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ORIGINAL ARTICLE

Perception of genetic testing among patients with inherited retinal disease: Benefits and challenges in a Japanese population

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Abstract

Inherited retinal disease (IRD) is clinically and genetically heterogeneous. Awareness of the importance of genetic testing for IRD in the clinical setting is increasing with the recent development of new therapeutic strategies, such as gene therapy. Here, the perception of genetic testing, including its benefits and potential challenges, among patients with IRD was investigated to establish strategies for IRD genetic testing and counseling practices that can meet the requirements of the patients in Japan. An anonymous self-administered questionnaire was distributed to 275 patients with IRD who underwent genetic testing after clinical consultation and genetic counseling to investigate the motivations for genetic testing, benefits, challenges, status of communication of results to family, and attitude to timing of genetic testing. In total, 228 (82.9%) responses were analyzed. Several major motivations for genetic testing were identified, including gaining information on future treatment options and clarification of the inheritance pattern, among others. No association was found between the sharing of results with family members and the results of genetic testing. Moreover, according to patients who received positive results, the benefits of genetic testing included information on the inheritance pattern, additional information on the diagnosis, and mental preparation for the future. Even patients who received negative or inconclusive (variant of uncertain significance) results reported certain informative and psychological benefits. Altogether, these findings suggest that provisions for genetic testing and genetic counseling are necessary within a certain period after clinical diagnosis and it is necessary to facilitate appropriate family communication about genetic testing results while paying attention to the background of family relationships. Moreover, the benefits of genetic testing can be influenced by the careful interpretation and information provided on the test results during genetic counseling and consultation.

KEYWORDS
genetic counseling, genetic testing, inherited retinal disease, retinitis pigmentosa

1 | INTRODUCTION

Inherited retinal disease (IRD) is the most common inherited ophthalmic disease that is characterized by the progressive degeneration of photoreceptor cells and retinal pigment epithelial cells (Berger et al., 2010). Patients with retinitis pigmentosa, the most common type of IRD, generally develop night blindness, constriction of the visual field, and impairment of visual acuity. It is the second leading cause of visual impairment among adults in Japan (Morizane et al., 2019). The process of treatment development has progressed considerably, and even though IRD is regarded as intractable, the approach to the disease has undergone major changes (Hafler, 2017; Maeda et al., 2019).

IRD is genetically heterogeneous, and more than 200 causal genes have been identified (RetNet [https://sph.uth.edu/retnet/] by November 9, 2020). The genetic evaluation of individuals with IRD can be helpful for molecular diagnosis, prediction of prognosis and risk to other organs, and therapeutic applications. With the discovery and development of new treatment strategies, such as gene therapy, the importance of genetic testing in the clinical setting is increasing (Duncan et al., 2018). IRD can follow any of the different inheritance patterns, such as autosomal dominant, autosomal recessive, and X-linked (Méjécase et al., 2020). The prediction of the causal gene based on clinical symptoms or family history is also difficult in almost all cases. The accurate inheritance pattern and causal gene can only be revealed by genetic testing.

Genetic counseling is recommended along with IRD genetic testing (Duncan et al., 2018; Méjécase et al., 2020). The clinical and personal implications of identifying the IRD causal gene and the implications of revealing information about the risks to family members should also be discussed with patients in IRD genetic counseling practices. Previous studies in UK and China on patients who have undergone IRD genetic diagnosis reported that the patients considered genetic testing to be a beneficial and important step, and this is thus potentially beneficial for the family members of the patients as well (McVeigh et al., 2019; Zhang et al., 2019). These reports revealed diversity among the experience of a small number of subjects. However, the knowledge of the motivations and benefits, as well as the patient background that could influence these perceptions is still scarce; this also includes the current status of sharing genetic testing results with family members, which is also known to be a challenge for most patients with IRD from study in UK (McKibbin et al., 2014). There has been an increasing opportunity for IRD patients in Japan to receive their genetic counseling, however, genetic analyses of IRD are mostly performed in research settings in a limited facility. The practice of IRD genetic counseling with genetic testing has not been sufficiently discussed. In this study, we investigated the important motivations, benefits, potential challenges, status of result sharing with family, and attitude toward the timing of genetic testing in Japanese patients with IRD. This study could provide additional information that helps establish IRD genetic testing and genetic counseling practices in Japan.

What is known about this topic

The demand for genetic testing is high among patients with IRD, and individual experiences and their variations have been reported in qualitative studies.

What this paper adds to the topic

The tendencies underlying among individuals with IRD in Japan, the motivations and benefits of genetic testing, the association between patient background and these motivations, benefits, and the family communication situation were determined using quantitative analysis.

2 | METHODS

2.1 | Participants and recruitment

In this study, 275 patients with IRD who visited the IRD consultation and Genetic Counseling at the Kobe City Eye Hospital, located in Kobe, Hyogo prefecture, were recruited. Genetic evaluation was performed and the patients received the results between December 2017 and March 2020. All patients underwent a multigene panel analysis study including 39 or 50 causal genes associated with IRD (Maeda et al., 2018). Patients aged <21 years and those who refused to be contacted by the clinic or lived overseas were excluded. The questionnaire and study explanation documents were mailed to the patients. The return of an answered questionnaire was considered equivalent to providing consent for participation.

2.2 | Instrumentation

This cross-sectional study used an anonymous self-administered questionnaire that was sent to the participants via mail. A draft of the questionnaire was prepared based on our previous genetic counseling records and qualitative analyses (Akira Inaba et al., 2019), and other previous reports (McKibbin et al., 2014; McVeigh et al., 2019; Zhang et al., 2019). The questionnaire was completed after a pilot study was performed on 10 patients with IRD, which consisted of questions on motivation for genetic testing (one question), benefits (one question), and challenges (two questions) experienced, status of sharing results with family (four questions), attitude toward the timing of the test (one question), characteristics (nine questions), additional comments (one question), and analysis results (one question only for patients who received positive results).

2.3 | Procedure

The response period was during June, 2020. For patients who had difficulty writing their own answers to the questionnaire, answers

could be provided by their family member, caregiver, or the investigator on behalf of them. The participants decided whether to provide their names to facilitate further contact and acquire additional information. A reminder letter was sent 2 weeks before the response deadline to all patients, except those who had already returned a signed questionnaire. This study was approved by the institutional review board of Kobe City Eye Hospital (Protocol no. E19002 and Permit no. ezh200501).

2.4 | Data analysis

Data used for the analysis was validated by two investigators. Statistical analysis was performed using JMP 15.1.0 (SAS Institute Inc., Cary, NC, USA). The frequency distribution and percentage of responses to each question were investigated. The Chi-square test and Student's *t*-test were used to compare the characteristics in each group of patients with positive (E+) or negative (E−)/variant of uncertain significance (VUS) results, and the Chi-square test and multivariate logistic regression analysis were used to investigate the factors associated with the attitude toward the timing of genetic analysis. *p*-values < 0.05 were considered statistically significant based on two-sided tests, except for the Chi-square test with Bonferroni correction ($p < 0.0038$), for comparing benefits and test results. The descriptive answers in open-ended question were analyzed supplementary using inductive thematic analysis (Braun & Clarke, 2006).

