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Peripherin/*RDS* Gene in Indonesian Patients with Retinitis Pigmentosa: Geographic Comparison of Polymorphic Variations

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ABSTRACT

To analyze peripherin/*RDS* (*retinal degeneration slow*) gene alterations in Indonesian patients with retinitis pigmentosa.

We examined the gene in 13 unrelated Indonesian patients with retinitis pigmentosa and in 24 normal individuals. Peripheral venous blood was extracted, and genomic DNAs were amplified by polymerase chain reaction (PCR). The PCR products were directly sequenced. Each subject underwent ocular examination. The prevalence of the gene alteration was compared to that reported in Japanese and Caucasian populations.

Among 13 patients, 3 concurrently had Glu304Gln and Gly338 Asp alterations at exon 3 of the peripherin/*RDS* gene. Two patients had heterozygous alterations and one had a homozygous variation. The prevalence of the alterations (23%) in Indonesian patients was similar to that in Japanese patients (26%) and was lower than that in Caucasian patients (30–70%). The alterations were also observed in 7 of 24 (29%) normal healthy Indonesian individuals.

Peripherin/*RDS* gene polymorphisms (Glu304Gln and Gly338Asp) were found in Indonesian patients with retinitis pigmentosa. The prevalence of alterations in Indonesian patients was similar to that in Japanese patients and lower than in Caucasian patients.

Key words: *Indonesian, Peripherin / RDS gene, Polymorphic alteration, Retinitis pigmentosa*

Retinitis pigmentosa is a group of inherited retinal diseases characterized by photoreceptor damage, night blindness, and visual field loss[18]. Several pathogenic gene mutations involving the peripherin/*RDS* gene, rhodopsin gene, cGMP-phosphodiesterase beta subunit gene, RP1 gene, and ROM-1 gene have been found in Caucasian patients[3,14].

Peripherin/*RDS* is an integral disk membrane protein that stabilizes photoreceptor outer segments of the retina[12,16]. Several mutations[1,4-6,9,11,15] and polymorphisms[1,2,7,8,10,13,17] of the peripherin/*RDS* gene have been identified in Caucasian patients with retinitis pigmentosa. Fujiki et al[7] and Budu et al[1] have reported that peripherin/*RDS* gene mutations were uncommon in Japanese patients. In the present study, we examined mutations and polymorphisms of the peripherin/*RDS* gene in Indonesian patients with retinitis pigmentosa.

MATERIALS AND METHODS

Subjects

We analyzed the peripherin/*RDS* gene in 13 patients (7 men and 6 women) with retinitis pigmentosa and in 24 normal healthy individuals (14 men and 10 women). Of 13 patients, 1 had retinitis pigmentosa of autosomal dominant inheritance, and the other 12 had no family history of retinal disease and night blindness. All 13 patients had progressive night blindness, gradual loss of visual acuity, ring scotoma or concentric constriction on Goldmann visual field testing, bone-spicule pigmentation and gray discoloration of the retina, and an elevated threshold curve on Goldmann-Weekers adaptometry. Laboratory test results, including Treponema pallidum hemaglutinin, were negative or within normal range in

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each patient. Normal individuals had good visual acuity and visual fields, normal ophthalmic and systemic findings, and no night blindness. Informed consent was obtained from each subject. The study was approved by the Review Board of Hasanuddin University.

Molecular Studies

Genomic DNAs were extracted from the peripheral venous blood of each subject. Three exons of peripherin/*RDS* gene were amplified by polymerase chain reaction (PCR) using 4 pairs of primers, as reported by Kohl et al[11]. The amplification of peripherin/*RDS* was performed using 100–200 ng of genomic DNAs in a 50-μl mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 200 μM each of dNTP, 1.25 U of *Taq* polymerase (Perkin Elmer, Norwalk, CT, USA), and 1- pmol of each primer. The PCR was performed by incubation at 94°C for 2 min followed by 30 cycles of 94°C for 30 sec (denaturation), of 55°C for 30 sec (annealing), and 72°C for 1 min (extention). The PCR products of exon 2 and 3 of peripherin/*RDS* gene were directly sequenced (ABI 310, Perkin Elmer, USA).

