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RESEARCH NOTE

Identification of a novel *GPR143* mutation in X-linked ocular albinism with marked intrafamilial phenotypic variability

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Abstract. Ocular albinism type 1 (OA1) is an X-linked inherited disease characterized by impaired visual acuity, congenital nystagmus, foveal hypoplasia, hypopigmentation of iris and fundus. It is caused by mutations in the G protein-coupled receptor 143 (*GPR143*) gene. The genetic characteristics of OA1 have not been well defined in Asians. In this study, six members from three consecutive generations of a Korean family with OA1 were enrolled. We performed whole-exome sequencing followed by validation and segregation analysis. Two affected patients underwent detailed ophthalmic examinations and eye movement recordings. Of the two affected males, the proband had all classical phenotypes of OA1, but the other showed isolated foveal hypoplasia without nystagmus. We identified a hemizygous missense (c.623C > A, p.Ala208Glu) mutation of *GPR143* in affected males. This mutation was also present as heterozygous in two obligate female carriers, and was not found in unaffected members. Our data expands the spectrum of phenotypes and genotype in *GPR143* in Asians, and highlights the phenotypic heterogeneity in OA1.

Keywords. ocular albinism; *GPR143* gene; whole-exome sequencing; phenotype; nystagmus.

Introduction

Ocular albinism type 1 (OA1, MIM: 300500) is the most common form of ocular albinism with an estimated prevalence of one in 60,000 live births (King *et al.* 2001). It is inherited as an X-linked trait, and most affected males present with impaired visual acuity, congenital nystagmus, foveal hypoplasia, hypopigmentation of iris and fundus. However, female carriers can also show iris transillumination and blotchy pattern of retinal pigmentation.

OA1 is caused by mutations in the G protein-coupled receptor 143 (*GPR143*) gene which is located at Xp22.2 (Schiaffino *et al.* 1995). *GPR143* encodes a protein that binds to heterotrimeric G proteins and is exclusively expressed by melanocytes and retinal pigment epithelium (Schiaffino *et al.* 1999). To date, more than 100 different mutations in *GPR143* have been identified in patients from different countries (Oetting 2002). However, the characteristics of OA1 have not been well defined in Asians. It is known that the genotype and phenotype spectrum depend on each ethnic group (Oetting 2002). Since iris and fundus hypopigmentation is not obvious among Asians, OA1 can be easily misdiagnosed as other congenital eye diseases, such as idiopathic infantile nystagmus syndrome, Leber congenital amaurosis, and achromatopsia (Shiono *et al.* 1995; Liu *et al.* 2007; Zhou *et al.* 2008).

In the present study, we described a Korean family showing completely a different phenotype of OA1 within the family, caused by a novel missense mutation in *GPR143*.

Materials and methods

Subjects and clinical assessment

Six members from three consecutive generations of a Korean family with OA were enrolled. They included two clinically affected (III-2 and III-4) and four unaffected (I-2, II-2, III-1 and III-3) individuals (figure 1a). Two affected patients underwent detailed ophthalmic examinations and eye movement recordings. The ophthalmic examinations included measurement of best-corrected visual acuity (BCVA), anterior segment observation with slit-lamp biomicroscopy and fundus examination. Eye movements were recorded binocularly using infrared video-oculography (SLMED, Seoul, Korea).

All experiments followed the tenets of the Declaration of Helsinki, and informed consent was obtained after the nature and possible consequences of this study had been explained to the participants. This study was approved by the Institutional Review Boards of Pusan National University Yangsan Hospital.

Mutation analysis

Genomic DNA was extracted from the blood sample of all members. Whole-exome sequencing (WES) was conducted on the proband (III-2) using the SureSelect Focused Exome kit (Agilent, USA). Briefly, the qualified genomic DNA sample was randomly fragmented by Covaris followed by adapter ligation, purification, hybridization and polymerase chain reaction (PCR). Captured libraries were subjected to Agilent 2100 Bioanalyzer to estimate the quality and were loaded on to the Illumina HiSeq2500 (TheragenEtex, Suwon, Korea) according to the manufacturer's recommendations. Raw image files were processed by HCS1.4.8 for base-calling with default parameters and the sequences of each individual were generated as 100 bp paired-end reads.

