Table 3. Summary of the possible non-pathogenic sequence alterations in the *EYS* gene identified in this study.

Gene exon	Nucleotide change	Predicted effect	Conservation in hu/o/m/ho/d/op/p/c/z/dr[a]	Patient frequency	Control frequency	SNP ID	Reference
Exon 1	c.-500A>G			13/200		rs1490127	Abd El-Aziz et al., 2010
Exon 4	c.334G>A	p.V112I	V/I/I/I/I/I/-/-/-/E	1/200	0/192	rs112609906	
	c.359C>T	p.T120M	T/T/T/T/T/A/-/-/-/I	60/200		rs12193967	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.525_527delGGA	p.176delE	E/E/E/E/E/A/-/-/-/G	1/200	1/192		This study
Intron 5	c.863-23_863-22insTT			53/200		rs34154043	Abd El-Aziz et al., 2010
	c.863-23_863-22insTTT			44/200			This study
Exon 6	c.1005G>T	p.E335D	E/E/D/-/-/-/-/-/-/-	3/200		rs80095433	
Exon 7	c.1146T>C	p.N382N	N/N/T/-/-/-/-/-/-/-	97/200		rs974110	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 8	c.1300-3C>T			117/200		rs1936439	Audo et al., 2010; Abd El-Aziz et al., 2010
Exon 9	c.1382G>A	p.C461Y	C/C/Y/-/-/-/-/-/-/-	8/200	4/192	rs76754818	Littink et al., 2010
Intron 9	c.1599+96A>C			200/200		rs1502963	Abd El-Aziz et al., 2010
Intron 10	c.1600-38G>A			12/200		rs1502965	Abd El-Aziz et al., 2010
Exon 11	c.1712A>G	p.Q571R	Q/Q/Q/-/-/-/-/-/-/-	26/200		rs61753610	Audo I et al., 2010
Exon 12	c.1809C>T	p.V603V	V/V/V/-/-/-/-/-/-/-	178/200		rs9345601	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1891G>A	p.G631S	G/S/E/C/C/-/-/-/-/-	178/200		rs9342464	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1922A>T	p.E641V	E/E/E/E/E/-/-/-/-/-	18/200		rs17411795	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1985G>T	p.R662M	R/R/R/S/S/-/-/-/-/-	8/200	3/96		This study
Intron 12	c.2023+6_2023+7insT			175/200		rs67504324	
	c.2024-14C>T			3/200		rs45628235	
Intron 15	c.2382-26C>G			106/200		rs9445437	
Exon 16	c.2490T>C	p.P830P	P/P/P/P/P/P/P/Q/P/-	2/200	1/392		This study
	c.2528G>A	p.G843E	G/G/G/G/G/G/G/G/A/G	16/200	9/192	rs74419361	
	c.2555T>C	p.L852P	L/P/P/-/S/P/S/P/-/E	106/200		rs9294631	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 18	c.2846+52_2846+53insTAAT			120/200		rs66504228	Abd El-Aziz et al., 2010
	c.2847-24C>T			178/200		rs7743515	
Exon 19	c.2980C>G	p.P994A	P/P/P/-/-/-/-/-/-/-	3/200	2/192		This study
Intron 22	c.3444-5C>T			69/200		rs9445051	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 23	c.3568+60delA			1/200			This study
Exon 25	c.3787A>G	p.I1263V	I/V/V/V/V/V/-/-/-/-/I	36/200		rs17404123	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3809T>G	p.V1270G	V/V/V/V/V/-/-/-/-/P	1/200	1/192		This study
Intron 25	c.3877+17_22delAGATA			36/200			Barragán I et al., 2010
Exon 26	c.3906C>T	p.H1302H	H/H/H/H/H/-/-/-/-/S	10/200		rs12663916	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3936A>G	p.T1312T	T/A/T/A/A/-/-/-/-/S	10/200		rs12662610	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3973C>G	p.Q1325E	Q/E/K/K/K/-/-/-/-/S	12/200		rs12663622	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4026C>T	p.S1342S	S/S/S/S/S/-/-/-/-/A	10/200		rs12663619	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4081A>G	p.I1361V	I/I/T/V/V/-/-/-/-/S	12/200		rs17403955	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4256T>C	p.L1419S	L/S/S/S/S/L/S/V/Q/V	137/200		rs624851	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4352T>C	p.I1451T	I/T/T/K/K/-/-/-/-/T	13/200		rs62415828	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4543C>T	p.R1515W	R/R/R/R/R/-/-/-/-/H	36/200		rs62415827	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4549A>G	p.S1517G	S/G/D/T/T/-/-/-/-/H	36/200		rs62415826	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4593G>A	p.E1531E	E/E/E/E/E/-/-/-/-/Q	36/200		rs62415825	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.5244A>C	p.L1748F	L/L/L/L/L/-/-/-/-/F	8/200		rs57312007	Audo I et al., 2010; Littink et al., 2010
	c.5617C>G	p.L1873V	L/L/L/P/P/-/-/-/-/I	38/200		rs16895517	Audo I et al., 2010
Exon 27	c.5705A>T	p.N1902I	N/N/N/N/N/P/-/R/-/A	90/200		rs9353806	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 28	c.5928-35T>C			118/200		rs587278	Abd El-Aziz et al., 2010
Intron 29	c.6078+68A>G			81/200		rs36133910	Abd El-Aziz et al., 2010
	c.6079-4_6079-3delTC			87/200		rs35395170	Audo I et al., 2010
Intron 34	c.6834+61T>G			60/200		rs66502009	Abd El-Aziz et al., 2010