3 | RESULTS

3.1 | Participants

In total, 275 patients with IRD were mailed the questionnaire, of which 234 (85.1%) answered and returned the questionnaire, and the responses provided by 228 (82.9%) patients were analyzed. Of these, 100 (100 of 228; 43.9%) patients received positive results, 87 (87 of 228; 38.2%) received negative results, and 41 (41 of 228; 18.0%) received VUS results. The characteristics of patients who received positive and negative/VUS results are presented in Table 1. No significant differences were observed with respect to age, gender, has at least one biological child, and best-corrected visual acuity between the two groups. The age of diagnosis was higher for patients who received negative/VUS results than for those who received positive results ($p < 0.001$), and the number of affected family members was higher among patients who received positive results than among those who received negative/VUS results ($p = 0.002$).

3.2 | Motivations for undergoing genetic testing

The motivations for undergoing genetic testing are presented in Figure 1. The important motivations included obtaining information on future treatment options (64 of 228; 28.1%), confirmation of the inheritance pattern (57 of 228; 25.0%), and identification of

TABLE 1 Characteristics of patients in this study

		Result of genetic testing	
		Positive, $n = 100$	Negative/VUS, $n = 128$
Age (years, mean ± *SD*, range)		55.2 ± 14.3, 20–88	56.2 ± 13.6, 21–80
Gender (*n*, %)	Male	52 (52.0)	51 (39.8)
	Female	48 (48.0)	77 (60.2)
Biological child[a] (*n*, %)	Yes	69 (69.0)	93 (72.7)
Family history	Yes	48 (48.0)	36 (28.1)
Age at diagnosis (years, mean ± *SD*, range)		33.5 ± 15.0, 3–75	41.1 ± 14.6, 8–70
Best-corrected visual acuity	≧0.7	26 (26.0)	33 (25.8)
	0.3–0.7	18 (18.0)	35 (27.3)
	0.1–0.3	20 (20.0)	14 (10.9)
	<0.1	29 (29.0)	37 (28.9)
	Not sure	7 (7.0)	9 (7.0)
Timing of genetic analysis	<1 year	12 (12.0)	19 (14.8)
	1–5 years	10 (10.0)	27 (21.1)
	5–10 years	12 (12.0)	14 (10.9)
	≧10 years	66 (66.0)	68 (53.1)
Inheritance pattern	AD	19 (19.0)	–
	AR	49 (49.0)	–
	XL	18 (18.0)	–
	not sure	14 (14.0)	–

[a]Excluding adopted children.

the cause of the disease (23 of 228; 10.1%). The patients selected six options or more on an average in a multiple-choice question on motivations.

3.3 | Timing of undergoing genetic testing for IRD

Eighty-eight (88 of 228; 38.6%) patients reported that they would have preferred to undergo genetic testing at an earlier time point. Bivariate and multivariate analyses were performed to investigate the association between attitude regarding the timing of genetic testing and factors, such as results and other characteristics. As shown in Table 2, receiving positive results and undergoing genetic testing more than 10 years after an initial clinical diagnosis were associated with the desire to undergo genetic testing at an earlier time point. Gender, has at least one biological child, and affected family members were not associated with this. None of the patients wanted to undergo genetic testing at a later time point.

3.4 | Communication of genetic testing results with family members

Next, the status of sharing genetic testing results with family members was investigated. Two hundred and twenty-six individuals provided a complete answer to this question. As shown in Figure 2, most of the patients shared the result with their partner (E+: 78 of 81; 96.3%, E−/VUS: 92 of 96; 95.8%) and approximately 50%–70% of patients shared the result with their parents (E+: 36 of 64; 56.3%, E−/VUS: 52 of 78; 66.6%), siblings (E+: 48 of 78; 61.5%, E−/VUS: 46 of 96; 47.9%), and children (E+: 45 of 69; 65.2%, E−/VUS: 65 of 92; 70.7%). As shown in Figure S1, the reasons that were selected most frequently for sharing and not sharing the genetic testing results with family members were 'I usually share information regarding my disease and heredity with my family' (E+: 77 of 94; 81.9%, E−/VUS: 99 of 123; 80.4%) and 'I felt my family members would feel burdened by the result, considering their age and conditions' (E+: 30 of 49; 61.2%, E−/VUS: 29 of 60; 48.3%), respectively. No significant association was found between the reasons for sharing or not sharing and the results.

3.5 | Benefits and challenges of genetic testing

The benefits 'information on inheritance pattern' [$\chi^2(1) = 12.13$, $p < 0.001$], 'additional information on diagnosis' [$\chi^2(1) = 19.409$, $p < 0.001$], 'psychological preparation for the future' [$\chi^2(1) = 9.93$, $p = 0.0016$], 'information for future treatment options' [$\chi^2(1) = 19.26$, $p < 0.001$], 'life planning of family member about marriage and reproduction' [$\chi^2(1) = 11.59$, $p < 0.001$], and 'life planning of family member about future steps' [$\chi^2(1) = 12.30$, $p < 0.001$] were selected at a significantly higher frequency by patients who received positive results than by those who received a negative/VUS result, as shown in Figure 3. However, no significant difference was observed between the selected benefits among those who received positive results and clarified their inheritance pattern, except for 'reduced concern regarding inheritance pattern.' In addition, more than 50% of the patients who received negative/VUS results recognized benefits, such as 'information on inheritance pattern' (93 of 128; 72.7%), 'acceptance of disease' (77 of 128; 60.2%), and 'psychological preparation for the future' (76 of 128; 59.4%). Eighty-four patients (84 of 226; 36.7%) expressed concerns regarding genetic testing. In the descriptive answer, the concerns were suggested to be associated with the duration between blood sample collection and result declaration, the uncertainty of the genetic information, clarification of the inheritance pattern, and communicating the genetic results to family members. In addition, suggestions for improvement, such as increasing access to ophthalmic genetic medicine, expansion of the analysis system at a national level, and continuation of support after genetic testing were also provided.

4 | DISCUSSION

To date, a genetic diagnosis of IRD is mostly obtained under research settings in Japan; however, it is expected to soon be performed in the clinic setting similar to the United States and European countries (Eden et al., 2016; Hafler, 2017). The findings of our study suggest that genetic testing for IRD has various benefits for patients, as seen in previous studies on other genetic diseases (Kohler et al., 2017). Thus, referral to genetic counseling and timely genetic diagnosis of IRD are needed.

Information for future treatment options, confirmation of the inheritance pattern, and identification of causal genes were found to be important motivators for genetic testing in this study. Moreover, a similar trend, in which multiple personal motivating factors drive such decisions on genetic testing, has also been reported in previous studies on other genetic disorders (Stafford et al., 2019; Withrow et al., 2008). The primary characteristics of IRD, which include intractability and difficulty in identification of the causal gene and inheritance pattern based on clinical symptoms and family history, seem to influence the need for information that is revealed only through genetic testing. A previous study reported that a prenatal diagnosis and reproduction planning are the objectives of genetic testing for IRD (Eden et al., 2016). Nonetheless, prenatal diagnosis for IRD is not performed in Japan, and motivation related to marriage and reproduction was rarely observed in this study.