Sequence reads were aligned to the human reference genome sequence (GRCh37.3, hg19) using the Burrows-Wheeler Aligner (BWA, v. 0.7.12). PCR duplicate reads were marked and removed with Picard tools (v. 1.92). Genome Analysis Toolkit (GATK, v. 2.3-9) was used for indel realignment and base recalibration. Variation annotation and interpretation analysis were performed using SnpEff (v. 4.2). To identify the pathogenic mutation, we selected 28 candidate genes associated with the albinism based on online Mendelian inheritance in man (OMIM) (table 1). Variants causing nonsynonymous amino acid changes, stop codons, in-frame insertions/deletions in coding regions, or changes to splice site sequences in exon/intron boundaries were identified. Common variants with minor allele frequency (MAF) > 0.01 that represented in dbSNP147, the Exome Aggregation Consortium (ExAC), and the 1000 Genomes Project were excluded. The variants screened by the above process were annotated for previously reported disease-causing variants using the Human Gene Mutation Database and Korean Personal Genome Project information. Nonsynonymous variants that were predicted as damaging by at least three of four prediction tools (SIFT, Polyphen2, LRT and MutationTaster), were considered as pathogenic mutation. The variants were confirmed by PCR-based direct sequencing, and were screened in the other family members and 150 Korean controls.

Protein structural modelling

Structural modelling of human wild-type and mutants *GPR143* were predicted using I-TASSER and the results saved in PDB file format. Then, the PDB files were visualized by Pymol to identify the structures of these proteins.

Results

Clinical characteristics

Detailed ophthalmic and oculomotor findings are described in table 2. All affected individuals were males. The proband (III-2) showed poor BCVA with 0.2 in both eyes. Examination of the iris revealed irregular ring hypopigmentation in the peripheral area. Fundus examination disclosed an absent foveal reflex and severe hypopigmentation with clearly visible choroid vessels in the entire retina. Eye movement recording showed a horizontal, conjugate pendular nystagmus with about 2 Hz. The nystagmus was attenuated in darkness or during convergence, and changed to jerky form during lateral gaze. Patient III-4 had relatively good BCVA with 0.5 in both eyes. He showed normal pigmentation in the iris. Although foveal hypoplasia was identified in fundus examination, retina hypopigmentation was much milder compared to that of the proband. Unlike the proband, he had no nystagmus.

Mutation analysis and protein structural modelling

WES was performed on the proband with a minimum of 77.41% of the on-target regions which were converted to a depth of at least x20 mean coverage. After variant filtering, annotation, and interpretation, we initially detected 24 variants in the protein-coding regions of the genes associated with the albinism. Among them, only two variants were considered as possible pathogenic mutations based on the absence in public databases and deleterious effect by prediction tools; a hemizygous missense (c.623C > A, p.Ala208Glu) mutation in *GPR143* and heterozygous