Table 3. Cont.

Gene exon	Nucleotide change	Predicted effect	Conservation in hu/o/m/ho/d/op/p/c/z/dr[a]	Patient frequency	Control frequency	SNP ID	Reference
Exon 35	c.6977G>A	p.R2326Q	R/R/R/L/L/L/L/L/I/L	95/200		rs4710457	Audo et al., 2010; Abd El-Aziz et al., 2010
Exon 37	c.7394C>G	p.T2465S	T/T/T/T/T/T/T/T/S/F	8/200	2/176		This study
Exon 39	c.7666A>T	p.S2556C	S/S/S/S/S/N/S/H/E/E	57/200		rs66462731	Audo et al., 2010; Abd El-Aziz et al., 2010; Barragán et al., 2010; Littink et al., 2010
Intron 41	c.8071+84T>G			53/200		rs4710257	Abd El-Aziz et al., 2010
Exon 44	c.8923T>C	p.F2975L	F/F/F/F/F/F/F/F/-/K	1/200	0/400	rs79036642	
	c.9300A>G	p.L3100L	L/L/L/L/L/L/L/L/V/I	4/200	2/192		This study

Fifty-four sequence alterations were identified in 100 patients. These alterations were predicted to be non-pathogenic for various reasons. Some have been reported as polymorphisms in previous reports. Newly identified alterations within the exons, except for c.334G>A and c.8923T>C, were also found in the control chromosome. The hyphen (-) indicates that genomic sequence of corresponding region in the species was reported to be unknown [5].

[a]hu/o/m/ho/d/op/p/c/z/dr denotes Human/Orangutan/Marmoset/Horse/Dog/Opossum/Platypus/Chicken/Zebrafish/Drosophila EYS orthologs, respectively.

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approximately 7% of arRP cases [9,10], whereas most other genes contribute to only 1% to 2% of arRP cases [11]. The estimated prevalence of very likely and possible pathogenic mutations of the *EYS* gene in our study was 26%, suggesting its major involvement in the pathogenesis of arRP in the Japanese population.

We found that 16% of Japanese arRP patients displayed at least one c.4957_4958insA or c.8868C>A mutation, which accounted for 57.1% (15+5/35) of the mutated alleles. Thus, these mutations seem to be frequent among Japanese arRP patients. Previous studies employing Indonesian, Pakistani, Chinese, Israeli, Spanish, French, British, Dutch, and Palestinian RP patient populations have not detected them [3–6,12–15]. Since the Japanese were divided into small semi-closed population groups among which intercommunication was quite less until the mid-20th century, obvious or latent consanguineous marriages were carried out more frequently, leading to relatively high inbreeding levels in those populations. The frequency of the c.4957_4958insA and c.8868C>A mutations may result from a founder effect like that of the 2299delG *USH2A* gene mutation, which accounts for 44% of disease alleles in Danish and Norwegian patients with Usher syndrome type II [16].