RESULTS

Of 13 patients with retinitis pigmentosa, 3 had polymorphisms at exon 3 of the peripherin/*RDS* gene. A substitution of base pair from G to C at the first nucleotide of codon 304 was seen, leading to the change of an amino acid glutamic acid to glutamine (GAG to CAG /Glu304Gln) (Fig. 1).

Near the end of exon 3, an alteration of base pair from G to A at the second nucleotide of codon 338 was seen, resulting in the change of an amino acid glycine to aspartic acid (GGC to GAC /Gly338Asp) (Fig. 2). Both alterations were concurrently found in 2 patients with heterozygous and in 1 case with homozygous DNA patterns (3/13, 23%). The variations of Glu304Gln and Gly338Asp were also identified in 7 of 24 normal individuals (29%).

DISCUSSION

Several mutations of peripherin/*RDS* gene have been found in Caucasian and Japanese patients with retinitis pigmentosa. These mutations were located specifically in the exon 2 (codon 200–219), known as the hot spot region of the gene[12]. Exons 1 and 3 of the gene reportedly contained silent mutation (Val106Val) and missense variations (Glu304 and Gly338Asp), respectively[2,8,10,12].

In the present study, no pathogenic mutations were found in the hot spot region of the gene. However, 2 polymorphic alterations (Glu304Gln and Gly338Asp) were found concurrently in exon 3. Both variations were found in 3 of 13 Indonesian patients with retinitis pigmentosa (23%) and in 7 of 24 (29%) normal individuals. The prevalence of the alterations is compared in the Table. Jordan et al[8] found Glu304Gln in 58% of

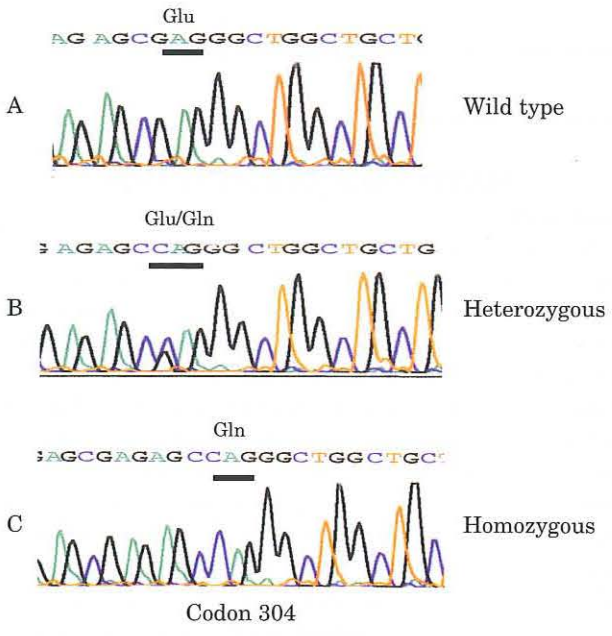

Fig. 1. Nucleotide sequences around codon 304 of the peripherin/RDS gene.
(A) Wild type DNA sequence, (B) heterozygous alteration, and (C) homozygous alteration. A substitution of guanine to cytosine at the first nucleotide of codon 304 (GAG to CAG) led to the change of amino acid glutamic acid to glutamine.