The definition of appropriate timing for genetic testing for IRD (McKibbin et al., 2014), as well as for other genetic diseases (Lesperance et al., 2018; Van de Beek et al., 2020), remains controversial. In our cohort, for those subjected to genetic testing more than 10 years after the initial clinical diagnosis and for those receiving positive results, there was an association with a preference to undergo genetic testing at an earlier time point. This study suggests that IRD patients should be provided with an opportunity for genetic counseling to consider genetic testing within 10 years after the

diagnosis, even in situations in which the utility of genetic results for medical intervention is limited.

Almost all patients shared the results with their partners, which was considerably higher compared with those who shared with first-degree relatives. Since IRD is a progressive disease and requires the support of the closest family members in daily life, it is presumed that sharing of information about the disease, including the results of genetic testing, is more common. In agreement with the findings of this study, a previous study on hereditary cancer reported that factors such as background of family relationships are relevant for family communications related to genetic diseases (Chivers Seymour et al., 2010), suggesting that a general tendency to share information on the disease with family members promotes sharing the results of genetic testing. Concurrently, concerns about the burden on family members are suggested to be critical factors affecting patients who hesitate to share the results. Considering that genetic testing results have limited effects on the willingness to share results with family, it is necessary to pay attention to the background of family relationships and to facilitate appropriate communication with family with respect to the genetic testing results from the stage of genetic counseling before genetic testing.

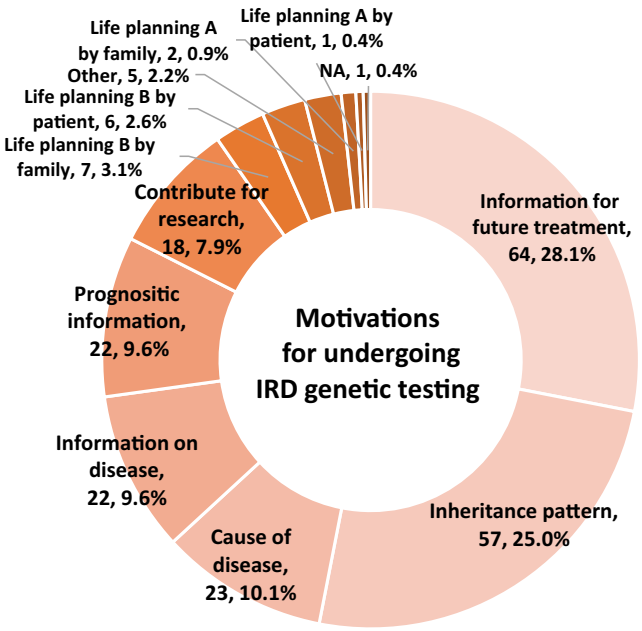

FIGURE 1 Prevalence of motivations for undergoing inherited retinal disease (IRD) genetic testing ($n = 228$). A: life planning by patient/family members, especially future step. B: life planning by patient/family members, especially with respect to marriage and reproduction. NA: No Answer

The genetic testing for IRD had been described as 'a significant milestone' (McVeigh et al., 2019) and patients in this study also recognized the benefits of 'preparation for the future' and 'disease acceptance.' Genetic testing might contribute to improving the quality of life of patients and preventing psychological problems, as illustrated in previous reports (Prem Senthil et al., 2017; Sainohira et al., 2018). Conversely, the benefits were recognized even in patients for whom the causal gene was not identified or those who received VUS results. The patients in this study presumably evaluated the genetic testing process based on pre- and post-clinical consultation and genetic counseling, not only based on the information derived from genetic analysis. The potential value of genetic testing results for patients includes a better understanding of their own prognosis and risk of disease in family members (Grosse & Khoury, 2006). Moreover, in IRD genetic counseling, it is necessary to interpret results and communicate the predicted results of genetic testing to patients, such as the prognosis and effects on daily life, the need for follow-up tests on other organs, possible approaches for future

TABLE 2 Bivariate and multivariate analysis for the attitude toward the timing of genetic analysis

		I wanted to undergo genetic testing at an earlier time		*p*-value	
		Yes $n = 88$ (38.6%)	No $n = 140$ (61.4%)	Bivariate analysis	Multivariate analysis
Timing[a]	≧10 years	62 (46.3)	72 (53.7)	**0.0045**	**0.0065**
	<10 years	26 (27.7)	68 (72.3)		
Result	Positive	48 (48.0)	52 (52.0)	**0.0099**	**0.0142**
	Negative/VUS	40 (31.3)	88 (68.8)		
Gender	Male	44 (42.7)	59 (57.3)	0.2458	0.5400
	Female	44 (35.2)	81 (64.8)		
Biological child[b]	Yes	64 (39.5)	98 (60.5)	0.6585	0.4591
	No	24 (36.4)	42 (63.6)		
Affected family	Yes	30 (35.7)	54 (64.3)	0.4947	0.1016
	No	58 (40.3)	86 (59.7)		

[a]Indicates the timing when patients underwent genetic analysis after clinical diagnosis.
[b]Excluding adopted children.

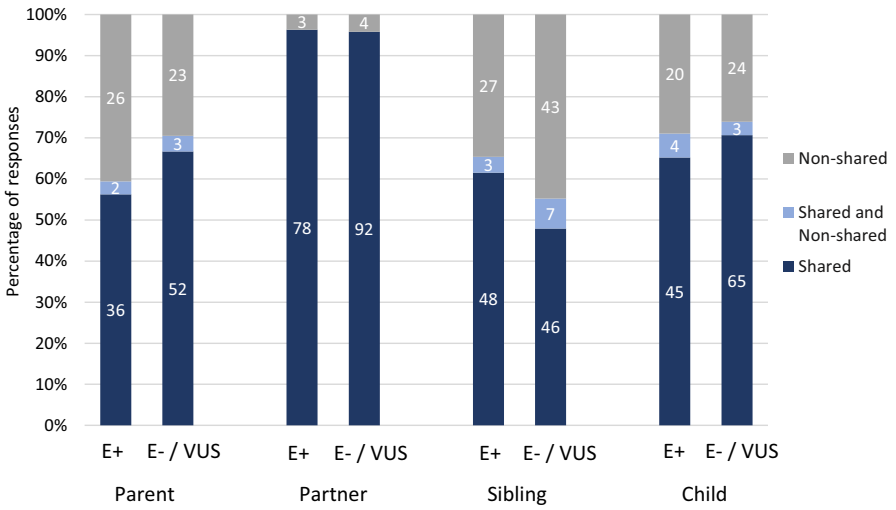

FIGURE 2 Distribution of patients who shared genetic analysis results with each family member after receiving positive (E+) or negative (E−)/variant of uncertain significance (VUS) result. The y-axis indicates the percentage of patients who shared/did not share information, and the x-axis indicates the genetic analysis results and represents family members as objects. The numeric values in each column indicate the number of responses. Total number of responses was 64 and 78 for parents, 81 and 96 for partner, 78 and 96 for sibling, and 69 and 92 for child in E+ and E−/VUS cases, respectively. The responses from the patients who have no corresponding family members are not included

