Eye (2015) 29, 972–977

www.nature.com/eye

LABORATORY STUDY

A novel truncation mutation in *GJA1* associated with open angle glaucoma and microcornea in a large Chinese family

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Abstract

Purpose **To identify genetic defects in a large family with open angle glaucoma (OAG) and microcornea.**

Methods **Genomic DNA was prepared from leukocytes of 15 individuals from three generations of a Chinese family, including seven individuals with OAG and microcornea, one with microcornea alone, and seven healthy individuals. Whole exome sequencing was performed on genomic DNA of the proband. Candidate variants were obtained through multiple steps of bioinformatics analysis and validated by Sanger sequencing and segregation analysis.**

Results **Exome sequencing detected a candidate variant in *GJA1,* a novel truncation mutation (c.791_792delAA, p.K264Ifs*43). This mutation was present in all seven individuals with OAG and microcornea and the individual with microcornea alone, but not in the seven unaffected relatives in the family. It was not present in 1394 alleles from 505 unrelated controls without glaucoma and 192 normal controls. Extraocular signs were not observed in seven out of the eight individuals; only one was affected with dental enamel hypoplasia and syndactyly.**

Conclusions **A novel truncation mutation in *GJA1* is associated with OAG and microcornea in a Chinese family. This suggests that *GJA1* should be included as a candidate gene for glaucoma.**

Eye (2015) **29,** 972–977; doi:10.1038/eye.2015.74; published online 15 May 2015

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Received: 17 June 2014
Accepted in revised form: 17 March 2015
Published online: 15 May 2015

Introduction

Glaucoma is a neurodegenerative disease characterised by typical visual dysfunction and structural damage to the optic nerve.[1] The prevalence of primary open angle glaucoma (OAG) was 2.6% in Chinese with age over 40 years.[2] It is widely accepted that reduced drainage of aqueous humor is the main cause for elevated intraocular pressure (IOP), which is associated with retina ganglion cell loss.[3] Both genetic and environmental factors are involved in the development of glaucoma.[3] A few genes are associated with primary OAG,[4–8] but mutations in these genes only account for 5–7.8% of cases.[9,10] The molecular basis of OAG in most patients has yet to be identified.

We sampled individuals from a large Chinese family with OAG and microcornea. In this study, the genetic defect in the family was investigated by whole exome sequencing and a novel truncation mutation in *GJA1* was identified.

Materials and methods

The proband from a Chinese family with OAG and microcornea was collected from the Zhongshan Ophthalmic Center as part of an ongoing program to identify the genes responsible for glaucoma. An additional 14 family members were collected for segregation analysis thereafter. Written informed consent was obtained from the participants or their guardians before the study. Genomic DNA was prepared from the peripheral venous leukocytes of 15 subjects from the family by using procedures described in a previous study,[11] including seven individuals with OAG and microcornea, one individual with microcornea alone, and seven unaffected relatives. The diagnostic criteria for OAG is as follows:[12] IOP over 21 mm Hg; glaucomatous optic neuropathy (vertical cup-to-disc ratio ≥ 0.7, asymmetry ≥ 0.2, rim width ≤ 0.1 or disc hemorrhage); glaucomatous visual field defect meeting the Hodapp–Parrish–Anderson criteria for early defect;[13] and open anterior

chamber angle on gonioscopy (Shaffer grades III or IV). Microcornea refers to a horizontal corneal diameter <10 mm.[14] The clinical data of four patients (II:1, II:6, II:8, and III:6) were in consensus with the diagnostic criteria of OAG and microcornea; one patient (III:3) reached the diagnostic criteria of microcornea alone, and the other three patients (II:3, III:4, and III:5) were diagnosed as OAG and microcornea by the same ophthalmologist, although detailed clinical data were not available. Our study was consistent with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. We certified that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Genomic DNA from the proband was initially analyzed by whole exome sequencing using a procedure similar to that described in our previous study.[9] The variants detected in proband III:4 were filtered using the following steps: (1) variations predicted to affect the coding residues or mRNA splicing were selected; (2) after comparison with the 1000 Genomes Project database, variants with a minor allele frequency (MAF) <0.01 were selected; (3) variants in genes associated with ocular diseases were selected; (4) the remaining variants were compared with ethnicity-matched regional controls (195 patients with high myopia and 310 patients with hereditary retinal diseases but not glaucoma based on our unpublished data of whole exome sequencing on patients with hereditary eye diseases) to exclude ethnicity-specific polymorphisms (MAF ≥ 0.01); (5) effects of the remaining variants on coding residues of the remaining variants were analyzed using Polyphen-2 and SIFT;[15,16] (6) the remaining variants predicted to have damaging effects on coding sequence and to affect splicing were confirmed by Sanger sequencing and validated in family members, as well as in 192 normal controls. A pair of primers used to confirm the novel variant in *GJA1* were designed using the Primer3 online tool (http://frodo.wi.mit.edu/primer3/):[17] *GJA1*-Forward 5′-aaaagagatccctgcccaca-3′ and *GJA1*-Reverse 5′-aggctgttgagtaccacctc-3′. The polymerase chain reaction was used to amplify the target variants, and the amplicons were analyzed with an ABI BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI3130 Genetic Analyzer (Applied Biosystems) as described in a previous study.[9]

