to that of his older sister, Patient 1 (Fig. 3, 4). The fundi appeared reticulated before the age 10 years. The macular degeneration gradually spread, and a posterior staphyloma developed and progressed in both eyes (Fig. 4). His central vision decreased to hand motion in his late teens (Fig. 2). He is now 22-year old, and he still has some peripheral vision but no cataracts in both eyes.

The full-field ERGs, OCT, and ultrasonographic findings were similar to those of his older sister (Patient 1), namely, non-recordable single-bright flash ERGs, barely recordable flicker ERGs, and deep excavation and thin retina at the posterior pole of both eyes (Fig. 4). The axial length at age 22 years was 23.82 ± 0.05 mm in the right eye and 24.06 ± 0.02 mm in the left eye.

Genetic analysis revealed a homozygous A126V substitution in *RDH12* gene, the same as his sister (Patient 1).

Patient 3 (Fig. 5, kinki-1076 in Fig. 1): Patient 3 was a girl who was 3-year old when we first examined her in 2004. She was a member of a family (kinki-F33) unrelated to that of Patients 1 and 2 (Fig. 1). She was brought to our clinic because of esotropia and nystagmus. Her decimal BCVA was 0.07 with +6.0 DS and −1.0 DC ax 115° in the right eye and 0.07 with +5.5 DS and −1.5 DC ax 175° in the left eye. Ophthalmoscopy showed diffuse retinal degeneration with pigmentation in the macular area (Fig. 5). Her fundi appeared reticulated before the age 10 years. She was followed until the age of 13 years, and her vision gradually decreased to light perception in both eyes (Fig. 2).

Single-bright flash full-field ERGs were non-recordable, and flicker ERGs were barely recordable at age 11 years (Fig. 5). OCT and ultrasonography performed at 11 and 13 years of age revealed excavation of the posterior pole of both eyes (Fig. 5). The axial length at age 13 years was 20.92 ± 0.37 mm in the right eye and 21.22 ± 0.93 mm in the left eye.

When the sequence of her whole exome was compared with the reference human genome (hs37d5), 1,488,313 mutations were found. After excluding common mutations, 406 mutations remained. We filtered the remaining mutations by the pattern of inheritance with her parents and found 16 genes as causal candidates. Finally, they were compared to that of Patients 1 and 2, and only *RDH12* was shared between three patients. As a result, genetic analysis showed a homozygous c.377C>T transition in exon 4 resulting in alanine126 to valine substitution (A126V) in the *RDH12* gene. Genetic analyses on her non-symptomatic parents (kinki-1077 & 1078, Fig. 1) showed heterozygous A126V substitution in the *RDH12* gene.

Discussion

ERG findings in carrier relatives

The *RDH12* gene is located at 14q 24.1 and encodes a photoreceptor cell retinol dehydrogenase. Mutation of the *RDH12* gene is estimated to account for <4 % of all autosomal recessive LCA/EORD patients [5, 8]. To date, 16 different mutations have been reported in this gene [6]; however, the homozygous substitution of A126V in the *RDH12* gene has never been reported except in a highly consanguineous Arabic family [13] and our patients. In the Arabic family, a non-symptomatic relative who was a heterozygous carrier of A126V had markedly reduced rod ERGs, and the cone ERGs were at the lower limits of normal [13]. Another study reported that heterozygous mutations in the *RDH12* gene can cause a late-onset, relatively mild autosomal dominant retinitis pigmentosa [24].

The parents of our patients were non-symptomatic, and their fundi were normal. The rod and cone ERGs performed on three of them (kinki-1047, kinki-1077, and kinki-1078 in Fig. 1) were normal.

Clinical course of visual acuity

The initial visual disturbance in our patients was noticed at age 2–5 years, and there was a progressive decrease thereafter (Fig. 2). Their central vision decreased to light perception in the teens. Patients 2 and 3 maintained some peripheral vision at age 22 and 13 years although Patient 1 lost vision in the entire visual field at age 17 years (Figs. 3, 4, 5).

The vision in patients with LCA/EORD was investigated by Fulton et al. [25] and Walia et al. [26]. Walia et al. [26] related the vision of patients with LCA/EORD to their causative genes and reported that LCA/EORD caused by *RPE65* (LCA2), *CRB1* (LCA8), and *RDH12* (LCA13) mutations led to a wide variations in visual disturbances, whereas LCA/EORD caused by *GUCY2D* (LCA1), *AIPL1* (LCA4), *RPGRIP1* (LCA6), and *CRX* (LCA7) gene mutations had severe visual disturbances which began in the first year of life. Other studies on LCA/EORD associated

with *RDH12* mutations reported an initial vision reduction occurring between birth to 20 years with most of them at age 3–7 years [7–16].

These results are consistent with our patients who had decreased vision at age 2–5 years and loss of their central vision in their teens (Fig. 2).

Coloboma/posterior staphyloma and LCA/EORD

The fundus of our three patients appeared similar; namely, they showed diffuse retinal degeneration and macular atrophy (Figs. 3, 4, 5). The fundi also had a reticulated appearance (Figs. 3, 4, 5). These findings are similar to the phenotype reported for *RDH12*-associated LCA/EORD [7–16].

In our patients, the macular degeneration progressed to atrophic macula with the formation of a posterior staphyloma which resembled a coloboma (Figs. 3, 4, 5). The relationships between LCA and macular coloboma have been discussed in several papers [27–29], before the causative genes for LCA/EORD were discovered. Recently, a macular coloboma/posterior staphyloma was reported in patients with *LCA5* (LCA5) [30], *CRX* (LCA7) [31], *CRB1* (LCA8) [32], *NMNAT1* (LCA9) [33], and *RDH12* (LCA13) mutations [7, 9–11, 14, 16]. A relationship between LCA/EORD and the macular coloboma/posterior staphyloma is still unknown. Single-gene mutation cannot explain the formation of a macular coloboma/posterior staphyloma because they are present in cases of LCA/EORD associated with several different causative genes.

In our patients, the reticulated appearance of the fundus was present in early childhood, and it became less apparent after the formation of the posterior staphyloma. Whether the reticulated appearance was related to the development of the staphyloma was not determined.

One limitation of this study is the small number of the patients. In addition, a more detailed screened investigation of the phenotypes and genotypes of patients with LCA/EORD is needed to confirm our results.

In conclusion, we report the longitudinal clinical course of three patients in two families with LCA/EORD who had homozygous A126V substitution in the *RDH12* gene. All of the patients had a progressive retinal degeneration and posterior staphyloma, and impairment of the central vision. This is the first report of Japanese patients with LCA/EORD which was caused by *RDH12* gene mutation.

Acknowledgments Authors express gratitude to Professor Toshifumi Otori, M.D., who transferred the longitudinal clinical data to us and gave us suggestive advice on this study. The authors wish to acknowledge RIKEN GeNAS for the sequencing of the Exome enriched libraries. This research was supported by the research grants to T.I. and K.K. from the Ministry of Health, Labour and Welfare, Japan (13803661), to K.T. and K.K. from the Ministry of Health, Labour and Welfare, Japan (23164001), Y.S from the Ministry of Health, Labour and Welfare, Japan (82259921), S.K. and K.K. from Japan Society for the Promotion of Science, Japan (23592597), and to M.F. from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) for RIKEN Omics Science Center.

Conflict of interest All authors have no commercial interests related to this research.

References

1. Leber T (1869) Ueber Retinitis pigmentosa und angeborene Amaurose. Graefes Arch Klin Exp Ophthalmol 15:1–25
2. Franceschetti A, Dieterlé P (1954) Importance diagnostique et pronostique de l' électrorétinogramme (ERG) dans les dégénérescences tapéto-rétiniennes avec rétrécissement du champ visuel et héméralopie. Confin Neurol 14:184–186
3. Leber T (1916) Die Pigmentdegeneration der Netzhaut und die mit ihr verwandten Erkrankungen. In: Saemisch T, Elschnig A (eds) Graefe-Saemisch-Hess Handbuch der gesamten Augenheilkunde. Zweite Hälfte. Verlag von Wilherm Engelmann, Leipzig, pp 1076–1225
4. Weleber RG & Gregory-Evans K (2006) Leber congenital amaurosis. In: Hinton DR (ed), Ryan SJ (ed in chief) Retina, 4th edn, vol 1. Elsevier Inc., Philadelphia, pp 455–457
5. Weleber RG, Francis PJ, Trzupek KM & Beattie C (2014) Leber congenital amaurosis. In: Pagon RA (ed in chief), Adam MP, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds) GeneReviews™. NCBI Bookshelf. http://www.ncbi.nlm.nih.gov/books/NBK1298/. Accessed 12 Mar 2014
6. Daiger SP, The University of Texas Health Science Center (2014) RetNet™. Retinal information network. Updated 10 Mar 2014. https://sph.uth.edu/retnet. Accessed 12 March 2014
7. Jenecke AR, Thompson DA, Utermann G, Becker C, Hübner CA, Schmid E, McHenry CL, Nair AR, Rüschendorf F, Heckenlively J, Wissinger B, Nürnberg P, Gal A (2004) Mutations in *RDH12* encoding a photoreceptor cell retinol dehydrogenase cause childhood-onset severe retinal dystrophy. Nat Genet 36:850–854
8. Perrault I, Hanein S, Gerber S, Barbet F, Ducroq D, Dollfus H, Hamel C, Dufier JL, Munnich A, Kaplan J, Rozet JM (2004) Retinal dehydrogenase 12 (*RDH12*) mutations in Leber congenital amaurosis. Am J Hum Genet 75:639–646
9. Jacobson SG, Cideciyan AV, Aleman TS, Sumaroka A, Schwartz SB, Windsor EAM, Roman AJ, Heon E, Stone EM, Thompson DA (2007) *RDH12* and *RPE65*, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. Invest Ophthalmol Vis Sci 48:332–338

