (106-1, 106-3, and 106-5) except for the unaffected member (106-6), which suggested that a heterozygous large deletion might have occurred in *OPA1*. To further analyze the CNV in the *OPA1* gene, the normalized depth of 29 exons in the *OPA1* gene was obtained (Figure 2), demonstrating that the normalized depth of exons 1-9, as well as exons 1-2, of the *OPA1* gene in the three patients showed similar low levels when compared with those in individual 106-6 and with other exons.

**Table 3.** Summary statistics for targeted NGS on 4 samples.

| | 106-1 | 106-3 | 106-5 | 106-6 |
|---|---|---|---|---|
| Number of targeted regions | 5,403 | 5,403 | 5,403 | 5,403 |
| Total length of targeted regions | 1,505,650 | 1,505,650 | 1,505,650 | 1,505,650 |
| Total number of reads | 8,564,664 | 12,216,712 | 7,719,174 | 12,091,644 |
| Number of aligned reads | 8,521,008 | 12,168,171 | 7,681,211 | 11,991,291 |
| Percentage of aligned reads | 0.994903 | 0.996027 | 0.995082 | 0.991701 |
| Number of reads of targets | 4,319,546 | 6,157,701 | 3,870,659 | 6,204,260 |
| Percentage of reads of targets | 50.69% | 50.60% | 50.39% | 51.74% |
| Total number of bases (Mb) | 770.8198 | 1,099.504 | 694.7257 | 1,088.248 |
| Number of bases of targets (Mb) | 408.27 | 273.25 | 365.5 | 587.8 |
| Percentage of bases of targets | 53.24% | 53.38% | 52.87% | 54.47% |
| Number of reads near targets | 801,522 | 10,099,955 | 6,489,280 | 10,140,445 |
| Percentage of reads near targets | 84.68% | 83.00% | 84.48% | 84.57% |
| Mean depth of targets | 271.16 | 388.29 | 242.76 | 390.4 |
| Target coverage 4X | 98.80% | 97.90% | 97.95% | 98.19% |
| Target coverage 10X | 98.54% | 97.49% | 97.49% | 97.88% |
| Target coverage 20X | 98.32% | 97.01% | 96.96% | 97.47% |

NGS = next generation sequencing.

**Table 4.** Summaries of SNVs.

| | 106-1 | 106-3 | 106-5 | 106-6 |
|---|---|---|---|---|
| Total number of SNVs | 2651 | 2743 | 2528 | 3109 |
| Missense SNVs | 369 | 395 | 401 | 499 |
| Nonsense SNVs | 14 | 10 | 9 | 11 |
| SNVs in splice sites | 123 | 117 | 96 | 121 |
| Readthrough | 1 | 1 | 2 | 2 |
| SNVsinCDS: synonymous | 398 | 367 | 332 | 470 |
| SNVs in 5'-UTR | 135 | 126 | 125 | 189 |
| SNVs in introns | 1129 | 1226 | 1029 | 1243 |
| Intergenic SNVs | 47 | 53 | 53 | 54 |

SNV = single nucleotide variant; CDS: coding DNA sequence.

**Table 5.** Summaries of indels.

| | 106-1 | 106-3 | 106-5 | 106-6 |
|---|---|---|---|---|
| Total number of indels | 772 | 749 | 722 | 756 |
| Frameshift indels | 0 | 2 | 1 | 2 |
| CDS_Indel | 3 | 2 | 2 | 2 |
| Indels in splice sites | 16 | 14 | 19 | 16 |
| Indels in 5'-UTR | 7 | 7 | 7 | 7 |
| Indels in 3'-UTR | 75 | 74 | 76 | 72 |
| Indels in introns | 562 | 526 | 531 | 544 |
| Intergenic indels | 109 | 124 | 86 | 113 |

CDS = coding DNA sequence.

**Table 6.** Gene mutations detected in patients with ADOA.

| | 106-1 | 106-3 | 106-5 |
|---|---|---|---|
| Total (SNVs + indels) | 3423 | 3492 | 3250 |
| Detected in patients only; not in controls | | 133 | |
| Rare variants | | 22 | |
| Functional + synonymous | | 8 | |
| Functional | | 8 | |

ADOA = autosomal dominant optic atrophy; SNV = single nucleotide variant.

**Table 7.** Gene mutations detected in patients with ADOA.

| Gene | Mut_name | Mut_type | NM_ID |
|---|---|---|---|
| GPR98 | c.11581-2A>C | Het | NM_032119 |
| RP1L1 | p.Glu1351Ala | Het | NM_178857 |
| RP1L1 | p.Gln1349Lys | Het | NM_178857 |
| RP1L1 | p.Gly1348Arg | Het | NM_178857 |
| RP1L1 | p.Glu1347Lys | Het | NM_178857 |
| RP1L1 | p.Thr1341Ala | Het | NM_178857 |
| RP1L1 | p.Gln1302Arg | Het | NM_178857 |
| FKRP | p.Glu343Gln | Het | NM_024301 |

All the mutations in this table were absence in dbSNP (database of single nucleotide polymorphisms), HapMap database, 1000 genomes database and local database; ADOA = autosomal dominant optic atrophy; Mut = mutation.