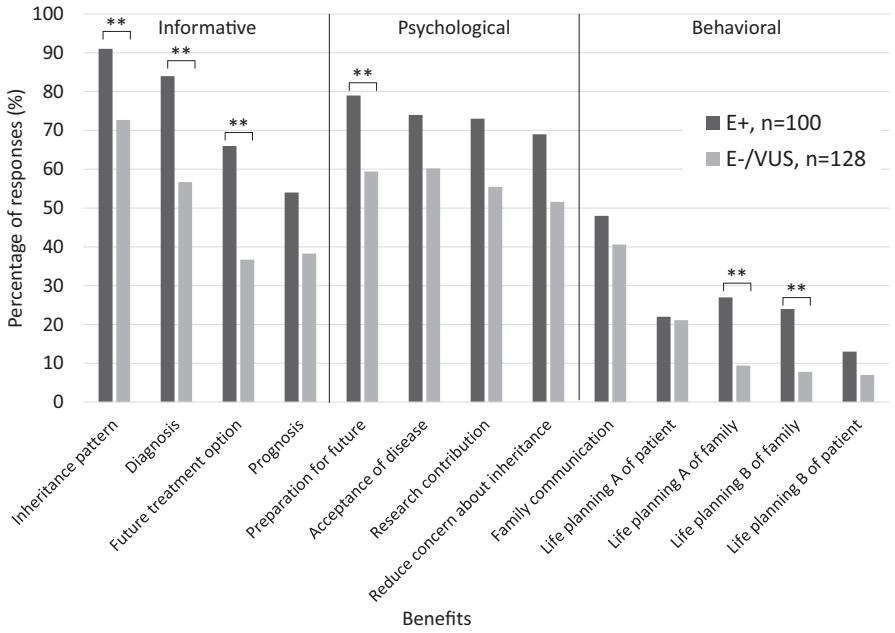

FIGURE 3 Distribution of the benefits experienced from genetic analysis reported by patients with positive (E+) and negative (E−)/variant of uncertain significance (VUS) results. The y-axis represents the percentage of responses and the x-axis lists the benefits. The total number of responses were 100 and 128 for patients who received E+ and E−/VUS results, respectively. A: life planning by patient/family members, especially future step. B: life planning by patient/family members, especially with respect to marriage and reproduction. ** indicates significant difference found using Chi-square test with Bonferroni correction ($p < 0.0038$)

treatment, and the presence or absence of relatives at risk of developing the disease. Alterations to some genes can be responsible for syndromic visual impairment accompanied by systemic conditions such as sensorineural hearing loss (e.g., Usher syndrome), developmental delay (e.g., Joubert syndrome), and kidney disease (e.g., Bardet-Biedl syndrome) (Adams et al., 2007). A comprehensive understanding of the patient's conditions and referral to the relevant department might change their clinical management. Furthermore, it is important to support the patients and families and help them understand the information and that their actual behavior will help them to benefit greatly from the genetic testing results, regardless of the nature of such results. Considering the previous studies that pointed out disappointment with the result and misunderstanding and anxiety regarding disease conditions persisting in patients who received negative/VUS results (McVeigh et al., 2019; Stafford et al., 2019), continuous support and involvement of patients are necessary before and after genetic diagnosis.

Regarding suggestions for future research, expanding the scope of research participants will help to more accurately understand the overall trend for IRD patients. Understanding of backgrounds, concerns, and expectations of the patients with IRD who decided not to undergo the genetic analysis will enable us to appropriately support a wider range of IRD patients. Comparing our findings with those of patients from different countries who have undergone a genetic

testing process, the characteristics of Japanese patients with IRD regarding their attitude toward ophthalmic genetic medicine could become clearer. Whereas the demand for IRD genetic counseling with genetic testing has increased, there is not a sufficient supply system for ophthalmic genetic counseling in Japan. Understanding challenges in constructing the tele-genetics system for IRD patients and verifying the usefulness of this system will improve access to advanced ophthalmic genetic medicine.

4.1 | Study limitations

Our investigation had the following limitations. First, this was a single-center study, and whether our data accurately represent the trend in Japanese patients at large cannot be confirmed. Second, our clinic is one of the institutions that are actively conducting clinical research and patients visit from across the country; as such, our patients could have a positive attitude toward receiving medical care, information acquisition, and research participation compared with other patients with IRD. Third, this was a cross-sectional study, and the causal relationships with each result are unknown. In addition, we included patients who received negative and VUS results in a single group; however, the two groups of patients are not completely identical in other genetic disorders (Mighton et al., 2020). Finally, we only included those patients who decided to undergo genetic testing after medical consultation and genetic counseling; however, there were other patients in our clinic who decided against genetic testing.

4.2 | Practice implications

This study demonstrated that even in situations where the clinical utility of genetic testing results for medical intervention is limited, Japanese patients often ask for information on the potential causal genes, future treatment options, and inheritance patterns, which can only be obtained using genetic testing. Therefore, referral to a facility of genetic medicine within 10 years of the initial clinical diagnosis is necessary. Careful interpretation of the genetic analysis results based on patient conditions and family history is important. The communication of genetic testing results with family members is influenced by the family situation and the general habits of sharing disease-related information than by the result itself.

AUTHOR CONTRIBUTIONS

A.I. and A.Y. conceptualized and designed the study. A. I., A. Y., and K. K. prepared and mailed the questionnaire. A.I. and K.K entered and checked the data used for analysis. A.I. performed the data analysis. A.I. and A.Y. confirm that they had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis. A.I. and A.Y. drafted the manuscript. A.M., K.K., S.K., and M.T. assisted in designing the survey and critically revising the manuscript. All authors gave final approval of this version to be published and agree to be accountable for all aspects of the work.

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COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

A.I., A.Y., A.M., K.K., S.K., and M.T. declare no conflict of interests.

HUMAN STUDIES AND INFORMED CONSENT

All procedures were performed in accordance with the Helsinki Declaration following ethical standards of the institutional review board of Kobe City Eye Hospital (Protocol no. E19002 and Permit no. ezh200501). Informed consent was obtained from individuals who voluntarily completed the mail survey and submitted their responses.