10. Schuster A, Janecke AR, Wilke R, Schmid E, Thompson DA, Utermann G, Wissinger B, Zrenner E, Gal A (2007) The phenotype of early-onset retinal degeneration in persons with *RDH12* mutations. Invest Ophthalmol Vis Sci 48:1824–1831
11. Sun W, Gerth C, Maeda A, Lodowski DT, van der Kraak L, Saperstein DA, Héon E, Palczewski K (2007) Novel RDH12 mutations associated with Leber congenital amaurosis and cone-rod dystrophy: biochemical and clinical evaluations. Vision Res 47:2055–2066
12. Valverde D, Pereiro I, Vallespín E, Ayuso C, Borrego S, Baiget M (2009) Complexity of phenotype-genotype correlations in Spanish patients with *RDH12* mutations. Invest Ophthalmol Vis Sci 50:1065–1068
13. Benayoun L, Spiegel R, Auslender N, Abbasi AH, Rizel L, Hujeirat Y, Salama I, Garzozi HJ, Allon-Shalev S, Ben-Yosef T (2009) Genetic heterogeneity in two consanguineous families segregating early onset retinal degeneration: the pitfalls of homozygosity mapping. Am J Med Genet Part A 149A:650–656
14. Sodi A, Caputo R, Passerini I, Bacci GM, Menchini U (2010) Novel *RDH12* sequence variations in Leber congenital amaurosis. J AAPOS 14:349–351
15. Weleber RG, Michaelides M, Trzupek KM, Stover NB, Stone EM (2011) The phenotype of severe early childhood onset retinal dystrophy (SECORD) from mutation of *RPE65* and differentiation from Leber congenital amaurosis. Invest Ophthalmol Vis Sci 52:292–302
16. Mackay DS, Borman AD, Moradi P, Henderson RH, Li Z, Wright GA, Waseem N, Gandra M, Thompson DA, Bhattacharya SS, Holder GE, Webster AR, Moore AT (2011) *RDH12* retinopathy: novel mutations and phenotypic description. Mol Vis 17:2706–2716
17. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M (2009) ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 118:69–77
18. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760
19. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303
20. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w^{1118}; *iso-2*; *iso-3*. Fly 6:80–92
21. Kyoto University (2014) Human genetic variation browser. http://www.genome.med.kyoto-u.ac.jp/SnpDB/. Accessed 12 Mar 2014
22. Adzhubei I, Jordan DM, Sunyaev SR (2013) Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 76:7.20.1–7.20.41
23. National Institute of Genetics (NIG) (2014) Cell innovation program. http://cell-innovation.nig.ac.jp/index_en.html. Accessed 12 Mar 2014
24. Fingert JH, Oh K, Chung M, Scheetz TE, Andorf JL, Johnson RM, Sheffield VC, Stone EM (2008) Association of a novel mutation in the retinol dehydrogenase 12 (*RDH12*) gene with autosomal dominant retinitis pigmentosa. Arch Ophthalmol 126:1301–1307
25. Fulton AB, Hansen RM, Mayer DL (1996) Vision in Leber congenital amaurosis. Arch Ophthalmol 114:698–703
26. Walia S, Fishman GA, Jacobson SG, Aleman TS, Koenekoop RK, Traboulsi EI, Weleber RG, Pennesi ME, Héon E, Drack A, Lam BL, Allikmets R, Stone EM (2010) Visual acuity in patients with Leber's congenital amaurosis and early childhood-onset retinitis pigmentosa. Ophthalmology 117:1190–1198
27. Leighton DA, Harris R (1973) Retinal aplasia in association with macular coloboma, keratoconus and cataract. Clin Genet 4:270–274
28. Margolis S, Scher BM, Carr RE (1977) Macular colobomas in Leber's congenital amaurosis. Am J Ophthalmol 83:27–31
29. Heckenlively JR, Foxman SG, Parelhoff ES (1988) Retinal dystrophy and macular coloboma. Doc Ophthalmol 68:257–271
30. Mohamed MD, Topping NC, Jafri H, Raashed Y, McKibbin MA, Inglehearn CF (2003) Progression of phenotype in Leber's congenital amaurosis with a mutation at the LCA5 locus. Br J Ophthalmol 87:473–475
31. Nakamura M, Ito S, Miyake Y (2002) Novel de novo mutation in *CRX* gene in a Japanese patient with Leber congenital amaurosis. Am J Ophthalmol 134:465–467
32. Lotery AJ, Jacobson SG, Fishman GA, Weleber RG, Fulton AB, Namperumalsamy P, Héon E, Levin AV, Grover S, Rosenow JR, Kopp KK, Sheffield VC, Stone EM (2001) Mutations in the *CRB1* gene cause Leber congenital amaurosis. Arch Ophthalmol 119:415–420
33. Koenekoop RK, Wang H, Majewski J, Wang X, Lopez I, Ren H, Chen Y, Li Y, Fishman GA, Genead M, Schwartzentruber J, Solanki N, Traboulsi EI, Cheng J, Logan CV, McKibbin M, Hayward BE, Parry DA, Johnson CA, Nageeb M, FORGE Canada Consortium, Poulter JA, Mohamed MD, Jafri H, Rashid Y, Taylor GR, Keser V, Mardon G, Xu H, Inglehearn CF, Fu Q, Toomes C, Chen R (2012) Mutations in *NMNAT1* cause Leber congenital amaurosis and identify a new disease pathway for retinal degeneration. Nat Genet 44:1035–1039

Case Reports in Ophthalmology

Case Rep Ophthalmol 2014;5:318–324

DOI: 10.1159/000368298
Published online: October 2, 2014

1663–2699/14/0053–0318$39.50/0
www.karger.com/cop

Intravitreal Injection of Bevacizumab for Retinopathy of Prematurity in an Infant with Peters Anomaly

Tsuyoshi Minami[a] Kazuki Kuniyoshi[a] Shunji Kusaka[c] Koji Sugioka[a] Hiroyuki Sakuramoto[a] Masuo Sakamoto[a] Akane Izu[b] Norihisa Wada[b] Yoshikazu Shimomura[a]

Departments of [a]Ophthalmology and [b]Pediatrics, Kinki University Faculty of Medicine, and [c]Department of Ophthalmology, Sakai Hospital, Osaka, Japan

Key Words

Retinopathy of prematurity · Peters anomaly · Vascular endothelial growth factor · Anti-VEGF therapy · Bevacizumab

Abstract

Purpose: To report our findings in an infant with Peters anomaly type II whose retinopathy of prematurity (ROP) was treated with an anti-VEGF agent and surgeries. ***Case Report:*** A male infant weighing 548 g was born prematurely at 23 weeks and 1 day with corneal opacity and shallow anterior chambers in both eyes. At the postmenstrual age of 35 weeks and 3 days, the infant was tentatively diagnosed with stage 3 ROP because of a dilated tunica vasculosa lentis and ultrasonographic findings. The boy was treated with bilateral intravitreal injections of bevacizumab (IVB) because laser photocoagulation of the retina could not be performed due to the corneal opacity. The retina in the right eye detached 3 times, namely 5 days, 16 days, and 7 months after the IVB; encircling the scleral buckle and a vitrectomy with endolaser photocoagulation were therefore required. In his left eye, the retina was reattached after the initial IVB, and no additional treatment was required. ROP was not reactivated in both eyes until the last examination at the age of 2 years and 6 months. ***Conclusions:*** Our results showed that IVB is a useful treatment for ROP in patients with Peters anomaly. However, a retinal detachment can be a complication after IVB. The optimal timing of IVB for ROP in infants with hazy media needs to be determined.

Kazuki Kuniyoshi, MD
Department of Ophthalmology, Kinki University Faculty of Medicine
377-2 Ohno-Higashi, Osaka-Sayama 589-8511 (Japan)
E-Mail kuniyoshi-kazuki@umin.net

Case Rep Ophthalmol 2014;5:318–324
DOI: 10.1159/000368298

Introduction

Peters anomaly is characterized by congenital corneal opacities, defects of the Descemet's membrane, shallow anterior chambers, cataracts, and glaucoma [1, 2]. The corneal opacities in patients with Peters anomaly make the diagnosis and treatment of retinal disorders such as retinopathy of prematurity (ROP) difficult. Recently, the intravitreal injection of antivascular endothelial growth factors, including bevacizumab (IVB), was reported to be effective as a treatment for ROP [3, 4]. One advantage of IVB over traditional treatments for ROP, e.g., retinal photocoagulation, is that it can be performed on infants with hazy media.

We present our findings in an infant with Peters anomaly accompanied by ROP that was treated with IVB. The research protocol was approved by the Ethics Review Board of the Kinki University Faculty of Medicine in November 2011, and the procedures conformed to the tenets of the Declaration of Helsinki of the World Medical Association.

Case Report

Our patient, a male infant, was born prematurely at 23 weeks and 1 day and weighed 546 g. He was noted to have corneal opacities with shallow anterior chambers in both eyes soon after birth (fig. 1). He was diagnosed with Peters anomaly type II because he had corneal-lenticular adhesions in addition to a central corneal opacity with iridocorneal adhesions [1]. Details of the fundi could not be obtained because of the corneal opacity.

An ophthalmic examination performed at the postmenstrual age of 34 weeks and 2 days showed a dilated tunica vasculosa lentis, and ultrasonography showed an abnormal echo, which suggested ridge formation (white triangles in fig. 2). Although these findings suggested stage 3 ROP, which requires treatment [5], laser photocoagulation would have been difficult to perform because of the corneal opacity, dilated tunica vasculosa lentis, and the fixed pupils.

