Research Article

Coding Region Mutation Screening in Optineurin in Chinese Normal-Tension Glaucoma Patients

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Purpose. To study the roles of sequence alterations in the optineurin (OPTN) gene-coding region in normal-tension glaucoma (NTG) among Chinese patients.

Methods. Genomic DNA was extracted from 190 NTG patients and 201 control subjects. The thirteen exons of OPTN were amplified by polymerase chain reaction and analyzed by direct sequencing. Detected sequence changes were compared between NTG patients and control subjects.

Results. Seven sequence changes in OPTN were identified in both NTG patients and control subjects. Among them, c.464G>A (T34T), c.509C>T (T49T), c.806G>A (V148V), and c.959T>C (P199P) were synonymous codon changes, whilst c.655T>A (M98K), c.1996G>A (R545Q), and c.1582T>C (I407T) were missense changes. Two previously reported heterozygous mutations, c.458G>A (E50K) in exon 4 and c.691_692insAG in exon 6, were not found in this study. Out of these seven OPTN sequence variants, c.464G>A (T34T) was significantly associated with NTG in both the allelic and genotypic association analyses (allelic association: \( p = 0.0001, \text{OR} = 2.20, 95\% \text{ CI: 1.46-3.31}; \) genotypic association: \( p = 0.0001 \)), whereas the association of other variants with NTG did not reach statistical significance (\( p > 0.05 \)). Variants c.1582 T>C (I407T) and c.806G>A (V148V) were identified in one and two NTG patients, respectively, but not in the control subjects.

Conclusions. This study confirmed the association of the OPTN T34T variant with NTG, suggesting that OPTN is a susceptibility gene for NTG in Chinese. Moreover, a variant with amino acid change (I407T) was identified in NTG but not in controls. Further studies are warranted to assess whether this variant is a causative mutation for NTG.

1. Introduction

Glaucoma, a leading cause of irreversible blindness worldwide [1], is a heterogeneous group of optic neuropathies characterized by progressive degeneration of the optic nerve, and it could be detected clinically as cupping of the nerve head, thinning of the retinal nerve fiber layer, and typical visual field defect [2]. According to the morphology of the anterior chamber angle, primary glaucoma can be classified into primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG). According to the highest intraocular pressure (IOP), POAG has been conventionally divided into high-tension POAG (HTG, IOP > 21 mmHg) and normal-tension POAG (NTG, IOP ≤ 21 mmHg).

Currently, the molecular basis of NTG is not fully understood. Previous studies have reported gene mutations in NTG patients, like endothelin-1 (EDN1), optic atrophy type 1 (OPA1), and optineurin (OPTN) [3–5]. In 2002, Rezaie et al. identified OPTN mutations that were responsible for some of the hereditary NTGs [5]. There are 16 exons in the OPTN gene. Exons 1-3 are noncoding sequence, and exons 4-16 code for a 577-amino acid protein. Four mutations in OPTN, c.458G>A (E50K), c.655T>A (M98K), c.1996G>A (R545Q), and c.691_692insAG (2 bp “AG” insertion) were detected from 54 families with adult-onset POAG in which most families displayed normal IOP [5]. Subsequently, disease-associated variants have been found by other investigators. One of the rare mutations, E50K, showed a strong association with POAG, particularly with NTG [6].

The prevalence of NTG in Asia is higher compared that in Caucasian and African populations [7]. In China, the proportion of NTG in POAG was as high as 85% in Guangzhou, a city in southern China, and 90% in the Handan Eye study in northern China [8, 9]. It is important to identify gene
mutations and genetic association with NTG in Asians. In this study, we sought to identify OPTN coding sequence alterations in Chinese population and their association with NTG in order to compare findings from other populations and to identify novel OPTN associations in NTG.

2. Subjects and Methods

2.1. Case and Control Study Subjects. Unrelated patients with NTG were recruited from the Eye Centre, Prince of Wales Hospital, and Hong Kong Eye Hospital, Hong Kong. The study protocol was approved by the Ethics Committee for Human Research of the Chinese University of Hong Kong and adhered to the tenets of the Declaration of Helsinki. Informed consents were obtained from all study subjects. Diagnosis was based on meeting all the following criteria: exclusion of secondary cause (steroid-induced glaucoma, neovascular glaucoma, uveitis, or trauma); anterior chamber angle open (grades III or IV on gonioscopy); characteristic optic disc changes (vertical cup-to-disc ratio is >0.5, disc hemorrhage, or thin/notched neuroretinal rim); and characteristic visual field changes with reference to Anderson’s criteria for minimal abnormality in glaucoma [10]. Visual field was evaluated by a perimeter (Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA, USA) using the Glaucoma was evaluated by a perimeter (Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA, USA) using the Glaucoma.