ANIMAL STUDIES

No non-human animal studies were carried out by the authors for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Perception of genetic testing among patients with inherited retinal disease: Benefits and challenges in a Japanese population

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Running head: Perception of genetic testing in patients with IRD

Abstract

Inherited retinal disease (IRD) is clinically and genetically heterogeneous. Awareness of the importance of genetic testing for IRD in the clinical setting is increasing with the recent development of new therapeutic strategies, such as gene therapy. Here, the perception of genetic testing, including its benefits and potential challenges, among patients with IRD was investigated to establish strategies for IRD genetic testing and counseling practices that can meet the requirements of the patients in Japan. An anonymous self-administered questionnaire was distributed to 275 patients with IRD who underwent genetic testing after clinical consultation and genetic counseling to investigate the motivations for genetic testing, benefits, challenges, status of communication of results to family, and attitude to timing of genetic testing. In total, 228 (82.9%) responses were analyzed. Several major motivations for genetic testing were identified, including gaining information on future treatment options and clarification of the inheritance pattern, among others. No association was found between the sharing of results with family members and the results of genetic testing. Moreover, according to patients who received positive results, the benefits of genetic testing included information on the inheritance pattern, additional information on the diagnosis, and mental preparation for the future. Even patients who received negative or inconclusive (variant of uncertain significance) results reported certain informative and psychological benefits. Altogether, these findings suggest that provisions for genetic testing and genetic counseling are necessary within

a certain period after clinical diagnosis and it is necessary to facilitate appropriate family communication about genetic testing results while paying attention to the background of family relationships. Moreover, the benefits of genetic testing can be influenced by the careful interpretation and information provided on the test results during genetic counseling and consultation.

Keywords: genetic counseling, genetic testing, inherited retinal disease, retinitis pigmentosa

What is known about this topic: The demand for genetic testing is high among patients with IRD, and individual experiences and their variations have been reported in qualitative studies.

What this paper adds to the topic: The tendencies underlying among individuals with IRD in Japan the motivations and benefits of genetic testing, the association between patient background and these motivations, benefits, and the family communication situation were determined using quantitative analysis.

1. Introduction

Inherited retinal disease (IRD) is the most common inherited ophthalmic disease that is characterized by the progressive degeneration of photoreceptor cells and retinal pigment epithelial cells (Berger et al., 2010). Patients with retinitis pigmentosa, the most common type

of IRD, generally develop night blindness, constriction of the visual field, and impairment of visual acuity. It is the second leading cause of visual impairment among adults in Japan (Morizane et al., 2019). The process of treatment development has progressed considerably, and even though IRD is regarded as intractable, the approach to the disease has undergone major changes (Hafler et al., 2017; Maeda et al., 2019).

IRD is genetically heterogeneous, and more than 200 causal genes have been identified (RetNet [https://sph.uth.edu/retnet/] by November 9, 2020). The genetic evaluation of individuals with IRD can be helpful for molecular diagnosis, prediction of prognosis and risk to other organs, and therapeutic applications. With the discovery and development of new treatment strategies, such as gene therapy, the importance of genetic testing in the clinical setting is increasing (Duncan et al., 2018). IRD can follow any of the different inheritance patterns, such as autosomal dominant, autosomal recessive, and X-linked (Méjécase et al., 2020). The prediction of the causal gene based on clinical symptoms or family history is also difficult in almost all cases. The accurate inheritance pattern and causal gene can only be revealed by genetic testing.

Genetic counseling is recommended along with IRD genetic testing (Duncan et al., 2018; Méjécase et al., 2020). The clinical and personal implications of identifying the IRD causal gene and the implications of revealing information about the risks to family members should also be discussed with patients in IRD genetic counseling practices. Previous studies in

71 UK and China on patients who have undergone IRD genetic diagnosis reported that the patients
72 considered genetic testing to be a beneficial and important step, and this is thus potentially
73 beneficial for the family members of the patients as well (McVeigh et al., 2019; Zhang et al.,
74 2019). These reports revealed diversity among the experience of a small number of subjects.
75 However, the knowledge of the motivations and benefits, as well as the patient background that
76 could influence these perceptions is still scarce; this also includes the current status of sharing
77 genetic testing results with family members, which is also known to be a challenge for most
78 patients with IRD from study in UK (McKibbin et al., 2014). There has been an increasing
79 opportunity for IRD patients in Japan to receive their genetic counseling, however, genetic
80 analyses of IRD are mostly performed in research settings in a limited facility. The practice of
81 IRD genetic counseling with genetic testing has not been sufficiently discussed. In this study,
82 we investigated the important motivations, benefits, potential challenges, status of result
83 sharing with family, and attitude toward the timing of genetic testing in Japanese patients with
84 IRD. This study could provide additional information that helps establish IRD genetic testing
85 and genetic counseling practices in Japan.
86
87 **2. Methods**
88 **2.1 Participants and Recruitment**

In this study, 275 patients with IRD who visited the IRD consultation and Genetic Counseling at the Kobe City Eye Hospital, located in Kobe, Hyogo prefecture, were recruited. Genetic evaluation was performed and the patients received the results between December 2017 and March 2020. All patients underwent a multi-gene panel analysis study including 39 or 50 causal genes associated with IRD (Maeda et al., 2018). Patients aged < 21 years and those who refused to be contacted by the clinic or lived overseas were excluded. The questionnaire and study explanation documents were mailed to the patients. The return of an answered questionnaire was considered equivalent to providing consent for participation.

2.2 Instrumentation

This cross-sectional study used an anonymous self-administered questionnaire that was sent to the participants via mail. A draft of the questionnaire was prepared based on our previous genetic counseling records and qualitative analyses (unpublished data), and other previous reports (McKibbin et al., 2014; McVeigh et al., 2019; Zhang et al., 2019). The questionnaire was completed after a pilot study was performed on 10 patients with IRD, which consisted of questions on motivation for genetic testing (one question), benefits (one question), and challenges (two questions) experienced, status of sharing results with family (four questions), attitude toward the timing of the test (one question), characteristics (nine questions), additional comments (one question), and analysis results (one question only for patients who received positive results).

2.3 Procedure

The response period was during June, 2020. For patients who had difficulty writing their own answers to the questionnaire, answers could be provided by their family member, caregiver, or the investigator on behalf of them. The participants decided whether to provide their names to facilitate further contact and acquire additional information. A reminder letter was sent 2 weeks before the response deadline to all patients, except those who had already returned a signed questionnaire. This study was approved by the institutional review board of Kobe City Eye Hospital (Protocol no. E19002 and Permit no. ezh200501).

2.4 Data Analysis

Data used for the analysis was validated by two investigators. Statistical analysis was performed using JMP 15.1.0 (SAS Institute Inc., Cary, NC, USA). The frequency distribution and percentage of responses to each question were investigated. The Chi-square test and Student's *t*-test were used to compare characteristics in each group of patients with positive (E+) or negative (E−)/variant of uncertain significance (VUS) results, and the Chi-square test and multivariate logistic regression analysis were used to investigate the factors associated with the attitude toward the timing of genetic analysis. p-values < 0.05 were considered statistically significant based on two-sided tests, except for the Chi-square test with Bonferroni correction ($p < 0.0038$), for comparing benefits and test results. The descriptive answers in open-ended

question were analyzed supplementary using inductive thematic analysis (Braun & Clarke, 2006).