Thus, we selected to treat the eye with IVB [3, 4] (Avastin®; Genentech Inc., South San Francisco, Calif., USA). A detailed clinical information and treatment options, including cryotherapy and IVB, were given to the family, and the parents agreed and signed an informed consent to proceed with the IVB.

At the postmenstrual age of 35 weeks and 3 days, 0.25 mg/0.01 ml of bevacizumab were injected into the vitreous cavity of both eyes. Five days later, the dilatation of the tunica vasculosa lentis was reduced in both eyes, but a retinal detachment was detected by ultrasonography in the right eye (stage 4A ROP; black arrow in fig. 2). An encircling scleral buckle was placed on both eyes on the same day, and the retina of the right eye was detached again 11 days after the buckling surgery. We performed lens-sparing vitrectomy in the right eye because the tunica vasculosa lentis was reduced after the IVB and buckling surgery. The temporal area of the fundus, where the proliferation was most severe and the retina was partially detached, was barely observable, and vitrectomy with endolaser photocoagulation was performed. Since other areas of the fundus could not be observed due to the corneal opacity, we performed additional transscleral cryopexy in the right eye.

At 4 months after the IVB, a cataract with an increase of the intraocular pressure (IOP, 33 mm Hg) was detected in the right eye. The cataract was removed and the IOP was normalized. Seven months after the IVB, the corneal opacity was reduced in both eyes, allowing a better observation of the fundus (fig. 1, fig. 3). Ophthalmoscopy showed a third retinal detachment in the right eye (arrow in fig. 3), and fluorescein angiography showed an

Case Rep Ophthalmol 2014;5:318–324
DOI: 10.1159/000368298

avascular zone in both eyes and some leakage of fluorescein in the right eye (fig. 3). The fovea of the right eye was displaced temporally (fig. 3).

A second vitrectomy was performed in the right eye, and no progression or reactivation of the ROP or retinal detachment was noticed at the last examination at the age of 2 years and 9 months. In his left eye, the IOP was normal and the retina was attached after the initial IVB, and no additional treatment was required. No apparent systemic side effects of the IVB were observed.

Discussion

The treatment for ROP in infants with corneal opacity is challenging. Although partial corneal opacity, which is occasionally observed in neonatal infants, allows clinicians to observe and treat ROP, dense and bilateral corneal opacities, which were present in our patient, obstructed the detection and treatment of the ROP.

We had several treatment options for our patient: IVB, combination of penetrating keratoplasty with laser photocoagulation, and endoscopic vitrectomy with laser photocoagulation. We selected IVB as the first treatment because it could be performed even in an eye with a corneal opacity. Penetrating keratoplasty was not considered before the treatment for ROP because we knew the results are not favorable in patients with Peters anomaly type II [2].

There are 3 problems in managing ROP in eyes with dense and bilateral corneal opacities: (1) how do we detect ROP, (2) when do we treat ROP, and (3) how do we treat ROP.

According to the results of the Early Treatment for Retinopathy of Prematurity (ETROP) study [5], the treatment for ROP should be performed within 72 h after a diagnosis of type 1 ROP. The most important finding required to make a decision on the treatment of the ROP is the 'plus disease' condition [5], which is difficult to recognize in infants with hazy media. Therefore, clinicians need to decide on the treatment for ROP in infants with hazy media without any information regarding the status of the fundus.

The ETROP Cooperative Group also reported that stage 3 ROP was present in 48.1% of infants whose birth weight was <750 g and in 43.3% of infants whose gestational age was ≤27 weeks [6]. In our institution, the first treatment is performed between the postmenstrual age of 30 and 35 weeks [7]. From the results of these treatments, the infants who had a high risk for ROP, mainly those with a gestational age of ≤27 weeks or a birth weight of <750 g, were recommended to be treated between the postmenstrual age of 30 and 35 weeks.

Our results indicated that IVB appeared to be effective and led to a regression of the ROP; however, additional surgeries were needed for the tractional retinal detachments. Although a retrospective analysis of our findings indicated that earlier IVB treatments may not lead to retinal detachments, the systemic condition of our patient did not allow an earlier treatment for the ROP.

A late reactivation of ROP [4, 8] as well as incomplete vascularization after IVB [4, 9] have recently been reported. The results of fluorescein angiography in our patient suggested that the ROP had not completely regressed even 7 months after the IVB (fig. 3). Longitudinal and careful observations are needed after IVB treatment for ROP.

In conclusion, IVB can be a treatment for ROP in infants with Peters anomaly. However, the diagnosis of ROP and the timing of the IVB in infants with hazy media need more investigation.

Acknowledgements

The authors express gratitude to Kyutaro Ito, MD, working at the Department of Ophthalmology, Tottori Prefectural Central Hospital, for referring the patient to our clinic. We also thank Masahiko Fukuda, MD, and Chota Matsumoto, MD, in the Department of Ophthalmology, Kinki University Faculty of Medicine, for their suggestions regarding this paper.

This research was supported by the research grants to S.K. and K.K. from the Japan Society for the Promotion of Science, Japan (23592597) and to Y.S. from the Ministry of Health, Labour and Welfare, Japan (82259921).

Disclosure Statement

The authors have no proprietary or commercial interests related to this research.

References

1 Zaidman GW, Flanagan JK, Furey CC: Long-term visual prognosis in children after corneal transplant surgery for Peters anomaly type I. Am J Ophthalmol 2007;144:104–108.
2 Bhandari R, Ferri S, Whittaker B, Liu M, Lazzaro DR: Peters anomaly: review of the literature. Cornea 2011;30:939–944.
3 Mintz-Hittner HA, Kennedy KA, Chuang AZ; the BEAT-ROP Cooperative Group: Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N Engl J Med 2011;364:603–615.
4 Kuniyoshi K, Sugioka K, Sakuramoto H, Kusaka S, Wada N, Shimomura Y: Intravitreal injection of bevacizumab for retinopathy of prematurity. Jpn J Ophthalmol 2014;58:237–243.
5 Early Treatment for Retinopathy of Prematurity Cooperative Group: Revised indications for the treatment of retinopathy of prematurity. Arch Ophthalmol 2003;121:1684–1696.
6 Early Treatment for Retinopathy of Prematurity Cooperative Group: The incidence and course of retinopathy of prematurity: findings from the Early Treatment for Retinopathy of Prematurity study. Pediatrics 2005;116:15–23.
7 Kayazawa M, Kuniyoshi K, Sugioka K, Tsujioka D, Abe K, Matsumoto C, Wada N, Shimomura Y: Review of retinopathy of prematurity in very low birth weight infants and extremely low weight infants. Folia Jpn Ophthalmol Clin (Ganka Rinsho Kiyo) 2011;4:448–454.
8 Hu J, Blair MP, Shapiro MJ, Lichtenstein SJ, Galasso JM, Kapur R: Reactivation of retinopathy of prematurity after bevacizumab injection. Arch Ophthalmol 2012;130:1000–1006.
9 Tahija SG, Hersetyati R, Lam GC, Kusaka S, McMenamin PG: Fluorescein angiographic observations of peripheral retinal vessel growth in infants after intravitreal injection of bevacizumab as sole therapy for zone I and posterior zone II retinopathy of prematurity. Br J Ophthalmol 2014;98:507–512.

Case Rep Ophthalmol 2014;5:318–324
DOI: 10.1159/000368298

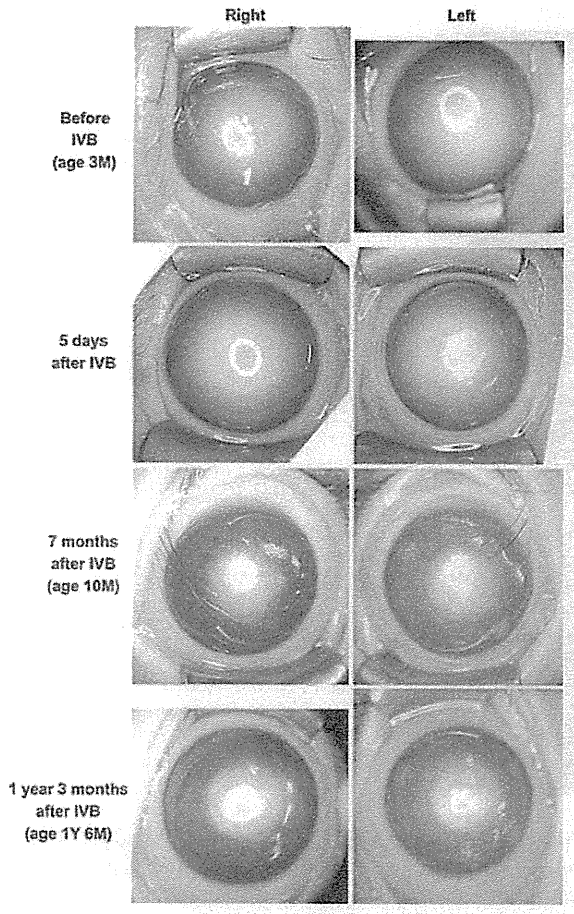

Fig. 1. Photographs of the anterior segment of eyes with Peters anomaly type II and ROP. The dense corneal opacities are gradually reduced. These photographs were taken with a RetCam® 3 (Clarity Medical Systems, Pleasanton, Calif., USA). Y=Year; M = month.