Results

Overall, 74 740 single nuclear variants, insertions, and deletions were captured by whole exome sequencing on the proband. After filtering the whole exome sequencing data, one novel candidate heterozygous variant (c.791_792delAA, p.K264Ifs*43) in *GJA1* was identified and subsequently confirmed in the proband by Sanger sequencing. This variant was neither present in databases (1000 Genomes Project and Exome Variant Server) nor in 1394 unrelated ethnicity-matched control alleles (192 normal controls, 195 patients with high myopia, and 310 patients with hereditary retinal diseases based on our unpublished data of whole exome sequencing on patients with hereditary eye diseases). It was present in the seven individuals with OAG and microcornea and the one with microcornea alone but absent in the seven unaffected relatives (Figure 1). By using this mutation as a marker, two-point linkage analysis yielded a maximum lod score of 2.709 at $\theta=0$, theoretically the maximum lod score for this type of family.

Clinical data for the family members participating in this study are listed in Table 1. The c.791_792delAA mutation was present in all eight individuals with microcornea, in whom the cornea diameter ranged from 9.0 to 9.5 mm. Of these eight individuals, seven had OAG, as diagnosed based on increased IOP, degenerative changes to the optic disc and the retina ganglion cell layers, and/or typical glaucomatous visual field defects (Figure 2). The individual with microcornea alone had no detectable signs of glaucoma when examined at the age of 29 years, but was at risk of developing glaucoma. Of these eight individuals, none had a remnant pupillary membrane, porous and spongy iris tissue, aniridia, or posterior embryotoxon. Systemic examination of the eight individuals identified syndactyly, camptodactyly, and dental enamel hypoplasia in one individual (III:6) and palmoplantar keratodermas in another individual (II:8). None of the eight individuals were observed to have paralysis, mental retardation, congenital cardiovascular defects, or hearing loss.

Discussion

In this study, we report on a large Chinese family with OAG and microcornea. For these conditions, glaucoma is the most prominent sign that brings the individuals to the hospital for treatment. Except for OAG and microcornea, most patients in the studied family do not have other noticeable ocular or systemic anomalies. A novel truncation mutation (c.791_792delAA, p.K264Ifs*43) in *GJA1* that cosegregates with the disease has been identified in this family. This mutation is predicted to result in complete loss of function of the coded protein by the mutant allele and is absent in 1394 ethnicity-matched control alleles.

Previously, based on the HGMD database (http://www.hgmd.org/, accessed on 22 May 2014), 92 mutations have been identified in *GJA1*, including 82 missense mutations, two nonsense mutations, four in-frame insertion/deletion mutations, three frameshift mutations, and one splicing mutation.[18] Of the 92 mutations, 71 are associated with oculodentodigital dysplasia (ODDD); 13 are associated with heart malformations; four are associated with deafness; two