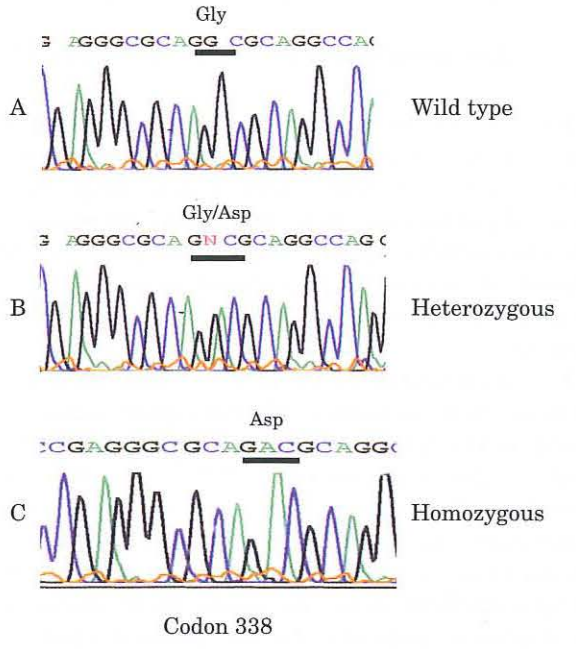

Fig. 2. Nucleotide sequences around codon 338 of the peripherin/RDS gene.
(A) Wild type DNA sequence, (B) heterozygous alteration, and (C) homozygous alteration. A substitution of guanine to adenine at the second nucleotide of codon 338 (GGC to GAC) led to a change of amino acid glycine to aspartic acid.

Table. Comparison of the prevalence of peripherin/RDS polymorphisms (Glu304Gln and Gly338Asp)

Authors	Nation	Glu304Gln		Gly338Asp	
		Patients No. (%)	Normal No. (%)	Patients No. (%)	Normal No. (%)
Jordan et al	Ireland	35/60 (58)	82/160 (51)	42/60 (70)	94/160 (59)
Trujillo et al	Spain	9/21 (45)	21/ 56 (37)	8/21 (37)	20/ 56 (36)
Dryja et al	U.S.A.	—	12/ 60 (22)	—	14/ 60 (23)
Kucinkas et al	Lithuania	5/14 (36)	—	5/14 (36)	—
Budu et al	Japan	11/42 (26)	29/100 (29)	11/42 (26)	29/100 (29)
Budu et al (this study)	Indonesia	3/13 (23)	7/ 24 (29)	3/13 (23)	7/ 24 (29)

No. indicates number of subjects with positive polymorphism per number of individuals examined.

patients and in 51% of normal individuals and Gly338Asp in 70% of patients and in 59% of normal individuals in Ireland. Trujillo et al[17] reported on Glu304Gln in 45% of patients and in 37% of normal individuals and Gly338Asp in 37% of patients and in 36% of normal individuals in Spain. Dryja et al[2] reported on Glu304Gln in 22% of normal individuals and Gly338Asp in 23% of normal individuals in the United States of America. Kucinkas et al[13] observed Glu304Gln and Gly338Asp in 36% of patients with retinitis pigmentosa in Lithuania. Budu et al[1] reported that these polymorphisms occurred in 26% of patients and in 29% of healthy individuals in Japan.

Jordan et al[8] reported that mutant amino acid substitutions in the C-terminal domain of exon 3 of peripherin/RDS, including Glu304Gln and Gly338Asp alterations may not seriously disrupt the existing function of the protein, unlike mutations in the transmembrane domain (Farrar et al, 1991)[5] and in the transdiscal loop (Kajiwara et al, 1991)[9], which have been implicated in patients with autosomal dominant retinitis pigmentosa.

The Glu304Gln and Gly338Asp alterations can not be correlated to a disease phenotype, since they occur within normal individuals. In addition, individuals who were homozygous for the two mutations were also found among subjects who did not suffer from retinitis pigmentosa[1,8].

Jordan et al[8] have studied the nucleotide polymorphisms of exon 3 in several species (human, mouse, rat, and bovine). Glu304 was completely conserved among the species. Interestingly, the amino acid in rat, mouse, and cow cDNA sequences corresponding to Gly338 in humans is an aspartate. This suggests that the identification of Gly338Asp alteration increases the conservation between species.

Our present findings and those reported by investigators previously suggest that the prevalence of Glu304Gln and Gly338Asp in the Indonesian population may be similar to that in the Japanese and lower than that in Caucasians. The frequency of CYP2C19 alleles to participate in drug metabolism is reportedly different geographically[19]. It is possible that different patterns of peripherin/*RDS* gene polymorphisms may reflect a gene flow among populations, as demonstrated in CYP2C19 alleles.

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