Case Rep Ophthalmol 2014;5:318–324
DOI: 10.1159/000368298

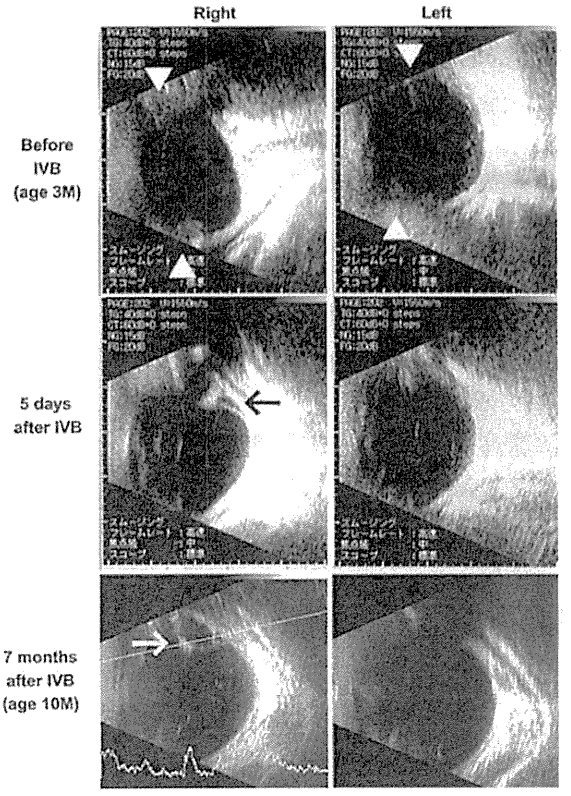

Fig. 2. Ultrasonographic findings of eyes with Peters anomaly and ROP. Before the IVB, abnormal echo was detected which suggested ridge formation (triangles in the upper row, stage 3 ROP). Five days after the IVB, a retinal detachment was suspected in the right eye (black arrow in the middle row, stage 4A ROP). Seven months after the IVB, a third retinal detachment is suspected in the right eye (white arrow in the lower row, stage 4A ROP).

Case Rep Ophthalmol 2014;5:318–324
DOI: 10.1159/000368298

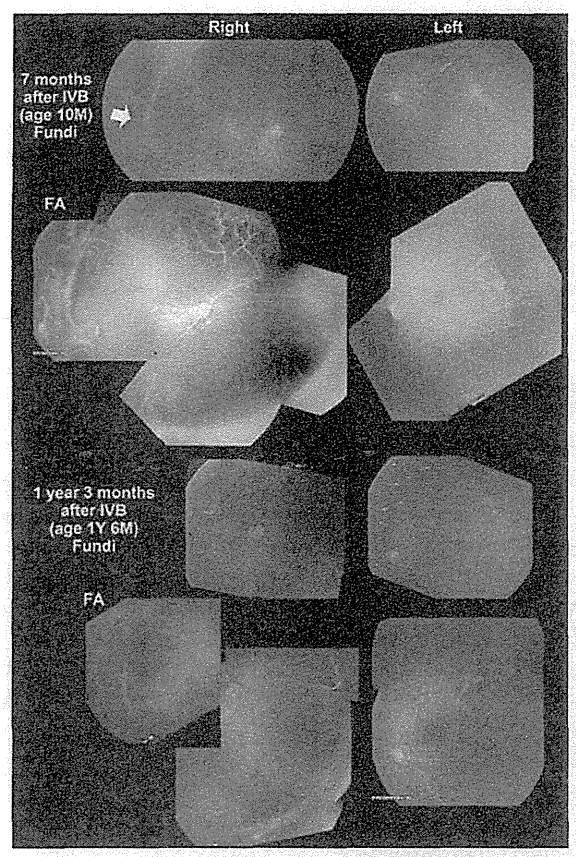

Fig. 3. Fundus photographs and fluorescein angiograms. A retinal detachment can be seen in the right eye 7 months after the IVB (arrow). An avascular zone and some leakage of fluorescein are still present in the periphery 1 year and 3 months after IVB. Photography was difficult because of the residual corneal opacity in both eyes. Fundus photography and fluorescein angiograms were performed using RetCam® 3.

Jpn J Ophthalmol (2014) 58:237–243
DOI 10.1007/s10384-014-0310-z

THE OFFICIAL INTERNATIONAL JOURNAL OF THE JAPANESE OPHTHALMOLOGICAL SOCIETY

CLINICAL INVESTIGATION

Intravitreal injection of bevacizumab for retinopathy of prematurity

Kazuki Kuniyoshi · Koji Sugioka · Hiroyuki Sakuramoto · Shunji Kusaka · Norihisa Wada · Yoshikazu Shimomura

Received: 17 July 2013 / Accepted: 31 January 2014 / Published online: 26 February 2014

Abstract
Purpose To evaluate the outcomes of intravitreal injection of bevacizumab (IVB) for retinopathy of prematurity (ROP).
Methods IVB was selected to be the first treatment for type 1 ROP in 8 eyes (4 patients). Bevacizumab (0.25 mg/eye) was injected into the vitreous cavity under either general anesthesia or sedation. Fundus photography and fluorescein angiography were performed before the IVB. One infant was observed to the age of 1 year 6 months, the second to 1 year 9 months, the third to 1 year 10 months, and the fourth to 2 years 0 month.
Results Before the IVB, 6 eyes (3 patients) had ROP in zone II and 2 eyes (one patient) had ROP in zone I. The 3 infants with ROP in zone II weighed 652, 476, and 579 g with gestational ages of 24, 27, and 24 weeks at birth, respectively. The infant with ROP in zone I weighed 972 g with a gestational age of 26 weeks at birth. IVB was performed at postmenstrual ages of 33–37 weeks. The IVB was effective in all eyes with ROP in zone II and additional treatment was not required, whereas vitreous hemorrhage and cataract were found at 19 weeks and 5 months after the initial IVB in the two eyes with ROP in zone I. These two eyes required additional IVB, laser photocoagulation, and surgery.
Conclusions Our findings suggest that eyes with type 1 ROP in zone II can be treated with IVB. Further studies are needed with a larger number of eyes.

Keywords Retinopathy of prematurity · Zone I ROP · Bevacizumab · Vascular endothelial growth factor · Anti-VEGF therapy

K. Kuniyoshi (✉) · K. Sugioka · H. Sakuramoto · Y. Shimomura
Department of Ophthalmology, Kinki University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
e-mail: kuniyoshi-kazuki@umin.net

S. Kusaka
Department of Ophthalmology, Sakai Hospital, Kinki University Faculty of Medicine, 2-7-1 Harayama-Dai, Minami-Ku, Sakai 590-0132, Osaka, Japan

N. Wada
Department of Pediatrics, Kinki University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama 589-8511, Osaka, Japan

Introduction

The mainstay treatment for retinopathy of prematurity (ROP) has been ablation of the avascular retina by either cryotherapy or laser photocoagulation since the Nagata et al. [1] publication, the Cryotherapy for Retinopathy of Prematurity study [2], and the Early Treatment for Retinopathy of Prematurity (ETROP) study [3]. The ablation of the avascular retina is reasonable treatment for ROP because vascular endothelial growth factor (VEGF) is expressed by cells in the avascular retina and plays an important role in the development of ROP [4].

In the last decade, there has been an increase in the use of VEGF inhibitors to treat chorioretinal vascular disorders [5]. Although it is an off-label use, bevacizumab (Avastin®; Genentech Inc, South San Francisco, CA, USA), a humanized anti-VEGF monoclonal antibody, is used extensively for diabetic retinopathy and other chorioretinal vascular diseases [5]. In eyes with ROP, an intravitreal injection of bevacizumab (IVB) was first used as salvage therapy in eyes that had undergone laser photocoagulation or used in combination with laser photocoagulation [6–13]. Preoperative IVB was also used to improve the surgical outcome of severe ROP [12, 14, 15].

Table 1 Summary of clinical findings of the patients

	PM age/weight	ROP at IVB zone/ stage/proliferation/ TVL dilatation	Number of times of IVB/ Additional treatments	ROP after IVB/ Age at vessels reached the ora serrata	Age at the last examination	Systemic complications
Patient 1/girl						
Birth	24W4D/652 g	OD p-zone II/stage 2+/none/++	1/none	Regressed/11M	1Y6M	IR (ileostomy)
IVB	34W6D	OS p-zone II/ stage 2+/none/++	1/none	Regressed/11M		
NICU discharge	56W0D					
Patient 2/boy						
Birth	27W0D/476 g	OD p-zone II/stage 3+/4 clocks/+	1/none	Regressed/4M	1Y9M	
IVB	36W2D	OS p-zone II/stage 3+/4 clocks/+	1/none	Regressed/4M		
NICU discharge	43W0D					
Patient 3/girl						
Birth	24W2D/579 g	OD p-zone II/stage 2+/none[a]/+++	1/none	Regressed/(−)[b]	1Y10M	PDA (operation)
IVB	33W3D	OS p-zone II/stage 2+/none[a]/+++	1/none	Regressed/(−)[b]		IR (ileostomy)
NICU discharge	52W0D					
Patient 4/boy						
Birth	26W6D/972 g	OD zone I/stage 1+/none/+++	2/PC+vitrectomy	Reactivated/(−)[b]	2Y0M	IR (ileostomy)
IVB	37W2D	OS zone I/stage 1+/none/+++	3/PC+cataract removal	Reactivated/(−)[b]		ICH
NICU discharge	(still hospitalized)					HC (VP shunt)

PM postmenstrual, *ROP* retinopathy of prematurity, *IVB* intravitreous injection of bevacizumab, *TVL* tunica vasculosa lentis, *W* week(s), *D* day(s), *Y* year(s), *M* month(s), *p-zone II* posterior zone II, *PC* laser photocoagulation, *IR* intestinal rupture, *PDA* patent ductus arteriosus, *ICH* intracranial hemorrhage, *HC* hydrocephalus, *VP* ventriculoperitoneal, *OD* oculus dexter, *OS* oculus sinister

[a] Details were hardly observed due to TVL dilatation

[b] Avascular zone still present

In 2011, the Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity (BEAT-ROP) Cooperative Group reported that IVB as the first treatment for zone I and zone II stage 3+ ROP was effective equally or more than laser ablation (BEAT-ROP study) [16]. Other studies of anti-VEGF for ROP report similar results, i.e., IVB is effective for ROP especially in the early stage of ROP [10, 11, 13, 17–20].