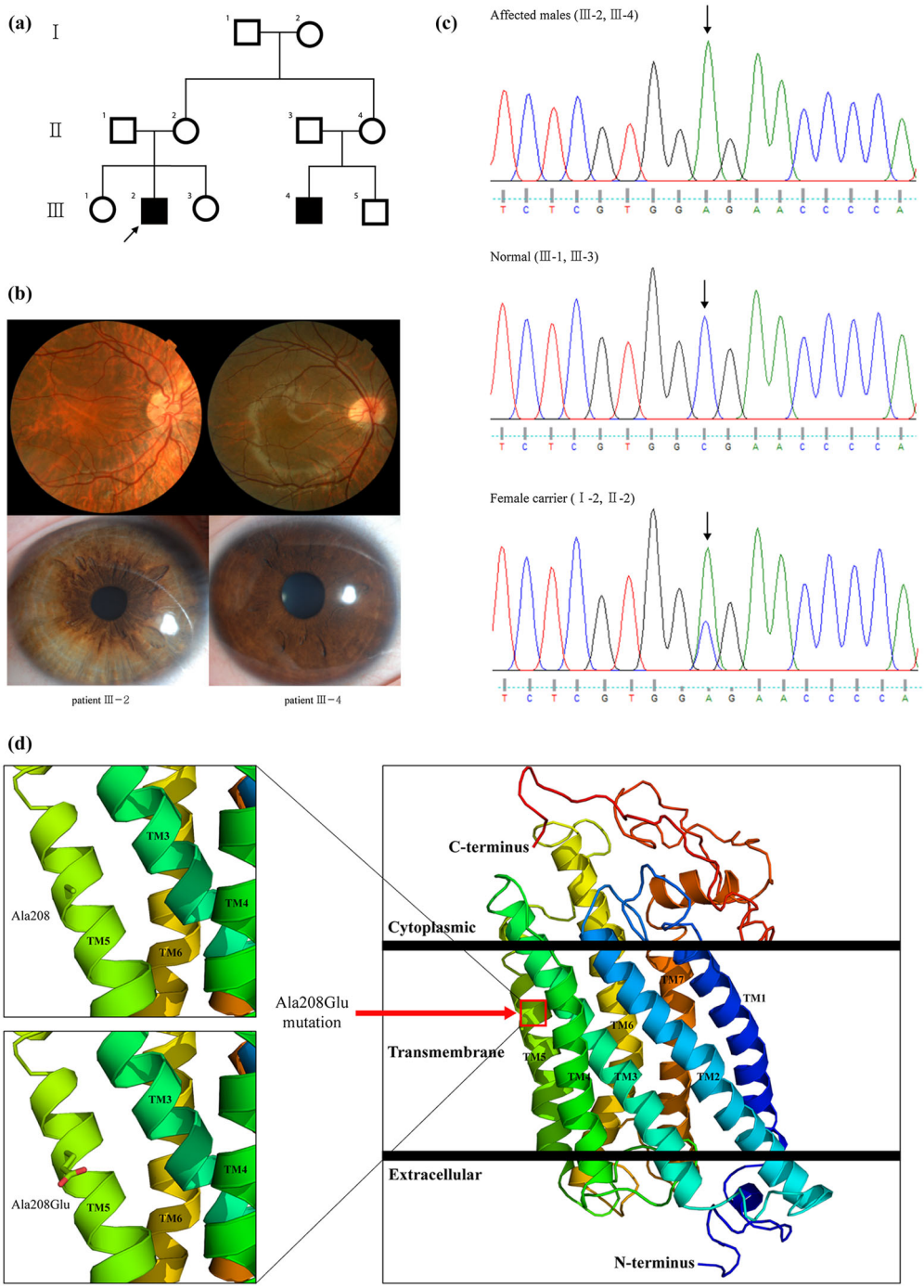

Figure 1. (a) Pedigree of the family. Solid symbols (squares, males; circles, females) indicate clinically affected individuals; open symbols, unaffected individuals. (b) Iris and fundus examinations of the patients. The proband (III-2) shows a foveal hypoplasia with the entire loss of retinal pigments (upper image) and an irregular ring depigmentation in the peripheral iris (lower image). Patient III-4 has a foveal hypoplasia, but the retinal hypopigmentation is not obvious (upper image). The iris also exhibits normal shape and pigments. (c) Sequencing results of affected and unaffected individuals**.** The chromatograms show a missense mutation (c.623C > A, p.Ala208Glu) in two hemizygous males (III-2 and III-4) and heterozygous female carriers (I-2 and II-3). (d) The structural modelling using an I-TASSER predicts that the A208 residue may be localized at the centre of transmembrane helix 5, and the side chain is projected toward a membrane rather than the interior of the protein. Therefore, change of Ala into Glu, a hydrophilic amino acid may be highly unstable in hydrophobic environment of membrane and eventually cause a structural instability.

Table 1. Target genes associated with albinism.