2. Subjects and Methods

2.2. Polymerase Chain Reaction. Genomic DNA was extracted from whole blood by the QIAamp Blood Kit (Qiagen, Hilden, Germany). 13 pairs of PCR and sequencing primers were designed according to all coding sequence of OPTN, including intron-exon boundaries (Table 1). PCR was performed on a PCR machine (C1000 Touch, Bio-Rad, Hercules, CA, USA), with an initial denaturation step of 5 minutes at 95°C, followed by 45 cycles of 95°C for 30 seconds, annealing temperature of 55–62°C for 30 seconds, 72°C for 1 minute, and a final extension step of 72°C for 5 minutes. Each 25 μL reaction contained 1.5–2.5 mM MgCl₂, 1 U Tag polymerase (AmpliTag Gold, Biosystems, Foster City, CA, USA), 200 μM dNTPs, 1 μL genomic DNA, and 400 pM primers. Agarose gel electrophoresis was performed after PCR to confirm the amplification efficiency.

2.3. DNA Sequencing. PCR products were sequenced using a cost-saving protocol on an automated DNA sequencer (model: ABI 377XL; Applied Biosystems, Foster City, CA, USA) [13]. Sequence data were aligned with sequence-analysis software (BioEdit, version 7.0.5.3, Carlsbad, CA, USA) and compared with the published OPTN gene sequence (Ensemble transcript ID: ENST00000378748.7, provided in the public domain by European Molecular Biology Laboratory’s European Bioinformatics Institute, Hinxton, Cambridge, UK; NCBI reference sequence: NM_001008211, provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, USA).

2.4. Statistical Analysis. The frequencies of various alleles and genotypes between NTG patients and control subjects were compared using the χ² test or Fisher’s exact test. p < 0.05 was considered statistically significant.

3. Results

3.1. OPTN Variants Detected in the Study Subjects. Seven sequence changes in OPTN were identified in this study: four were synonymous codon changes and three were missense changes (Table 2). We found c.655T>A (M98K), c.1996G>A (R545Q), c.464G>A (T34T), c.509C>T (T49T), and c. 959T>C (P199P) in both NTG patients and control subjects. Among these mutations, some of them were also identified as homozygotes in NTG subjects: M98K was found in 6 NTG patients and 4 controls, while T34T was observed in 9 NTG patients. Two other mutations, c.1582T>C (I407T) and c.806G>C (P269P) in both NTG patients and control subjects.

3.2. Distribution of OPTN Variants in NTG Patients and Control Subjects. T34T was found to be significantly associated with NTG when comparing the allelic and genotypic frequencies between NTG patients and control subjects (allelic association: p = 0.0001, OR = 2.20, 95% CI: 1.46-3.31; genotypic association: p = 0.0001), whereas the association of other variants with NTG did not reach a statistical significance (p > 0.05).

4. Discussion

Optineurin is a receptor protein of autophagy. There is a LC3-interacting region (LIR) and an ubiquitin-binding domain (UBD) in OPTN, which could guide the transportation of ubiquitylated cargos into autophagosome, a
specialized organelle to degrade targeted molecules. Autophagy is a dynamic process delivering cytosolic materials to lysosome for degradation which is a key process in maintaining cellular and tissue homeostasis [14]. Autophagy was reported to be associated with the pathogenesis of glaucoma [15]. The decrease of autophagic activity was observed in porcine trabecular meshwork (TM) cells which may represent the progressive failure of cellular TM function and contributes to the pathogenesis of POAG [16]. Accumulation of autophagic vacuoles and autophagosomes was also found in retinal ganglion cells (RGCs) of glaucoma animal models which may be related to the pathogenesis of glaucoma [17, 18].

Three variants found in the present study involve codon changes. M98K has been reported to be associated with POAG [5]. However, our data showed no significant difference in the prevalence between NTG patients and controls. M98K is located in the Tank-binding kinase 1 (TBK1) binding domain of OPTN. Binding between the OPTN and

Table 1: Oligonucleotide primers used for PCR and sequencing of OPTN in NTG patients.