The purpose of this study was to determine the effectiveness of IVB as the first choice treatment for type 1 ROP [3]. We defined type 1 ROP according to the revised ETROP study [3], namely, eyes with zone I, any stage ROP with plus disease; zone I, stage 3 ROP with or without plus disease; zone II, stages 2 or 3 ROP with plus disease.

Patients and methods

The research protocol was approved by the Ethics Review Board of Kinki University Faculty of Medicine in November 2011, and the procedures conformed to the tenets of the Declaration of Helsinki. All clinical procedures were performed after obtaining a signed informed consent form from all parents of the infants.

IVB was selected as the first choice treatment for ROP in 2 boys and 2 girls with type 1 ROP born in the Kinki University Hospital from December 2011 to June 2013 [3]. IVB was performed either in the operating room under general anesthesia with intubation or in the neonatal intensive care unit (NICU) under general sedation. Slit-lamp and fundus examinations, fundus photography and fluorescein fundus angiography (FA) were performed prior to the IVB. Bevacizumab (0.25 mg/0.01 ml) was injected into the vitreous cavity with a 30-gauge needle inserted 0.5 mm posterior to the corneal limbus of both eyes. After the IVB, general ophthalmic examinations were repeated once or twice a week, and examinations under general anesthesia or sedation including fundus photography and FA were performed every 2–3 months. Fundus photography and FA were performed using RetCam® III (Clarity Medical Systems, Pleasanton, CA, USA). FA was performed with an intravenous injection of 10 mg/kg body weight of fluorescein sodium.

When the ROP was found to have signs of reactivation, e.g., a re-dilatation of the retinal vessels, re-proliferation on the ridge, or retinal/vitreous hemorrhages, we performed another IVB, laser photocoagulation, or surgery after detailed information was given to the family.

Results

Of the 8 eyes, 6 eyes of 3 patients had ROP in zone II and 2 eyes of 1 patient had ROP in zone I. The patient characteristics and clinical results are shown in Table 1. In infants that had ROP in zone II (patients 1–3), the dilatation of retinal vessels and the tunica vasculosa lentis was reduced within 1 week after the IVB (Fig. 1). In patient 2, the proliferation on the ridge was absent 1 week after the IVB. After these regressions, no reactivation of the ROP was seen, and no additional treatment was needed. In patients 1 and 2, the retinal vessels had extended to the edge of the ora serrata at 9 and 2 months after the IVB, whereas in patient 3, an avascular zone was still present in the temporal periphery with a width of 4 disc diameters from the ora serrata at age 1 year 10 months. The capillary formation between the retinal vessels appeared to be normal except in the periphery where arteriovenous (AV) shunts and poorly developing capillaries were seen in FA (Fig. 2).

On the other hand, the 2 eyes with ROP in zone I (patient 4) had a regression of the ROP after the initial IVB; however, capillaries were not found between the retinal vessels in the FA, and vitreous hemorrhage was detected arising from the optic disc after all (Fig. 3).

No notable systemic side effects were found in all patients. A summary of the clinical course of two representative patients is presented below.

Patient 1

This girl was born prematurely at 24 weeks and 4 days weighing 652 g, and was noted to have stage 2+ ROP in posterior zone II with dilatation of the tunica vasculosa lentis oculus utro (OU) at postmenstrual age of 34 weeks 3 days (Figs. 1, 2). The eyes were treated with IVB at postmenstrual age of 34 weeks 6 days. Ten days after the IVB, the tunica vasculosa lentis was markedly reduced (Fig. 1), and the retinal vascular tortuosity was decreased (Fig. 2). However, 5 months after the IVB, an avascular zone still remained in both eyes with an AV shunt. The

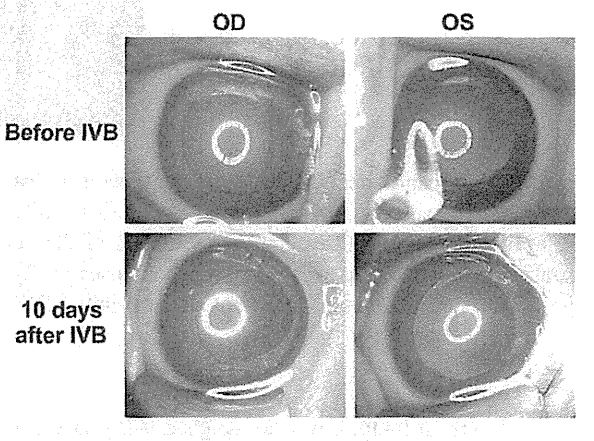

Fig. 1 Photographs of the anterior segment of the eye of patient 1 with ROP in zone II (Table 1). Before the intravitreal injection of bevacizumab (IVB) (*upper row*) and 10 days after the IVB (*lower row*)

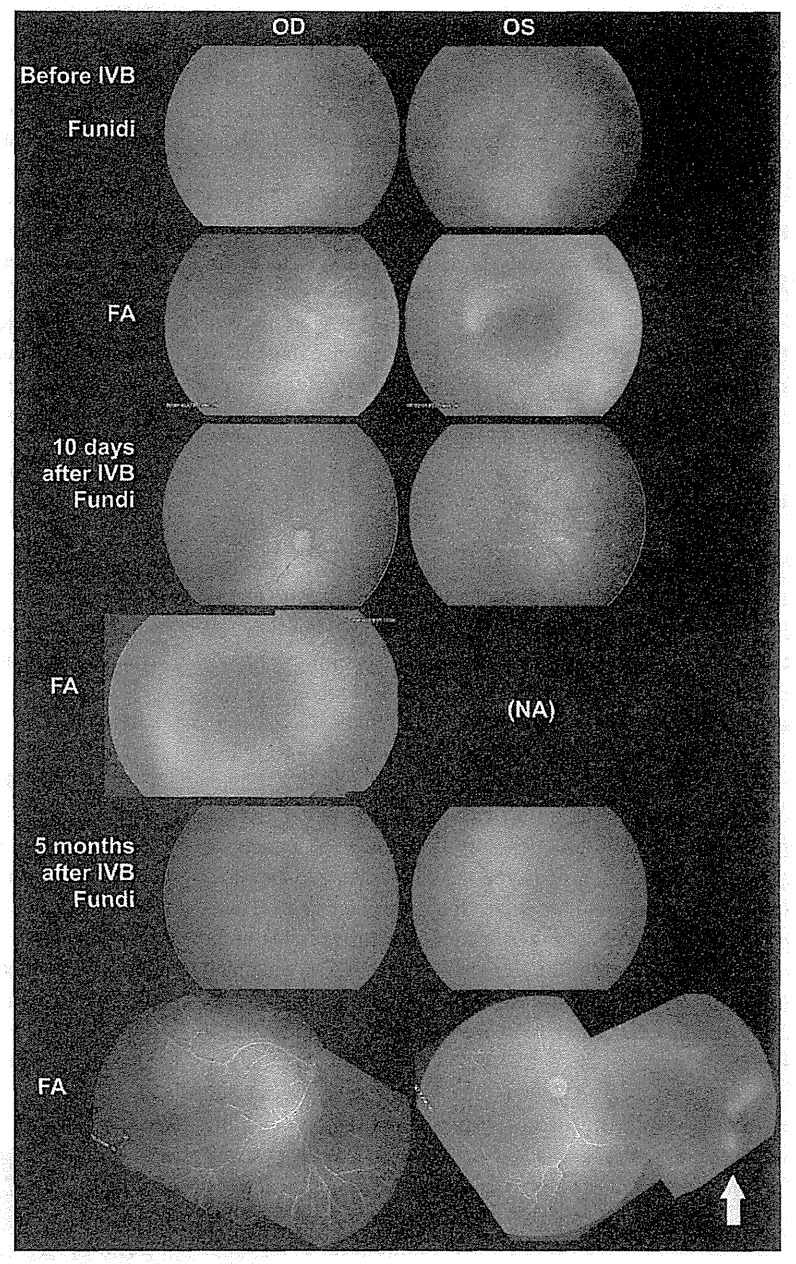

Fig. 2 Fundus photographs and fluorescein fundus angiograms of patient 1 with ROP in zone II. Avascular zones still remained 5 months after the IVB, and some leakage of fluorescein can be seen at the late stage of FA (*arrow*). *IVB* intravitreal injection of bevacizumab, *FA* fluorescein fundus angiography, *NA* not available

formation of capillaries between the peripheral retinal vessels was poor (Fig. 2, bottom right). At 9 months after the IVB, the vessels had extended to the edge of the ora serrata and no AV shut was found ophthalmoscopically. She did not need additional treatment after the initial IVB.