To test whether the deletion mutation led to a loss of expression of the nine exons in the *OPA1* gene, a real-time PCR assay was performed to amplify exons 1-9 of *OPA1* in the three patients and two unaffected relatives. The results (Figure 3) showed that no difference in expression of *OPA1* exons 1-9 in two normal individuals (106-4 and 106-6) was observed compared to YH1000 and YH200 (two normal control samples) while patients 106-1, 106-3, and 106-5 had decreased *OPA1* exon 1-9 expression compared to the normal individuals (106-4 and 106-6). This finding suggested that exons 1-9 were deleted in the *OPA1* gene of patients in accordance with their clinical phenotype.

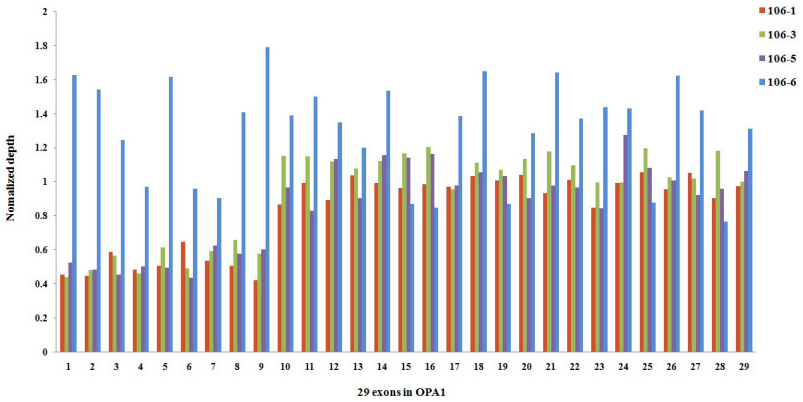


**Figure 2.** Sequencing depth of 29 OPA1 exons in *OPA1*. Copy number variation was analyzed by a homemade pipeline of BGI-Shenzhen. The depth was normalized twice; first, the sequencing depth of sample M was normalized:

$$ND_{sampleM} = \frac{\text{sequencing depth of sampleM}}{\text{average sequencing depth of all 20 samples from the same batch}}$$

Second, the depth of exon N in sample was transformed according to $ND_{sampleM}$:

$$TD_{exonN} = \frac{\text{sequencing depth of exonN in sampleM}}{ND_{sampleM}}$$

Finally, the sequencing depth of exon N was normalized:

$$ND_{exonN} = \frac{TD_{exonN}}{\text{average of } TD_{exonN} \text{ in all 20 samples}}$$

ND: normalized depth; TD: transformed depth.

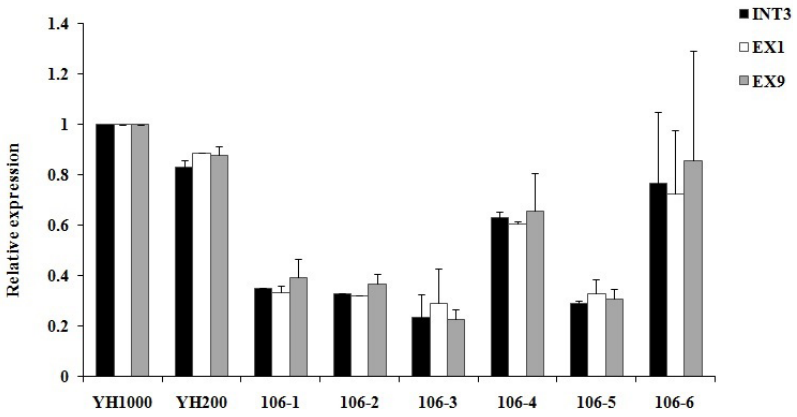


**Figure 3.** Expression of *OPA1* exons 1-9 in members of pedigree 106. Quantification of relative gene expression for exons 1-9 of *OPA1* was carried out in triplicate in an ABI 7500 real-time PCR system. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. Data are reported means ± SD of three independent experiments. PCR, polymerase chain reaction; SD, standard deviation. YH1000 and YH200 (two normal control samples).