Gene	Phenotype	Gene/locus MIM number
GPR143	Ocular albinism, type I	300808
	Nystagmus 6, congenital X-linked	
CACNA1F	Aland island eye disease	300110
	Cone–rod dystrophy, X-linked, 3	
	Night blindness, congenital stationary 2A, X-linked	
TYR	Albinism, oculocutaneous, type IA	606933
	Albinism, oculocutaneous, type IB	
	Waardenburg syndrome/albinism, digenic	
OCA2	Albinism, brown oculocutaneous	611409
	Albinism, oculocutaneous, type II	
TYRP1	Albinism, oculocutaneous, type III	115501
SLC45A2	Albinism, oculocutaneous, type IV	606202
SLC24A5	Albinism, oculocutaneous, type VI	609802
C10orf11	Albinism, oculocutaneous, type VII	614537
PAX3	Craniofacial-deafness-hand syndrome	606597
	Rhabdomyosarcoma 2, alveolar	
	Waardenburg syndrome type 1	
	Waardenburg syndrome type 3	
MITF	COMMAD syndrome	156845
	Tietz albinism-deafness syndrome	
	Waardenburg syndrome, type 2A	
	Waardenburg syndrome/ocular albinism, digenic	
SOX10	PCWH syndrome	602229
	Waardenburg syndrome, type 2E	
	Waardenburg syndrome, type 4C	
EDNRB	ABCD syndrome	131244
	Waardenburg syndrome, type 4A	
HPS1	Hermansky–Pudlak syndrome 1	604982
AP3B1	Hermansky–Pudlak syndrome 2	603401
HPS3	Hermansky–Pudlak syndrome 3	606118
HPS4	Hermansky–Pudlak syndrome 4	606682
HPS5	Hermansky–Pudlak syndrome 5	607521
HPS6	Hermansky–Pudlak syndrome 6	607522
DTNBP1	Hermansky–Pudlak syndrome 7	607145
BLOC1S3	Hermansky–Pudlak syndrome 8	609762
BLOC1S6	Hermansky–Pudlak syndrome 9	604310
AP3D1	Hermansky–Pudlak syndrome 10	607246
MYO5A	Griscelli syndrome, type 1	160777
RAB27A	Griscelli syndrome, type 2	603868
EPG5	VICI syndrome	615068
KIT	Piebaldism	164920
SNAI2	Piebaldism	602150
LYST	Chediak–Higashi syndrome	606897

missense (c.2060C > A, p.Ala687Glu) mutation in *OCA2*. Of the two, c.623C > A mutation of *GPR143* was segregated in another hemizygous male (III-4) (figure 1c). This mutation was also present as heterozygous in two obligate female carriers (I-2 and II-2), and was not found in unaffected members (III-1 and III-3) and 150 normal controls.

The structural modelling predicted that the GPR143 protein is an integral membrane protein consisting of seven transmembrane domains (figure 1d). In this model, the Ala208 residue might be localized at the centre of transmembrane helix 5, and the side chain might be projected toward a membrane rather than the interior of the protein. Therefore, change of Ala into Glu, a hydrophilic amino acid, might be highly unstable in hydrophobic environment of membrane and eventually cause a structural instability.

Discussion

In the present study, we identified a novel missense mutation, c.623C > A within the *GPR143* in a Korean family with OA1. Clinically, the proband had the complete classical phenotype of OA1, whereas the other affected member showed mild phenotype without nystagmus.

The *GPR143* maps to chromosome Xp22.2 and consist of 9 exons (Schiaffino *et al.* 1995; King *et al.* 2001).

It encodes a protein of 404 amino acids containing seven putative transmembrane domains. The protein is localized to the melanosomal membranes of the retinal pigment epithelium (RPE) and plays an important role in melanosome biogenesis as a ligand of L-DOPA, a precursor in melanin synthesis (Lopez *et al.* 2008). It also participates in the signal transduction system by binding heterotrimeric G proteins at the internal membrane (Schiaffino *et al.* 1995). Thus, mutations in *GPR143* could affect melanin synthesis in the RPE and result in abnormal maturation of the retina and optic nerve, which leads to ocular hypopigmentation, foveal hypoplasia, congenital nystagmus, reduced visual acuity, and optic misrouting.