Patient 4

Patient 4 was an infant boy who weighed 972 g at gestational age 26 weeks and 6 days. His intestines had ruptured, and he had severe intracranial hemorrhages and infection of the respiratory organs. At the first ophthalmological examination performed at postmenstrual age 32 weeks 5 days, the media were slightly hazy, and the retinal vessels were within zone I. However, the vessels were not dilated and no demarcation line was seen. At postmenstrual age 37 weeks 2 days, he weighed 1320 g and his pupils were fixed. The tunica vasculosa lentis was prominent, and the retinal vessels were still poorly developed in zone I with plus disease in both eyes (Fig. 3). Our examination indicated that he required treatment for ROP, and accordingly IVB was administered OU on the same

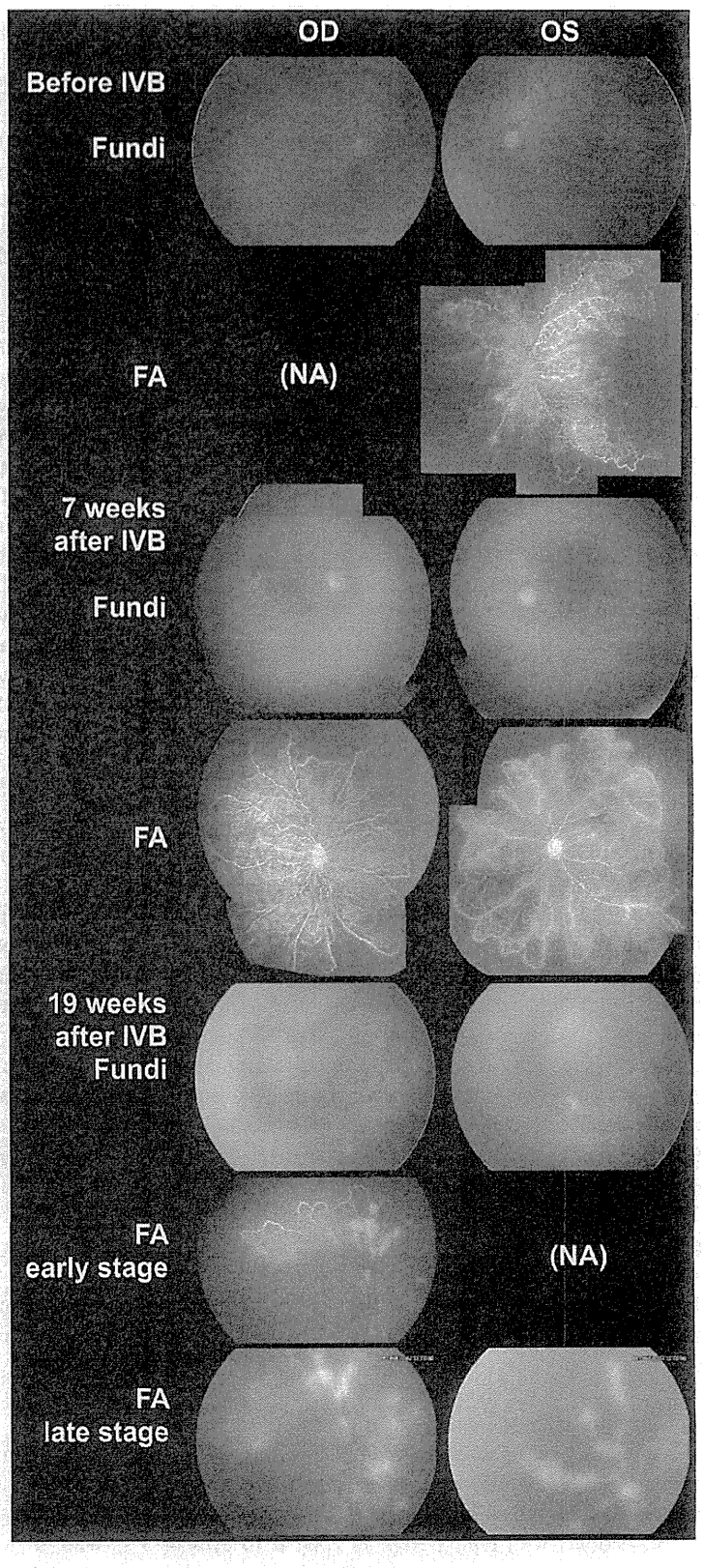

Fig. 3 Fundus photographs and fluorescein fundus angiograms of patient 4 with ROP in zone I. A vitreous hemorrhage can be seen at the posterior pole OD 19 weeks after the initial IVB, a reactivation of ROP

day. Nineteen weeks after the IVB, a vitreous hemorrhage was noticed at the posterior pole OD, and severe neovascularization and wide areas of avascular retina were found by FA OU (Fig. 3). A second IVB was administered together with laser photocoagulation OU, and vitreous surgery was performed OD. Because a mature cataract developed OS 8 months after the initial IVB, it was removed and a third IVB with endolaser photocoagulation was performed OS. After that, his ROP regressed until the age 2 years, although he is still hospitalized in the NICU because of his poor general condition.

Discussion

The results showed that IVB was effective and no additional treatment was required in 6 eyes (3 patients) with ROP in zone II. However, the 2 eyes (patient 4) with ROP in zone I required additional IVB, photocoagulation, and surgery.

The BEAT-ROP study reports that IVB and laser photocoagulation were effective treatments for stage 3+ ROP [16]. They studied 150 premature babies until postmenstrual age 54 weeks and reported that the recurrence rate of ROP was 42 % after laser photocoagulation and 6 % after IVB in infants with zone I ROP. The recurrence rate was 12 % after laser photocoagulation and 5 % after IVB in infants with zone II ROP [16]. From these results, IVB appears to be effective for stage 3+ ROP and had advantages over laser ablation for zone I stage 3+ ROP.

After the BEAT-ROP study, several studies reported late reactivations of the ROP following anti-VEGF treatment [21–25]. Jang et al. [21] report bilateral retinal detachments that developed 4 month after combined intravitreal injection of ranibizumab and laser photocoagulation in patients with zone I, stage 3+ ROP although the ROP completely regressed 3 months after the injection. Lee et al. [22] reported a late-onset vitreoretinal traction which resulted in retinal detachment 2.5–4 months after IVB with or without laser photocoagulation in cases with stage 3+ ROP. Hu et al. [24] reported late reactivations of ROP after IVB. They reported that the reactivations were seen at a mean postmenstrual age 49.3 ± 9.1 weeks, and the mean interval between the initial IVB and the reactivation was 14.4 weeks. In their report, 4 out of 17 eyes progressed to stage 5 ROP, and they concluded that laser treatment is a useful option for reactivated ROP after IVB [24].

Studies on a rat model of ROP demonstrated a late increase in erythropoietin and angiogenic signaling in the avascular retina after an intravitreal injection of anti-VEGF agents [26]. These results may be related to the late reactivation of ROP after IVB in humans [21–25].

Our patients with type 1 ROP in zone II (6 eyes of 3 patients) who were treated with IVB did not require any additional treatment after the initial IVB. However, in patients 1 and 2, it required 9 and 2 months, respectively, for the retinal vessels to reach the edge of the ora serrata after the IVB (Table 1; Fig. 2). Patient 3 still had an avascular area with an AV shunt at 1 year 8 months after the initial IVB. Capillary formation between retinal vessels appeared to be normal except for the peripheral AV shunt in eyes with ROP in zone II (Fig. 2, bottom right).

These findings are not consistent with the result of Lee et al. [9], who reported a rapid development of the retinal vessels after IVB combined with laser photocoagulation. The AV shunt with poorly developed capillaries in the periphery may be a risk factor for a late reactivation of ROP. However, additional studies are needed before a final conclusion can be made.

For zone I ROP, the BEAT-ROP and other studies reported favorable results after IVB [16–20]. However, patient 4 in the present study had ROP in zone I and was in poor general condition. This patient had a late reactivation OU of ROP 19 weeks after the initial IVB. He therefore required additional treatments OU. Retrospective analyses suggest that when FA detected poorly developed retinal vessels and no neovascularization, additional IVB and/or pan-retinal laser photocoagulation OU might be needed at 7 weeks after the initial IVB (Fig. 3).

Patient 4 developed a mature cataract, however, we do not know whether or not the cataract formation is related to the bevacizumab and/or ROP. The cataract does not seem to have been induced by lens injury as a complication of the IVB, because it developed 8 months after the injection.

IVB for ROP is effective and has several advantages over retinal ablation by laser or cryotherapy. First, IVB does not ablate the retina. Second, the procedures for IVB are less traumatic than retinal ablation and can be performed on babies who are in poor general condition. Third, IVB can be performed on eyes with hazy media.

However, the efficacy of IVB for type 1 ROP in zone II needs more investigation because it can be treated by laser photocoagulation. There are also other problems with IVB, e.g., IVB into premature babies in the NICU requires well-trained doctors; and retinal detachment, lens injury, or infection can be complications. The amount of bevacizumab for infants needs consideration. We used 0.25 mg of bevacizumab/eye, which was a lower dose than that used in the BEAT-ROP study [16], because we knew that unilateral IVB affects the fellow eyes [27]; however, we did not know the systemic safety dose of IVB in infants. In addition, the extent of the follow-up period following the IVB needs to be established because residual avascular retina with an AV shunt may develop with a severe reactivation of ROP [25]. It must be remembered that bevacizumab is also a drug approved for

cancer and is used as an intravenous injection, and that it has not been approved for intravitreal injections for either adults or infants.

In conclusion, IVB appears to be appropriate for treating eyes with ROP in zone II. Because only 8 eyes of 4 patients were studied, longitudinal and additional studies are needed to confirm our findings.

Acknowledgments The authors thank Daishi Tsujioka, MD, Masuo Sakamoto, MD, Tsuyoshi Minami, MD and Takuya Numata, MD in the Department of Ophthalmology, Kinki University Faculty of Medicine, and Shunsuke Minakata, MD and Akane Izu, MD in the Department of Pediatrics, Kinki University Faculty of Medicine, for their continuous clinical assistance and suggestive advice on this study.

This research was supported by research grants to S.K. Y.S. and K.K. from Japan Society for the Promotion of Science, Japan, and to Y.S. and K.K. from Ministry of Health, Labour and Welfare, Japan.

Conflicts of interest K. Kuniyoshi, None; K. Sugioka, None; H. Sakuramoto, None; S. Kusaka, None; N. Wada, None; Y. Shimomura, None.