## DISCUSSION

In this study, we examined patients from a Chinese pedigree with clinically diagnosed ADOA and screened pathogenic genes related to ADOA. We identified eight rare variants in three genes in all patients, none of which were known as pathogenic genes related to ADOA. However, using copy number variation analysis, we also found heterozygous mutations in exons 1-9 of the *OPA1* gene, which is the gene most commonly associated with ADOA, and further confirmed defective gene expression of exons 1-9 of the *OPA1* gene in patients with ADOA.

*OPA1* is the primary gene associated with ADOA, and numerous mutations have been reported in this gene in patients with ADOA. In Denmark, mutations in *OPA1* have been identified in 90% of families, including 15 novel mutations (Almind et al., 2012). *OPA1* mutations were also found in patients with ADOA from Japan, Greece, and Saudi Arabia (Hayashi et al., 2007; Galvez-Ruiz et al., 2013; Kamakari et al., 2014). In China, *OPA1* mutations in patients with ADOA have also been reported. Li et al. (2008) found a novel heterozygous splicing site mutation (c.985 -2A>G) in intron 9 in one family, and detected a novel heterozygous nonsense mutation (c.2197C>T; p.R733X) in another family after sequencing of the *OPA1* gene. In the report of Yen et al. (2010), an additional four *OPA1* gene mutations were detected. However, to our knowledge, our report represents the first case of ADOA caused by CNV of the *OPA1* gene in the Chinese population.

In this study we identified three rare variants in patients, which were located in the *GPR98*, *RP1L1*, and *FKRP* genes. It has previously been reported that mutations of *GPR98* are associated with Usher syndrome 2 and familial febrile seizures (Nakayama et al., 2002; Ebermann et al., 2009); *RP1L1* gene mutations cause occult macular dystrophy (Hayashi et al., 2012); and mutations in *FKRP* have been associated with congenital muscular dystrophy, mental retardation, and cerebellar cysts (Topaloglu et al., 2003). In previous studies, however, none of these genes were suggested to be involved in ADOA. In addition, after analysis of CNVs, we detected mutation in exons 1-9 of the *OPA1* gene in all patients; this finding was also confirmed by real-time PCR. In our study, patient 106-1 was considered as a suspected

case of optical atrophy. The clinical report of 106-1 showed VEP recordings with prolonged latency of the P100 wave, but no diminished amplitude as had been recorded in other patients. However, in the analysis of CNV, we found heterozygous deletion of exons 1-9 of the *OPA1* gene in patient 106-1, as had been found in the other patients. Furthermore, sample 106-1 had defective gene expression of *OPA1* exons 1 and 9. Accordingly, we confirmed 106-1 as an ADOA patient. Therefore, based on to this patient's clinical manifestation, a differential phenotype might be observed caused by the same mutation within the family. We also note that no obvious genotype-phenotype correlations have been detected between the degree of visual impairment and the type of mutation in *OPA1* (Puomila et al., 2005).

Rare CNVs primarily are responsible for hereditary diseases. In some genetic diseases, CNVs might lead to the deletion or amplification of specific genes, exons, or regulatory sequences (Cusco et al., 2008; Mefford et al., 2009). The general approach for detection of CNVs in genomes is array-based comparative genomic hybridization (array-CGH); however, in recent years, NGS has developed into a popular strategy for genotyping and has been applied to the identification of CNVs in patients and in healthy subjects (Zhao et al., 2013). In the study of Wei et al. (2014), a novel method to identify CNVs and SNVs at the same time using a targeted NGS approach was developed. Here, we used this targeted NGS approach to analyze CNVs and found a CNV resulting in the deletion of exons 1-9 of the *OPA1* gene. These results might suggest that the study of human disease by targeted NGS data should be supplemented by other approaches such as arrayCGH or genotyping arrays, extending the application of this useful technique for studying human disease. In the study of ADOA, little evidence of CNVs in patients has been reported. In a report of Danish patients with ADOA, it was found that ten in one hundred Danish families had *OPA1* deletions, including two with deletions of the entire coding region and eight with intragenic deletions (Almind et al., 2011). In accordance with these findings, we identified a deletion of exons 1-9 in the *OPA1* gene, but did not find any point mutations in *OPA1*. In contrast, in an Italian pedigree with ADOA, a new locus (*OPA8*) on chromosome 16q21-q22 was identified, but CNVs were not found (Carelli et al., 2011). The different results in the pedigrees with ADOA might be a consequence of the differences in the human species (genetic background) or the use of different methodologies to detect CNVs. However, CNVs might still provide a potential method to confirm the diagnosis of ADOA.

In summary, we evaluated CNVs in the *OPA1* gene in a Chinese pedigree with ADOA and found a deletion of exons 1-9 of the *OPA1* gene by targeted NGS. Our findings suggest that CNV detection might be useful in making the diagnosis of ADOA in the Chinese population and that targeted NGS might extend the ability to detect CNVs in ADOA.

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