We detected 13 different very likely and possible pathogenic mutations. Three were truncating mutations and accounted for 60% (21/35) of mutated alleles. Likewise, previous studies reported that most pathogenic mutations were truncated type (nonsense, deletion, insertion, or splicing) [3–6,12–15]. Furthermore, c.6557G>A was the only mutation that was common between the Japanese and other populations. This mutation has been found in Korean/American and Chinese patients [3,6]. These results indicate that the *EYS* gene mutation spectrum among Japanese patients largely differs from that among the previously mentioned non-Asian populations. The Japanese and Korean mutation spectrum may resemble each other, but an accurate comparison could not be made, because further *EYS* gene analysis of Korean RP patients is required to clarify this possibility.

A second mutant allele could not be detected by direct sequencing in 17 of 26 patients in our study. Previous studies reported 7 of 10 [3] and 9 of 17 [5] patients with heterozygous *EYS* gene mutation, implying that this finding could be due to relatively large heterozygous deletions [15]. The second mutation in these families may also have been located within the gene regulatory elements or unknown exons including alternative splicing areas. Although rare, a single *EYS* mutation in combination with another mutation on a second gene could also explain this phenotype [3].

The c.4957_4958insA and c.8868C>A mutations were not detected in Japanese patients with adRP or with LCA. Abd El-Aziz et al. reported that *EYS* gene mutation screening did not reveal any pathogenic mutations in 95 British and Chinese adRP patients [3]. Bandah-Rosenfeld et al. reported that no mutation was found in 2 Oriental Jewish and Israeli Muslim LCA patients who had a large homozygous region harboring the *EYS* gene [12]. Although further analysis of all *EYS* gene exons is required, *EYS* gene mutations may not be detected in Japanese patients with adRP and LCA. The c.4957_4958insA and c.8868C>A mutations were also detected in Korean patients with arRP and accounted for 6.3% (4/64 alleles) of the disease alleles. Similar to Japanese arRP results, the c.4957_4958insA mutation was more frequently detected than the c.8868C>A mutation. The fact that both c.4957_4958insA and c.8868C>A mutations were also detected in Korean patients suggests the possibility that the mutations occurred in an ancient common ancestor and spread throughout East Asia.

RP is a highly heterogeneous disease, with a reported prevalence rate of 1 in 4,000–8,000 people in Japan. Given the population of Japan, approximately a 100 million, the number of patients with RP can be estimated to be 12,500–25,000. The relative frequencies of RP inheritance patterns in Japanese patients were estimated as 25.2% for autosomal recessive, 16.9% for autosomal dominant, 1.6% for X-linked, and 56.3% for simplex, indicating that most Japanese RP patients represent arRP or isolated cases [17]. Autosomal recessive and simplex cases account over 80% of RP cases in Japan (approximately 10,000–20,000 people). Our results indicate that c.4957_4958insA and c.8868C>A mutations are possibly present in 1,600–3,200 Japanese patients with RP. These 2 novel mutations will be very useful for genetic diagnosis and counseling, and analysis of the mutated proteins may be helpful in the development of effective therapies for RP in Japan and Korea.

In conclusion, mutation screening of the *EYS* gene in 100 Japanese patients revealed 13 different pathogenic mutations, confirming that the mutation spectrum in Japanese patients differs from the previously reported spectrum in patients of non-Asian populations. Among these 13 mutations, 2 truncating mutations, c.4957_4958insA and c.8868C>A, were detected in at least one mutated allele in 16% of Japanese arRP patients and may be the

most frequent mutations causing RP in the Japanese populations. Screening for c.4957_4958insA and c.8868C>A mutations in the *EYS* gene is, therefore, very effective for the genetic testing and counseling of RP patients in Japan. Further analysis is necessary to obtain a more precise mutation spectrum and to identify other frequent mutations in other East Asian populations.

Supporting Information

Table S1 PCR primer sequences for human *EYS*.
(DOC)

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Author Contributions

Conceived and designed the experiments: KH MT SY MK YH. Performed the experiments: KH CI YZ. Analyzed the data: KH CI. Contributed reagents/materials/analysis tools: MT DHP YH HN SU TY AH TF SN JPS ITK SY NA HT MS MK YH. Wrote the paper: KH SM YH.