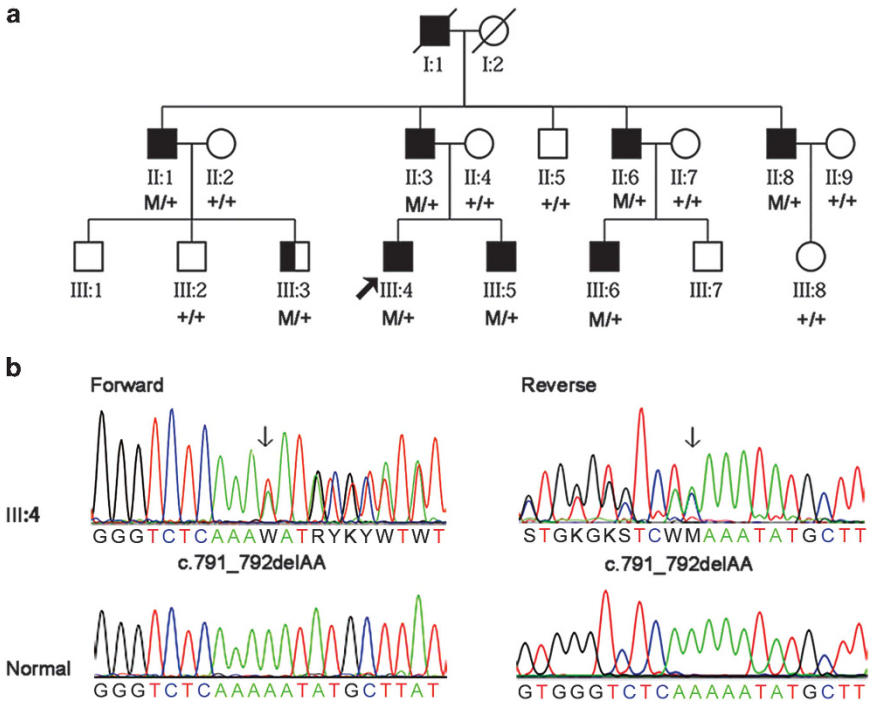

Figure 1 The *GJA1* mutation and the pedigree. (a) The mutation cosegregated with OAG and microcornea. Under each individual, + indicates a wild-type allele, and M indicates a mutant allele c.791_792delAA. Black symbols indicate individuals with OAG and microcornea; the black-and-white symbol indicates the individual with microcornea alone. (b) Sequencing chromatography demonstrated a heterozygous c.791_792delAA mutation in *GJA1*.

Table 1 Clinic data of the 15 family members in the study

Patient ID	*Gender*	*Age of onset*	*BCVA*	*Peak IOP*	*VCDR*	*Visual field MD (dB)*	*ØCornea (mm)*	*AL (mm)*	*ACD (mm)*	*ICD (mm)*	*PFL (mm)*
Affected											
II:1[a,b]	M	34 year	NLP/0.5	40/50	1.0/0.6	NA[d]	9.5/9.5	23.18/22.38	NA	40	25/25
II:3[a,b]	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
II:6[a,b]	M	19 year	NLP/0.2	NA[c]	1.0/0.7	NA/−21.42	9.0/9.3	NA	NA	34	24/23
II:8[a,b]	M	24 year	0.5/0.7	NA[c]	0.9/0.7	−29.53/−3.24	9.5/9.0	21.81/NA	2.41/2.30	38	26/23
III:3[a]	M	/	0.6/0.6	16/16	0.2/0.2	NA	9.0/9.0	20.63/20.48	2.41/2.39	37	22/20
III:4[a,b]	M	12 year	1.0/FC	NA	NA	NA[d]	9.0/9.0	23.19/20.80	2.63/2.78	37	22/22
III:5[a,b]	M	11 year	0.1/0.8	NA	NA	NA	9.0/9.0	20.58/24.28	2.86/2.96	NA	NA
III:6[a,b]	M	EC	NLP/1.0	NA[c]/45	NA/0.2	NA[d]	9.5/9.5	NA/22.20	NA/2.31	40	23/23
Unaffected											
II:2	F	/	1.0/1.0	13/13	0.2/0.2	NA	11.5/11.0	21.93/22.08	2.25/2.20	33	28/26
II:4	F	/	NA	NA	NA	NA	NA	NA	NA	NA	NA
II:5	M	/	1.0/1.0	15/14	0.2/0.3	NA	11.0/11.0	22.80/22.60	3.44/3.33	33	26/25
II:7	F	/	1.0/0.8	12/12	0.2/0.2	NA	11.5/11.5	22.70/22.75	2.63/2.48	NA	NA
II:9	F	/	1.0/1.0	11/12	0.2/0.2	NA	12.0/11.5	22.20/22.22	2.81/2.98	NA	NA
III:2	M	/	1.2/1.2	15/15	0.3/0.2	NA	11.5/11.5	25.04/25.04	NA	36	27/28
III:8	F	/	1.0/1.0	16/15	0.2/0.2	NA	11.5/11.5	21.75/22.28	3.10/3.00	33	27/27

Abbreviations: Ø, diameter; ACD, anterior chamber deepth; AL, axis length; BCVA, best corrected visual acuity; EC, early childhood; F, female; ICD, innercanthal distance; M, male; MD, mean deviation; NA, not available; NLP, no light perception; Peak IOP, peak intraocular pressure; PFL, palpebral fissure length.

[a] Micrcornea.

[b] Glaucoma.

[c] The introcular pressure of these patients was previously elevated, but the exact Peak IOP was not available.