3. Results

3.1 Participants

In total, 275 patients with IRD were mailed the questionnaire, of which 234 (85.1%) answered and returned the questionnaire, and the responses provided by 228 (82.9%) patients were analyzed. Of these, 100 (100 of 228; 43.9%) patients received positive results, 87 (87 of 228; 38.2%) received negative results, and 41 (41 of 228; 18.0%) received VUS results. The characteristics of patients who received positive and negative/VUS results are presented in Table 1. No significant differences were observed with respect to age, gender, has at least one biological child, and best-corrected visual acuity between the two groups. The age of diagnosis was higher for patients who received negative/VUS results than for those who received positive results ($p < 0.001$), and the number of affected family members was higher among patients who received positive results than among those who received negative/VUS results ($p = 0.002$).

3.2 Motivations for undergoing genetic testing

The motivations for undergoing genetic testing are presented in Figure 1. The important motivations included obtaining information on future treatment options (64 of 228; 28.1%), confirmation of the inheritance pattern (57 of 228; 25.0%), and identification of the cause of

the disease (23 of 228; 10.1%). The patients selected six options or more on an average in a multiple-choice question on motivations.

3.3 Timing of undergoing genetic testing for IRD

Eighty-eight (88 of 228; 38.6%) patients reported that they would have preferred to undergo genetic testing at an earlier time point. Bivariate and multivariate analyses were performed to investigate the association between attitude regarding the timing of genetic testing and factors such as results and other characteristics. As shown in Table 2, receiving positive results and undergoing genetic testing more than 10 years after an initial clinical diagnosis were associated with the desire to undergo genetic testing at an earlier time point. Gender, has at least one biological child, and affected family members were not associated with this. None of the patients wanted to undergo genetic testing at a later time point.

3.4 Communication of genetic testing results with family members

Next, the status of sharing genetic testing results with family members was investigated. Two hundred and twenty-six individuals provided a complete answer to this question. As shown in Figure 2, most of the patients shared the result with their partner (E+: 78 of 81; 96.3%, E−/VUS: 92 of 96; 95.8%) and approximately 50–70% of patients shared the result with their parents (E+: 36 of 64; 56.3%, E−/VUS: 52 of 78; 66.6%), siblings (E+: 48 of 78; 61.5%, E−/VUS: 46 of 96; 47.9%), and children (E+: 45 of 69; 65.2%, E−/VUS: 65 of 92; 70.7%). As shown in Supplementary Fig. S1, the reasons that were selected most frequently for sharing

and not sharing the genetic testing results with family members were “I usually share information regarding my disease and heredity with my family” (E+: 77 of 94; 81.9%, E−/VUS: 99 of 123; 80.4%) and “I felt my family members would feel burdened by the result, considering their age and conditions” (E+: 30 of 49; 61.2%, E−/VUS: 29 of 60; 48.3%), respectively. No significant association was found between the reasons for sharing or not sharing and the results.

3.5 Benefits and challenges of genetic testing

The benefits "information on inheritance pattern" [$\chi^2(1) = 12.13$, $p < 0.001$], "additional information on diagnosis" [$\chi^2(1) = 19.409$, $p < 0.001$], "psychological preparation for the future" [$\chi^2(1) = 9.93$, $p = 0.0016$], "information for future treatment options" [$\chi^2(1) = 19.26$, $p < 0.001$], "life planning of family member about marriage and reproduction" [$\chi^2(1) = 11.59$, $p < 0.001$], and "life planning of family member about future steps" [$\chi^2(1) = 12.30$, $p < 0.001$] were selected at a significantly higher frequency by patients who received positive results than by those who received a negative/VUS result, as shown in Figure 3. However, no significant difference was observed between the selected benefits among those who received positive results and clarified their inheritance pattern, except for “reduced concern regarding inheritance pattern.” In addition, more than 50% of the patients who received negative/VUS results recognized benefits such as "information on inheritance pattern" (93 of 128; 72.7%), "acceptance of disease" (77 of 128; 60.2%), and "psychological preparation for the future" (76

of 128; 59.4%). Eighty-four patients (84 of 226; 36.7%) expressed concerns regarding genetic testing. In the descriptive answer, the concerns were suggested to be associated with the duration between blood sample collection and result declaration, the uncertainty of the genetic information, clarification of the inheritance pattern, and communicating the genetic results to family members. In addition, suggestions for improvement such as increasing access to ophthalmic genetic medicine, expansion of the analysis system at a national level, and continuation of support after genetic testing were also provided.

4. Discussion

To date, a genetic diagnosis of IRD is mostly obtained under research settings in Japan; however, it is expected to soon be performed in the clinic setting similar to the United States and European countries (Eden et al., 2016; Hafler et al., 2017). The findings of our study suggest that genetic testing for IRD has various benefits for patients, as seen in previous studies on other genetic diseases (Kohler et al., 2017). Thus, referral to genetic counseling and timely genetic diagnosis of IRD are needed.

Information for future treatment options, confirmation of the inheritance pattern, and identification of causal genes were found to be important motivators for genetic testing in this study. Moreover, a similar trend, in which multiple personal motivating factors drive such decisions on genetic testing, has also been reported in previous studies on other genetic

202 disorders (Withrow et al., 2008; Stafford et al., 2019). The primary characteristics of IRD,
203 which include intractability and difficulty in identification of the causal gene and inheritance
204 pattern based on clinical symptoms and family history, seem to influence the need for
205 information that is revealed only through genetic testing. A previous study reported that a
206 prenatal diagnosis and reproduction planning are the objectives of genetic testing for IRD
207 (Eden et al., 2016). Nonetheless, prenatal diagnosis for IRD is not performed in Japan, and
208 motivation related to marriage and reproduction was rarely observed in this study.

209 The definition of appropriate timing for genetic testing for IRD (McKibbin et al., 2014),
210 as well as for other genetic diseases (Lesperance et al., 2018; Van de Beek et al., 2020), remains
211 controversial. In our cohort, for those subjected to genetic testing more than 10 years after the
212 initial clinical diagnosis and for those receiving positive results, there was an association with
213 a preference to undergo genetic testing at an earlier time point. This study suggests that IRD
214 patients should be provided with an opportunity for genetic counseling to consider genetic
215 testing within 10 years after the diagnosis, even in situations in which the utility of genetic
216 results for medical intervention is limited.

217 Almost all patients shared the results with their partners, which was considerably higher
218 compared to those who shared with first-degree relatives. Since IRD is a progressive disease
219 and requires the support of the closest family members in daily life, it is presumed that sharing
220 of information about the disease, including the results of genetic testing, is more common. In

221 agreement with the findings of this study, a previous study on hereditary cancer reported that
222 factors such as background of family relationships are relevant for family communications
223 related to genetic diseases (Chivers Seymour et al., 2010), suggesting that a general tendency
224 to share information on the disease with family members promotes sharing the results of
225 genetic testing. Concurrently, concerns about the burden on family members are suggested to
226 be critical factors affecting patients who hesitate to share the results. Considering that genetic
227 testing results have limited effects on the willingness to share results with family, it is necessary
228 to pay attention to the background of family relationships and to facilitate appropriate
229 communication with family with respect to the genetic testing results from the stage of genetic
230 counseling before genetic testing.