References

1. Nagata M, Kobayashi Y, Fukuda H, Suekane K. Photocoagulation for the treatment of the retinopathy of prematurity. Rinsho Ganka (Jpn). 1968;22:419–26.
2. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Multicenter trial of cryotherapy for retinopathy of prematurity. Preliminary results. Arch Ophthalmol. 1988;106:471–9.
3. Early Treatment for Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity. Arch Ophthalmol. 2003;121:1684–96.
4. Young TL, Anthony DC, Pierce E, Foley E, Smith LEH. Histopathology and vascular endothelial growth factor in untreated and diode laser-treated retinopathy of prematurity. J AAPOS. 1997;1:105–10.
5. Stewart MW. The expanding role of vascular endothelial growth factor inhibitors in ophthalmology. Mayo Clin Proc. 2012;87:77–88.
6. Chung EJ, Kim JH, Ahn HS, Koh HJ. Combination of laser photocoagulation and intravitreal bevacizumab (Avastin®) for aggressive zone I retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol. 2007;245:1727–30.
7. Quiroz-Mercado H, Martinez-Castellanos MA, Hernandez-Rojas ML, Salazar-Teran N, Chan RVP. Antiangiogenic therapy with intravitreal bevacizumab for retinopathy of prematurity. Retina. 2008;28(suppl):S19–25.
8. Lalwani GA, Berrocal AM, Murray TG, Buch M, Cardone S, Hess D, et al. Off-label use of intravitreal bevacizumab (Avastin) for salvage treatment in progressive threshold retinopathy of prematurity. Retina. 2008;28(suppl):S13–8.
9. Lee JY, Chae JB, Yang SJ, Yoon YH, Kim JG. Effects of intravitreal bevacizumab and laser in retinopathy of prematurity therapy on the development of peripheral retinal vessels. Grasefes Arch Clin Exp Ophthalmol. 2010;248:1257–62.
10. Axer-Siegel R, Snir M, Ron Y, Friling R, Sirota L, Weinberger D. Intravitreal bevacizumab as supplemental treatment or monotherapy for severe retinopathy of prematurity. Retina. 2011;31:1239–47.
11. Dani C, Frosini S, Fortunato P, Bertini G, Pratesi S, Pollazzi L, et al. Intravitreal bevacizumab for retinopathy of prematurity as first line or rescue therapy with focal laser treatment. A case series. J Matern Fetal Neonatal Med. 2012;25:2194–7.
12. Sun HJ, Choi KS, Lee SJ. Adjunctive effect of intravitreal bevacizumab prior to lens-sparing vitrectomy in aggressive posterior retinopathy of prematurity: a case report. Jpn J Ophthalmol. 2012;56:476–80.
13. Martínez-Castellanos MA, Schwartz S, Hernández-Rojas ML, Kon-Jara VA, Gracía-Aguirre G, Guerrero-Naranjo JL, et al. Long-term effect of antiangiogenic therapy for retinopathy of prematurity up to 5 years of follow-up. Retina. 2013;33:329–38.
14. Kusaka S, Shima C, Wada K, Arahori H, Shimojyo H, Sato T, et al. Efficacy of intravitreal injection of bevacizumab for severe retinopathy of prematurity: a pilot study. Br J Ophthalmol. 2008;92:1450–5.
15. Nonobe NI, Kachi S, Kondo M, Takai Y, Takemoto K, Nakayama A, et al. Concentration of vascular endothelial growth factor in aqueous humor of eyes with advanced retinopathy of prematurity before and after intravitreal injection of bevacizumab. Retina. 2009;29:579–85.
16. Mintz-Hittner HA, Kennedy KA, Chuang AZ, BEAT-ROP Cooperative Group. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N Engl J Med. 2011;364:603–15.
17. Mintz-Hittner HA, Kuffel RR Jr. Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina. 2008;28:831–8.
18. Wu WC, Yeh PT, Chen SN, Yang CM, Lai CC, Kuo HK. Effects and complications of bevacizumab use in patients with retinopathy of prematurity: a multicenter study in Taiwan. Ophthalmology. 2011;118:176–83.
19. Mintz-Hittner HA. Intravitreal pegaptanib as adjunctive treatment for stage 3+ ROP shown to be effective in a prospective, randomized, controlled multicenter clinical trial. Eur J Ophthalmol. 2012;22:685–6.
20. Wu WC, Kuo HK, Yeh PT, Yang CM, Lai CC, Chen SN. An updated study of the use of bevacizumab in the treatment of patients with prethreshold retinopathy of prematurity in Taiwan. Am J Ophthalmol. 2013;155:150–8.
21. Jang SY, Choi KS, Lee SJ. Delayed-onset retinal detachment after an intravitreal injection of ranibizumab for zone 1 plus retinopathy of prematurity. J AAPOS. 2010;14:457–9.
22. Lee BJ, Kim JH, Yu YS. Delayed onset atypical vitreoretinal traction band formation after an intravitreal injection of bevacizumab in stage 3 retinopathy of prematurity. Eye. 2012;26:903–10.
23. Patel RD, Blair MP, Shapiro MJ, Lichtenstein SJ. Significant treatment failure with intravitreous bevacizumab for retinopathy of prematurity. Arch Ophthalmol. 2012;130:801–2.
24. Hu J, Blair MP, Shapiro MJ, Lichtenstein SJ, Galasso JM, Kapur R. Reactivation of retinopathy of prematurity after bevacizumab injection. Arch Ophthalmol. 2012;130:1000–6.
25. Ittiara S, Blair MP, Shapiro MJ, Lichtenstein SJ. Exudative retinopathy and detachment: a late reactivation of retinopathy of prematurity after intravitreal bevacizumab. J AAPOS. 2013;17:323–5.
26. McCloskey M, Wang H, Jiang Y, Smith GW, Strange J, Hartnett ME. Anti-VEGF antibody leads to later atypical intravitreous neovascularization and activation of angiogenic pathways in a rat model of retinopathy of prematurity. Invest Ophthalmol Vis Sci. 2013;54:2020–6.
27. Karaca C, Oner AO, Mirza E, Polat OA, Sahiner M. Bilateral effect of unilateral bevacizumab injection in retinopathy of prematurity. JAMA Ophthalmol. 2013;131:1099–101.

OPEN ACCESS Freely available online

PLOS | ONE

Whole Exome Analysis Identifies Frequent *CNGA1* Mutations in Japanese Population with Autosomal Recessive Retinitis Pigmentosa

Satoshi Katagiri[1,2], Masakazu Akahori[1], Yuri Sergeev[3], Kazutoshi Yoshitake[4], Kazuho Ikeo[4], Masaaki Furuno[5], Takaaki Hayashi[2], Mineo Kondo[6], Shinji Ueno[7], Kazushige Tsunoda[8], Kei Shinoda[9], Kazuki Kuniyoshi[10], Yohinori Tsurusaki[11], Naomichi Matsumoto[11], Hiroshi Tsuneoka[2], Takeshi Iwata[1]*

1 Division of Molecular and Cellular Biology, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan, **2** Department of Ophthalmology, The Jikei University School of Medicine, Tokyo, Japan, **3** National Eye Institute, National Institutes of Health, Bethesda, MD, United States of America, **4** Laboratory of DNA Data Analysis, National Institute of Genetics, Shizuoka, Japan, **5** RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Life Science Accelerator Technology Group, Transcriptome Technology Team, Yokohama, Japan, **6** Department of Ophthalmology, Mie University Graduate School of Medicine, Tsu, Japan, **7** Department of Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Japan, **8** Laboratory of Visual Physiology, National Institute of Sensory Organs, Tokyo, Japan, **9** Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan, **10** Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka, Japan, **11** Department of Human Genetics, Yokohama City University, Yokohama, Japan

Abstract

Objective: The purpose of this study was to investigate frequent disease-causing gene mutations in autosomal recessive retinitis pigmentosa (arRP) in the Japanese population.

Methods: In total, 99 Japanese patients with non-syndromic and unrelated arRP or sporadic RP (spRP) were recruited in this study and ophthalmic examinations were conducted for the diagnosis of RP. Among these patients, whole exome sequencing analysis of 30 RP patients and direct sequencing screening of all *CNGA1* exons of the other 69 RP patients were performed.

Results: Whole exome sequencing of 30 arRP/spRP patients identified disease-causing gene mutations of *CNGA1* (four patients), *EYS* (three patients) and *SAG* (one patient) in eight patients and potential disease-causing gene variants of *USH2A* (two patients), *EYS* (one patient), *TULP1* (one patient) and *C2orf71* (one patient) in five patients. Screening of an additional 69 arRP/spRP patients for the *CNGA1* gene mutation revealed one patient with a homozygous mutation.

Conclusions: This is the first identification of *CNGA1* mutations in arRP Japanese patients. The frequency of *CNGA1* gene mutation was 5.1% (5/99 patients). *CNGA1* mutations are one of the most frequent arRP-causing mutations in Japanese patients.

Citation: Katagiri S, Akahori M, Sergeev Y, Yoshitake K, Ikeo K, et al. (2014) Whole Exome Analysis Identifies Frequent *CNGA1* Mutations in Japanese Population with Autosomal Recessive Retinitis Pigmentosa. PLoS ONE 9(9): e108721. doi:10.1371/journal.pone.0108721

Editor: Namik Kaya, King Faisal Specialist Hospital and Research center, Saudi Arabia

Received March 4, 2014; **Accepted** August 31, 2014; **Published** September 30, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript and its Supporting Information files.