| Primer    | Sequence (5′ to 3′) | Amplicon Size (bp) | MgCl₂ (mM) | Anneal Temp (°C) |
|-----------|---------------------|--------------------|------------|------------------|
| OPTN-3/4F | TTTCTGAAGCTACATATACCTTT | 445 | 2.5 | 62-55 (TD) |
| OPTN-3/4R | CTACCACCAAACGCCCTTACTTG | | | |
| OPTN-5F  | GCCATGCTTGCAAATCCCTTACCTTT | 362 | 1.5 | 62-55 (TD) |
| OPTN-5R  | AATCCCTGGTCTGTGGTGAACCTTATT | | | |
| OPTN-6F  | GTGCCCCACTGCTTGGATGCACTT | 336 | 1.5 | 58 |
| OPTN-6R  | CAGTTTAAATCTCCCTTCATTTT | | | |
| OPTN-7F  | CTCAGGTTCAACACATTGGAACCTT | 202 | 1.5 | 55 |
| OPTN-7R  | CTTGCGGCGTATGCGGAAATTTT | | | |
| OPTN-8F  | CAGTTTTTTAAATCTCCCTTACCT | 381 | 1.5 | 58 |
| OPTN-8R  | CCTGATCTCCCTTATCCCAATTTTG | | | |
| OPTN-9F  | GTAGTGCGGCTTGAATCTTTA | 244 | 1.5 | 58 |
| OPTN-9R  | GGCTACATAATGTGTTCTGAGCCTG | 239 | 1.5 | 62-55 (TD) |
| OPTN-10F | TGCCACAAAGCTGGGCTGAA | | | |
| OPTN-10R | TATTGGAATTTTCTCTTCAAAAC | | | |
| OPTN-11F | TGCCGATTTAAGGGAAGGACTTG | 230 | 1.5 | 62-55 (TD) |
| OPTN-11R | TCCGATCTGCCCTTCTGACCTCA | | | |
| OPTN-12F | TGGAGGCGCAAGACTATAAGGT | 221 | 1.5 | 55 |
| OPTN-12R | CGTTCAACACGTTCCTGCTTATT | | | |
| OPTN-13F | CAGGAGCAAGTATTTCCTCAAAAC | 273 | 1.5 | 62-55 (TD) |
| OPTN-13R | TTCCCATGGAACACATACAG | | | |
| OPTN-14F | CTCCTCATGCGATCCAAACACTGT | 267 | 1.5 | 58 |
| OPTN-14R | GGCACCTCTCTTCGCG GCC | | | |
| OPTN-15F | ACTTTCTGGACTCTTCTGCTC | 263 | 1.5 | 62-55 (TD) |
| OPTN-15R | TGATTTGGAATTCACCTTAGAG | | | |
| OPTN-16F | TGCCACATTTCCTTCATCAAGT | 272 | 1.5 | 58 |
| OPTN-16R | CACAAAAGCAGAACACTTGGGA | | | |

TD: touchdown PCR using a cycling program where the annealing temperature is gradually reduced by 0.2°C per cycle.

Table 2: OPTN variants observed in 190 NTG patients and 201 control subjects.

| Location | Sequence change | Codon change | Allele frequency (%) | Genotype frequency |
|----------|----------------|--------------|----------------------|-------------------|
| Exon 5   | c.655T>A       | M98K         | NTG 56 (14.7) Control 56 (13.9) p value 0.75 1.07 0.72-1.59 | NTG 6/44/140 Control 4/48/149 p value 0.76 |
| Exon 12  | c.1582T>C      | I407T        | NTG 1 (0.3) Control 0 (0.0) p value 0.49 N/A N/A | NTG 0/1/189 Control 0/0/201 p value 0.49 |
| Exon 16  | c.1996G>A      | R545Q        | NTG 12 (3.2) Control 15 (3.7) p value 0.66 0.84 0.39-1.82 | NTG 0/12/178 Control 0/15/186 p value 0.69 |
| Exon 3/4  | c.464G>A       | T34T         | NTG 76 (20) Control 41 (10.2) p value 0.0001 2.20 1.46-3.31 | NTG 9/58/123 Control 0/41/160 p value 0.0001 |
| Exon 3/4  | c.509C>T       | T49T         | NTG 7 (1.8) Control 7 (1.7) p value 0.92 1.06 0.37-3.05 | NTG 0/7/183 Control 0/7/194 p value 1.00 |
| Exon 6   | c.806G>A       | V148V        | NTG 2 (0.5) Control 0 (0.0) p value 0.24 N/A N/A | NTG 0/2/188 Control 0/0/201 p value 0.24 |
| Exon 7   | c.959T>C       | P199P        | NTG 2 (0.5) Control 1 (0.2) p value 0.61 2.12 0.19-23.50 | NTG 0/2/188 Control 0/1/200 p value 0.61 |

N/A: not applicable.
ubiquitin polypeptide chains could be enhanced by TBK1-mediated phosphorylation on optineurin [19]. Previous studies found that OPTN M98K could activate TBK1, which in turn enhanced the phosphorylation on Serine-177 on OPTN. The phosphorylated OPTN could enhance autophagosome formation and lead to retinal cell death [20, 21]. Previously, OPTN I407T was only found in HTG patients [22]. In this study, I407T was found in a NTG patient but not in control subjects. I407T is located in the coiled-coil region of OPTN. How I407T influences OPTN function is still not very clear. R545Q were located in exon 16 and are near the zinc finger domain within OPTN. Although it was reported in one NTG study before [5], our current study and two previous studies did not detect an association of R545Q with NTG [23, 24]. T34T was the only variant that showed significant association with NTG in our cohort. Moreover, it is the first time that I407T was found exclusively in a NTG patient. Future studies are needed to confirm more OPTN mutations and dissect the pathological roles of OPTN in NTG.

Data Availability

Data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare no conflict of interest.

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