231 The genetic testing for IRD had been described as “a significant milestone” (McVeigh
232 et al., 2019) and patients in also this study recognized the benefits of “preparation for the future”
233 and “disease acceptance”. Genetic testing might contribute to improving the quality-of-life of
234 patients and preventing psychological problems, as illustrated in previous reports (Prem Senthil
235 et al., 2017; Sainohira et al., 2018). Conversely, the benefits were recognized even in patients
236 for whom the causal gene was not identified or those who received VUS results. The patients
237 in this study presumably evaluated the genetic testing process based on pre- and post-clinical
238 consultation and genetic counseling, not only based on the information derived from genetic
239 analysis. The potential value of genetic testing results for patients includes a better

understanding of their own prognosis and risk of disease in family members (Grosse, et al., 2006). Moreover, in IRD genetic counseling, it is necessary to interpret results and communicate the predicted results of genetic testing to patients, such as the prognosis and effects on daily-life, the need for follow-up tests on other organs, possible approaches for future treatment, and the presence or absence of relatives at risk of developing the disease. Alterations to some genes can be responsible for syndromic visual impairment accompanied by systemic conditions such as sensorineural hearing loss (e.g. Usher syndrome), developmental delay (e.g. Joubert syndrome), and kidney disease (e.g. Bardet-Biedl syndrome) (Adams et al., 2007). A comprehensive understanding of the patient's conditions and referral to the relevant department might change their clinical management. Furthermore, it is important to support the patients and families and help them understand the information and that their actual behavior will help them to benefit greatly from the genetic testing results, regardless of the nature of such results. Considering the previous studies that pointed out disappointment with the result and misunderstanding and anxiety regarding disease conditions persisting in patients who received negative/VUS results (Stafford et al., 2019; McVeigh et al., 2019), continuous support and involvement of patients are necessary before and after genetic diagnosis.

Regarding suggestions for future research, expanding the scope of research participants will help to more accurately understand the overall trend for IRD patients. Understanding of backgrounds, concerns, and expectations of the patients with IRD who decided not to undergo

the genetic analysis will enable us to appropriately support a wider range of IRD patients. Comparing our findings with those of patients from different countries who have undergone a genetic testing process, the characteristics of Japanese patients with IRD regarding their attitude towards ophthalmic genetic medicine could become clearer. Whereas the demand for IRD genetic counseling with genetic testing has increased, there is not a sufficient supply system for ophthalmic genetic counseling in Japan. Understanding challenges in constructing the tele-genetics system for IRD patients and verifying the usefulness of this system will improve access to advanced ophthalmic genetic medicine.

4.1 Study Limitations

Our investigation had the following limitations. First, this was a single-center study, and whether our data accurately represent the trend in Japanese patients at large cannot be confirmed. Second, our clinic is one of the institutions that are actively conducting clinical research and patients visit from across the country; as such, our patients could have a positive attitude toward receiving medical care, information acquisition, and research participation compared to other patients with IRD. Third, this was a cross-sectional study, and the causal relationships with each result are unknown. In addition, we included patients who received negative and VUS results in a single group; however, the two groups of patients are not completely identical in other genetic disorders (Mighton et al., 2020). Finally, we only included

those patients who decided to undergo genetic testing after medical consultation and genetic counseling; however, there were other patients in our clinic who decided against genetic testing.

4.2 Practice Implications

This study demonstrated that even in situations where the clinical utility of genetic testing results for medical intervention is limited, Japanese patients often ask for information on the potential causal genes, future treatment options, and inheritance patterns, which can only be obtained using genetic testing. Therefore, referral to a facility of genetic medicine within 10 years of the initial clinical diagnosis is necessary. Careful interpretation of the genetic analysis results based on patient conditions and family history is important. The communication of genetic testing results with family members is influenced by the family situation and the general habits of sharing disease-related information than by the result itself.

Author Contributions

A.I. and A.Y. conceptualized and designed the study. A. I., A. Y., and K. K. prepared and mailed the questionnaire. A.I. and K.K entered and checked the data used for analysis. A.I. performed the data analysis. A.I. and A.Y. confirm that they had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis. A.I. and A.Y. drafted the manuscript. A.M., K.K., S.K., and M.T. assisted in designing the survey and critically revising the manuscript. All authors gave final approval of this version to be published

and agree to be accountable for all aspects of the work.

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Compliance with Ethical Standards

- **Conflict of Interest**

A.I., A.Y., A.M., K.K., S.K., and M.T. declare no conflict of interests.

- **Human Studies and Informed Consent**

All procedures were performed in accordance with the Helsinki Declaration following ethical standards of the institutional review board of Kobe City Eye Hospital (Protocol no. E19002 and Permit no. ezh200501). Informed consent was obtained from individuals who voluntarily completed the mail survey and submitted their responses.

- **Animal Studies**

No non-human animal studies were carried out by the authors for this article.

- **Data Availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1: Prevalence of motivations for undergoing inherited retinal disease (IRD) genetic testing (n = 228). A: life planning by patient/family members, especially future step. B: life planning by patient/family members, especially with respect to marriage and reproduction. NA: No Answer.

Figure 2: Distribution of patients who shared genetic analysis results with each family member after receiving positive (E+) or negative (E−)/variant of uncertain significance (VUS) result. The y-axis indicates the percentage of patients who shared/did not share information, and the x-axis indicates the genetic analysis results and represents family members as objects. The numeric values in each column indicate the number of responses. Total number of response were 64 and 78 for parents, 81 and 96 for partner, 78 and 96 for sibling, and 69 and 92 for child in E+ and E−/VUS cases, respectively. The responses from the patients who have no corresponding family members are not included.

Figure 3: Distribution of the benefits experienced from genetic analysis reported by patients with positive (E+) and negative (E−)/variant of uncertain significance (VUS) results. The y-axis represents the percentage of responses and the x-axis lists the benefits. The total number of responses were 100 and 128 for patients who received E+ and E−/VUS results, respectively.

411 A: life planning by patient/family members, especially future step. B: life planning by
412 patient/family members, especially with respect to marriage and reproduction. ** indicates
413 significant difference found using Chi-square test with Bonferroni correction ($p < 0.0038$).

Table 1 Characteristics of patients in this study

		Result of genetic testing	
		Positive, n=100	Negative/VUS, n=128
Age (years, mean ± SD, range)		55.2 ± 14.3, 20 - 88	56.2 ± 13.6, 21 - 80
Gender (n, %)	Male	52 (52.0)	51 (39.8)
	Female	48 (48.0)	77 (60.2)
Biological child [a] (n, %)	Yes	69 (69.0)	93 (72.7)
Family history	Yes	48 (48.0)	36 (28.1)
Age at diagnosis (years, mean ± SD, range)		33.5 ± 15.0, 3 - 75	41.1 ± 14.6, 8 - 70
Best corrected visual acuity	≧ 0.7	26 (26.0)	33 (25.8)
	0.3-0.7	18 (18.0)	35 (27.3)
	0.1-0.3	20 (20.0)	14 (10.9)
	< 0.1	29 (29.0)	37 (28.9)
	Not sure	7 (7.0)	9 (7.0)
Timing of genetic analysis	< 1 year	12 (12.0)	19 (14.8)
	1-5 years	10 (10.0)	27 (21.1)
	5-10 years	12 (12.0)	14 (10.9)
	≧ 10 years	66 (66.0)	68 (53.1)
Inheritance pattern	AD	19 (19.0)	-
	AR	49 (49.0)	-
	XL	18 (18.0)	-
	not sure	14 (14.0)	-

[a] exclude of adopted children.