[d] Visual acuity for the worst eye due to open angle glaucoma indicated end-stage glaucoma.

are associated with sudden infant death syndrome; and one is associated with craniometaphyseal dysplasia (Supplementary Table S1).[19–57] Glaucoma has been recorded in 33 of 161 patients with ODDD and *GJA1* mutations but in none of the 33 patients with other conditions and *GJA1* mutations.[23,26–31,37,40,46,47]

These glaucoma-associated mutations distribute randomly in the functional domains of GJA1. It total, glaucoma is reported in ~17% of patients (33/194) with *GJA1* mutations. Both OAG and angle closure glaucoma have been reported.[37] In nearly half of the previous reported 33 patients with glaucoma (48%, 16/33), the age

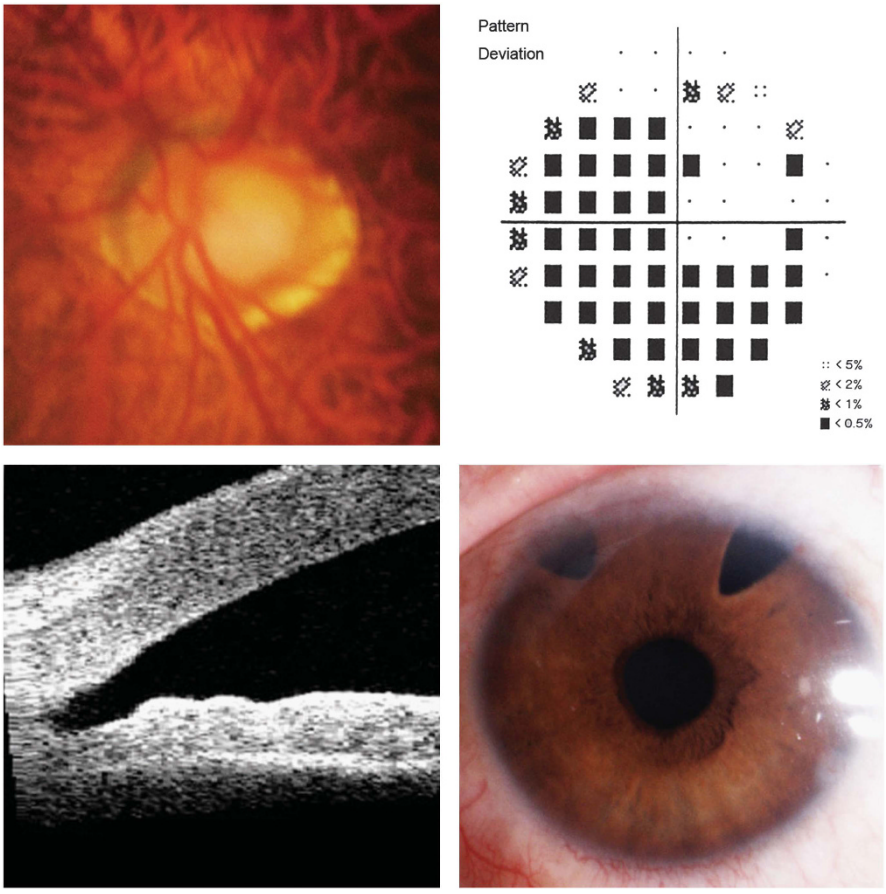

Figure 2 The enlarged cup-to-disc ratio, visual field defect and the open angle support the diagnosis of OAG. Anterior segment dysgenesis was absent, including remnant pupillary membrane, porous and spongy iris tissue, aniridia, and posterior embryotoxon.

at diagnosis of glaucoma was less than 35 years old (Supplementary Table S1).[23,26–29,40,46,47] In the present study, OAG is present in seven of the eight individuals (87.5%) with the *GJA1* mutation, which is a significantly higher association than that reported in previous studies.

Here we identify a novel truncation mutation in *GJA1* which is associated with OAG and microcornea in a Chinese family. The results suggest that *GJA1* mutations may frequently associate with OAG and, therefore, it should be considered as a causative gene for glaucoma.

Summary

What was known before

- Mutations in *GJA1* are associated with oculodentodigital dysplasia.

What this study adds

- A novel truncation mutation in *GJA1* was identified in a large family with open angle glaucoma and microcornea without typical systemic anormalies. This study suggested that *GJA1* may be included as a candidate gene for open angle glaucoma.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the patient and his family for their participation. This study was supported by the National Natural Science Foundation of China (81170881, U1201221), Guangdong Department of Science & Technology Translational Medicine Center grant 2011A080300002, and the Fundamental Research Funds of the State Key Laboratory of Ophthalmology.

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Supplementary Information accompanies this paper on Eye website (http://www.nature.com/eye)