To date, more than 100 different mutations of *GPR143* have been reported, and the mutation spectrum seems to depend on ethnic groups. In Chinese patients, a deletion/insertion mutation was the most common type (33.3%), whereas ~48% of reported *GPR143* mutations were intragenic deletions in Western population (Oetting 2002; Zou *et al.* 2017). Some authors also found a diverse prevalence of large deletions between European (<10%) and North American (>50%) patients with OA1 (Bassi *et al.* 2001). In Korean population, only six families including this case have been reported in the literature, and the truncated mutations such as large deletion, aberrant splicing and nonsense mutation are more common than missense mutations (table 2) (Kim *et al.* 2016; Rim *et al.* 2017). Our missense mutation is located at the centre of transmembrane domain 5, likely destabilizing the protein by the introduction of the hydrophilic amino acid (Glu) within the hydrophobic environment of membrane.

Intriguingly, two affected patients in our study exhibited a completely different phenotype. The proband had all classical phenotypes of OA1 including congenital nystagmus, iris translucency, fundus hypopigmentation, foveal hypoplasia, and poor visual acuity. On the other hand, another showed foveal hypoplasia and mild fundus hypopigmentation without nystagmus. Intrafamilial variations in the clinical phenotype have been previously described in OA1, but the phenotypic diversity was mostly limited to different extent of iris or fundus hypopigmentation (Schiaffino *et al.* 1999; Fang *et al.* 2008; Yan *et al.* 2012). The amount of ocular hypopigmentation is also related to ethnic origin (Zou *et al.* 2017). Caucasians mostly have iris translucency and albinotic fundus. In Chinese and Japanese patients, however, iris colour is usually brown with little iris translucency and the fundus hypopigmentation ranges from absent to severe. Since the hypopigmentation of iris and fundus is not apparent in Asian, OA1 could easily be misdiagnosed as another disease. Indeed, *GPR143* mutations have been identified in several Chinese families with X-linked congenital nystagmus without any typical signs of OA1 (Liu *et al.* 2007; Zhou *et al.* 2008).

Despite a different phenotype of OA1, only congenital nystagmus is the most prominent and consistent finding.

Table 2. Clinical and genetic characteristics of this study and reported Korean families with OA1.

Reference	*GPR143* mutation	Mutation type	BCVA	Iris hypopigmentation	Retinal hypopigmentation	Foveal hypoplasia	Nystagmus
This study	c.623C > A, p.A208E	Missense	0.2 (III-2)	Yes	Yes	Yes	HP
			0.5 (III-4)	No	Mild	Yes	–
Kim *et al.* (2016)	c.733C > T, p.R245X	Nonsense	0.3	Mild	No	Yes	HJ
Rim *et al.* (2017)	c.514G > C, p.G172R	Missense	0.2	No	Yes	Yes	HP
	c.659-3C > G	Aberrant splicing	0.2	No	No	Yes	HJ
	Loss of exon 2 and 3	Deletion	0.1	No	Yes	Yes	HJ
	Loss of exon 2 and 3	Deletion	0.4	No	Yes	Yes	HP

BCVA, best-corrected visual acuity; HJ, horizontal jerky; HP, horizontal pendular

To the best of our knowledge, our patient (III-4) is the first to be demonstrated of OA1 without congenital nystagmus by hemizygous *GPR143* mutation. Without the detection of foveal hypoplasia, it is difficult to consider this patient as clinically affected member. It remains unclear why our affected patients showed a complete heterogeneity of the clinical manifestations. Presumably, the other genes or environmental factors may modify the OA1 phenotype. In oculocutaneous albinism (OCA) that shares ocular and cutaneous hypopigmentation, the variations of *MC1R* gene are known to be associated with red hair, fair skin and poor tanning ability (King *et al.* 2003; Beaumont *et al.* 2005). Moreover, a single-nucleotide polymorphism (SNP) within the conserved region of *HERC2* gene, rs12913883, represented a regulatory region controlling constitutive expression of OCA2, and the C allele at rs129138832 led to decreased expression of OCA2 within iris melanocytes, resulting in different eye colour (Sturm *et al.* 2008). Similar to OCA, some SNPs within the unknown genes may bring the heterogeneity of the clinical phenotypes in OA1. A genetic or environmental basis modifying the OA1 phenotype may require further investigation.

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