Funding: This study was supported by the grants to T.I. from the Ministry of Health, Labor, and Welfare of Japan (13803661); to M.A. and T.H. from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Scientific Research C, 25462744 and 25462738); to T.H. from the Vehicle Racing Commemorative Foundation; and to M.F. from the Research Grant for RIKEN Omics Science Center MEXT. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: iwatatakeshi@kankakuki.go.jp

Introduction

Retinitis pigmentosa (RP; OMIM #268000) is a heterogeneous group of inherited disorders characterized by visual field loss, night blindness, abnormal color vision and fundus degeneration. The prevalence of RP is approximately 1 per 4,000 persons and more than 1 million individuals are affected worldwide [1]. The inheritance of RP shows various patterns including autosomal recessive (arRP), autosomal dominant, X-linked, sporadic (spRP), mitochondrial [2] and digenic [3] inheritance. Among the various patterns of RP inheritance, arRP is the most frequent inheritance pattern and accounts for approximately 50% to 60% of all RP patients [1]. To date, 42 arRP-causing genes and three loci have been reported in the Retinal Information Network (RetNet; https://sph.uth.edu/retnet/). Among these arRP-causing genes, mutations in Usher syndrome 2A (*USH2A*) are the most frequent and account for approximately 17% cases including cases with additional hearing loss [1]. In non-syndromic arRP, the most frequent arRP genes are eyes shut homolog (*EYS*), *USH2A* and ATP-binding cassette sub-family A member 4 (*ABCA4*), which

Table 1. Autosomal recessive retinitis pigmentosa (arRP)-causing mutations and potential arRP-causing variants found by exome sequencing.

Family ID	Gene Name	GenBank ID	Exon	Nucleotide Change	Amino Acid Change	State	Frequency*	SNP ID	Reference	Pathogenicity
RP#002	*CNGA1*	NM_000087	5	c.191delG	p.G64VfsX29	Homo	2		HGVB	Disease-causing
RP#004	*EYS*	NM_001142800	33	c.6714delT	p.P2238PfsX16	Hetero	0		Collin et al. 2008	Disease-causing
	EYS	NM_001142800	35	c.C7002A	p.C2334X	Hetero	0		This study	
RP#014	*EYS*	NM_001142800	4	c.A141T	p.E47D	Hetero	0		This study	Potential disease-causing
	EYS	NM_001142800	26	c.4957dupA	p.S1653KfsX2	Hetero	2		Iwanami et al. 2012	
RP#016	*TULP1*	NM_003322	1	c.G3A	p.M1I	Hetero	0		This study	Potential disease-causing
	TULP1	NM_003322	13	c.C1246T	p.R416C	Hetero	0	rs200769197	dbSNP	
RP#017	*EYS*	NM_001142800	26	c.4022delC	p.S1341FfsX11	Hetero	0		This study	Disease-causing
	EYS	NM_001142800	26	c.4957dupA	p.S1653KfsX2	Hetero	2		Iwanami et al. 2012	
RP#019	*CNGA1*	NM_000087	6	c.265delC	p.L89FfsX4	Hetero	2		Chen et al. 2013	Disease-causing
	CNGA1	NM_000087	11	c.1429delG	p.V477YfsX17	Hetero	0		This study	
RP#021	*CNGA1*	NM_000087	5	c.191delG	p.G64VfsX29	Homo	2		HGVB	Disease-causing
RP#023	*USH2A*	NM_206933	49	c.C9676T	p.R3226X	Hetero	0		This study	Potential disease-causing
	USH2A	NM_206933	55	c.T10859C	p.I3620T	Hetero	0		HGVB	
RP#026	*EYS*	NM_001142800	26	c.4957dupA	p.S1653KfsX2	Homo	2		Iwanami et al. 2012	Disease-causing
RP#027	*SAG*	NM_000541	11	c.926delA	p.T309TfsX12	Homo	6		Fuchs et al. 1995	Disease-causing
RP#028	*USH2A*	NM_206933	41	c.T7880C	p.I2627T	Hetero	0		This study	Potential disease-causing
	USH2A	NM_206933	55	c.C10931T	p.T3644M	Homo	1	rs185823130	dbSNP	
	USH2A	NM_206933	70	c.T15178C	p.S5060P	Hetero	0		This study	
RP#029	*CNGA1*	NM_000087	6	c.265delC	p.L89FfsX4	Homo	2		Chen et al. 2013	Disease-causing
RP#030	*C2orf71*	NM_001029883	1	c.C85T	p.R29W	Hetero	4	rs201706430	dbSNP	Potential disease-causing
	C2orf71	NM_001029883	2	c.C3748T	p.R1250C	Hetero	0		This study	

HGVB = Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.html); dbSNP = (http://www.ncbi.nlm.nih.gov/SNP/); Frequency* show the number of mutations or variants found in 1150 alleles of 575 controls.

doi:10.1371/journal.pone.0108721.t001

account for approximately 10%, 8% and 5% to 6% of cases, respectively [1,4–10].

Large scale screening of selective exons in 30 RP-causing genes was previously performed in 193 Japanese RP families [11]. Although it only targeted exons with known mutations, the study failed to identify high frequency RP genes [11]. Another study of the Japanese RP population focused on the RP genes *RHO* [12–14] and *EYS* [15,16], and found that *EYS* was a frequent arRP gene with a prevalence rate of 9% to 16% [15,16]. However, almost all reported *EYS* gene mutations in these studies have not been reported in Western populations suggesting that Japanese individuals have a different genetic background [15,16]. These results suggest that the genetic background of RP in the Japanese population is different from that in the Western population.

The recent technological development of exon capture with 99% coverage of all exons and its combination with next generation sequencing enables effective genetic studies for hereditary diseases [17–20] and the investigation of novel mutations in multiple candidate genes [21].

The purpose of this study was to find frequent arRP genes in the Japanese population. In this study, we performed whole exome analysis of 30 Japanese arRP/spRP patients with confirmation in an additional 69 arRP/spRP patients. We found frequent arRP-causing mutations in the cyclic nucleotide gated channel alpha 1 (*CNGA1*) gene.

Materials and Methods

Informed consent

The protocol of this study was approved by the Institutional Review Board at the six participating institutions (National Hospital Organization Tokyo Medical Center, Jikei University School of Medicine, Mie University School of Medicine, Nagoya University Graduate School of Medicine, Teikyo University

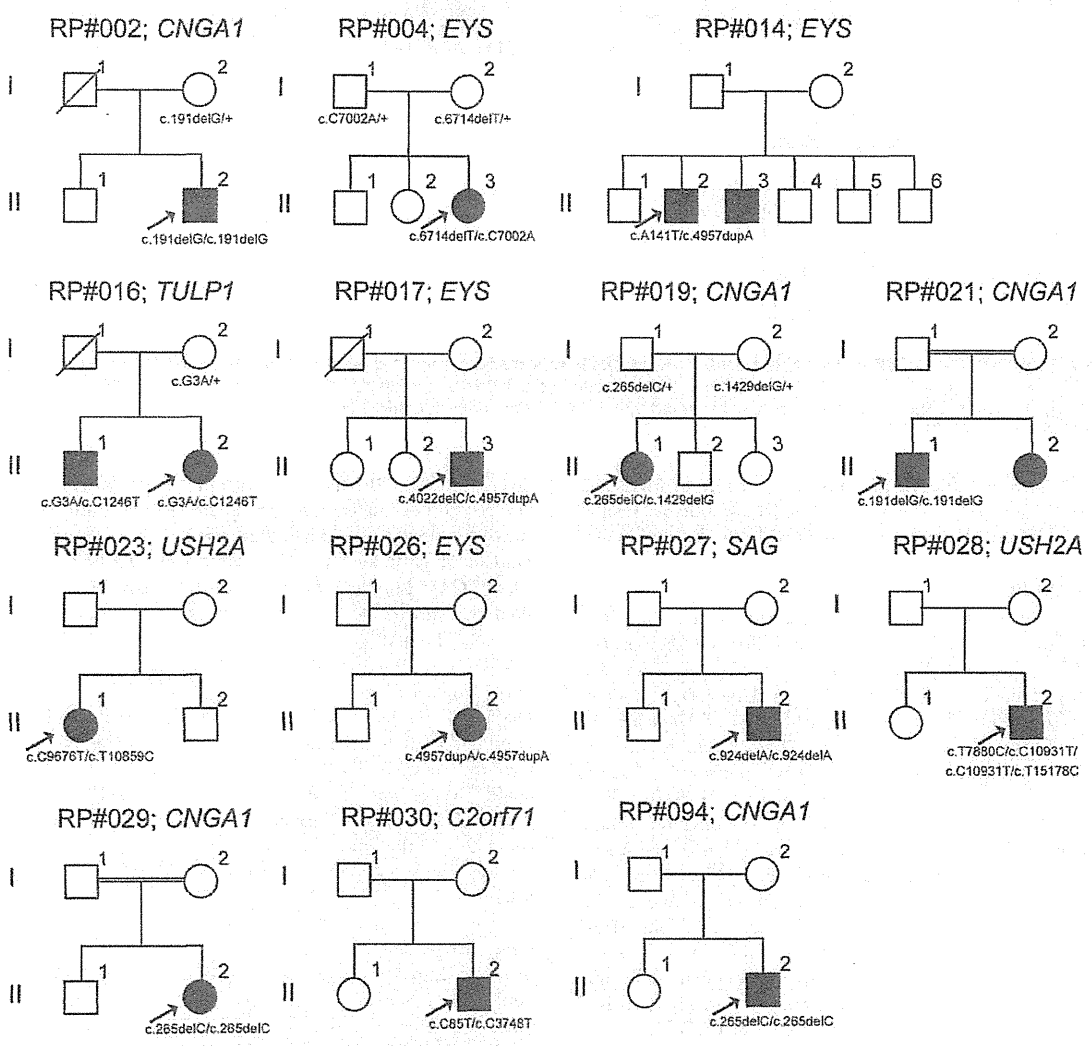

Figure 1. Pedigrees identified with arRP-causing mutations or potential arRP-causing variants. The solid squares (male) and circles (female) represent affected patients. The proband of each family is indicated by a black arrow. Unaffected family members are represented by white icons. The slash symbol indicates deceased individuals. The doubled line indicates consanguineous marriage. The generation number is shown on the left.

doi:10.1371/journal.pone.0108721.g001