Table 2 Bivariate and multivariate analysis for the attitude toward the timing of genetic analysis

		I wanted to undergo genetic testing an earlier time.		*p*-value	
		Yes	No	Bivariate	Multivariate
		n=88 (38.6%)	n=140 (61.4%)	Analysis	Analysis
Timing [a]	≧10 years	62 (46.3)	72 (53.7)	**0.0045**	**0.0065**
	< 10 years	26 (27.7)	68 (72.3)		
Result	Positive	48 (48.0)	52 (52.0)	**0.0099**	**0.0142**
	Negative/VUS	40 (31.3)	88 (68.8)		
Gender	Male	44 (42.7)	59 (57.3)	0.2458	0.5400
	Female	44 (35.2)	81 (64.8)		
Biological child [b]	Yes	64 (39.5)	98 (60.5)	0.6585	0.4591
	No	24 (36.4)	42 (63.6)		
Affected family	Yes	30 (35.7)	54 (64.3)	0.4947	0.1016
	No	58 (40.3)	86 (59.7)		

[a] indicates the timing when patients underwent genetic analysis after clinical diagnosis. [b] exclude of adopted children.

Figure 1

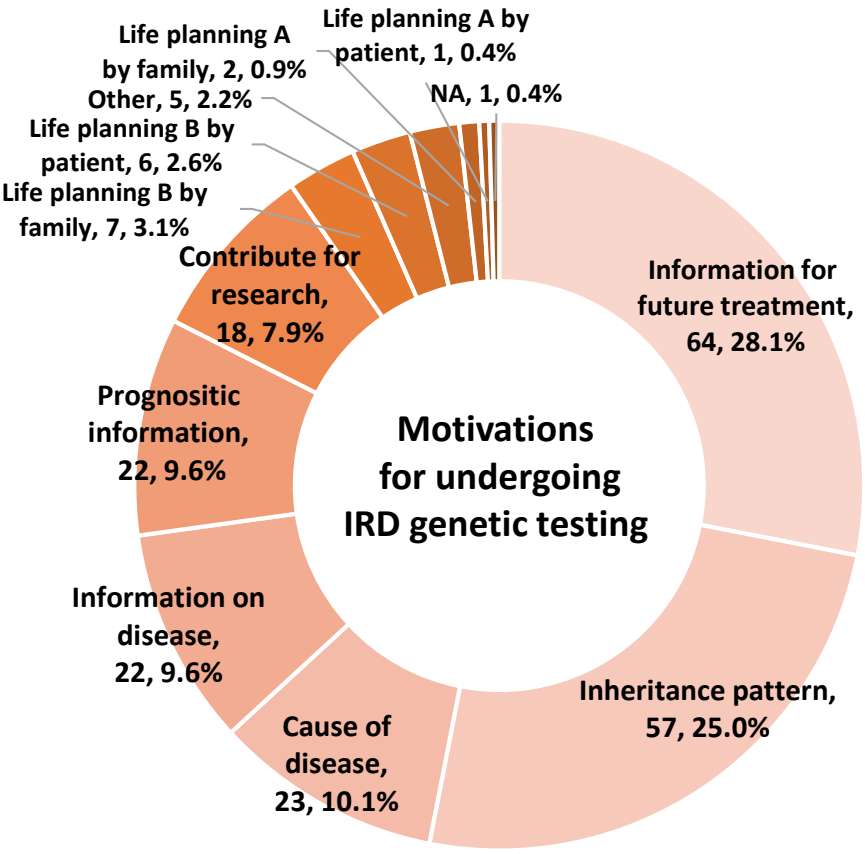

Motivations for undergoing IRD genetic testing
Information for future treatment, 64, 28.1%
Inheritance pattern, 57, 25.0%
Cause of disease, 23, 10.1%
Information on disease, 22, 9.6%
Prognositic information, 22, 9.6%
Contribute for research, 18, 7.9%
Life planning B by family, 7, 3.1%
Life planning B by patient, 6, 2.6%
Other, 5, 2.2%
Life planning A by family, 2, 0.9%
Life planning A by patient, 1, 0.4%
NA, 1, 0.4%

Figure 2

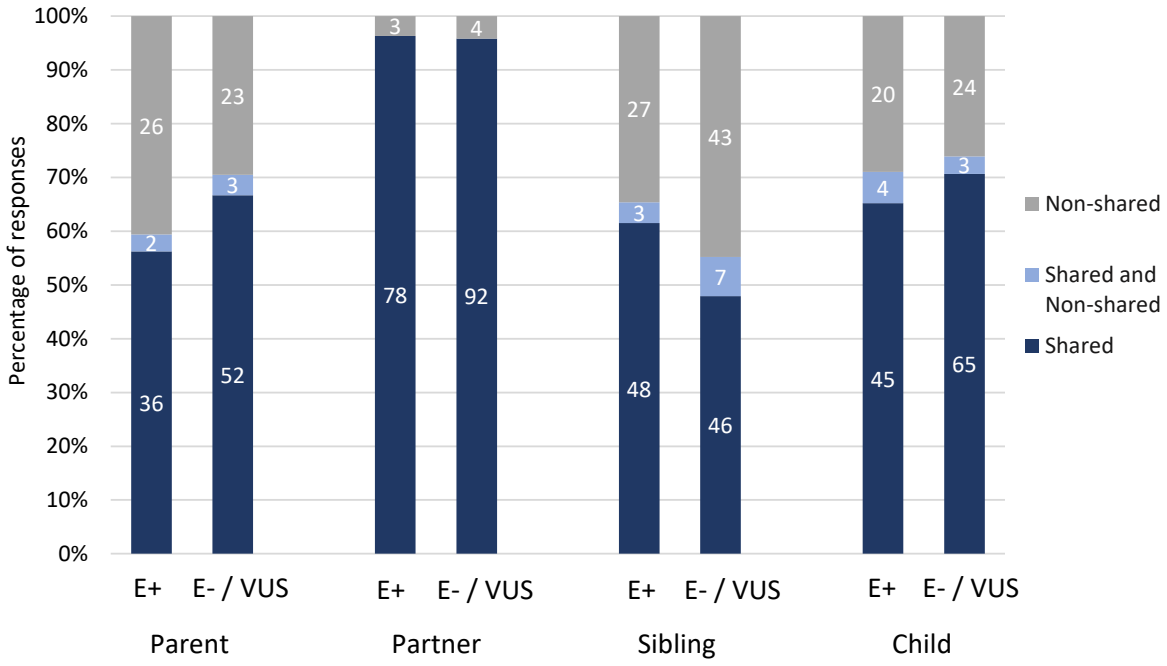
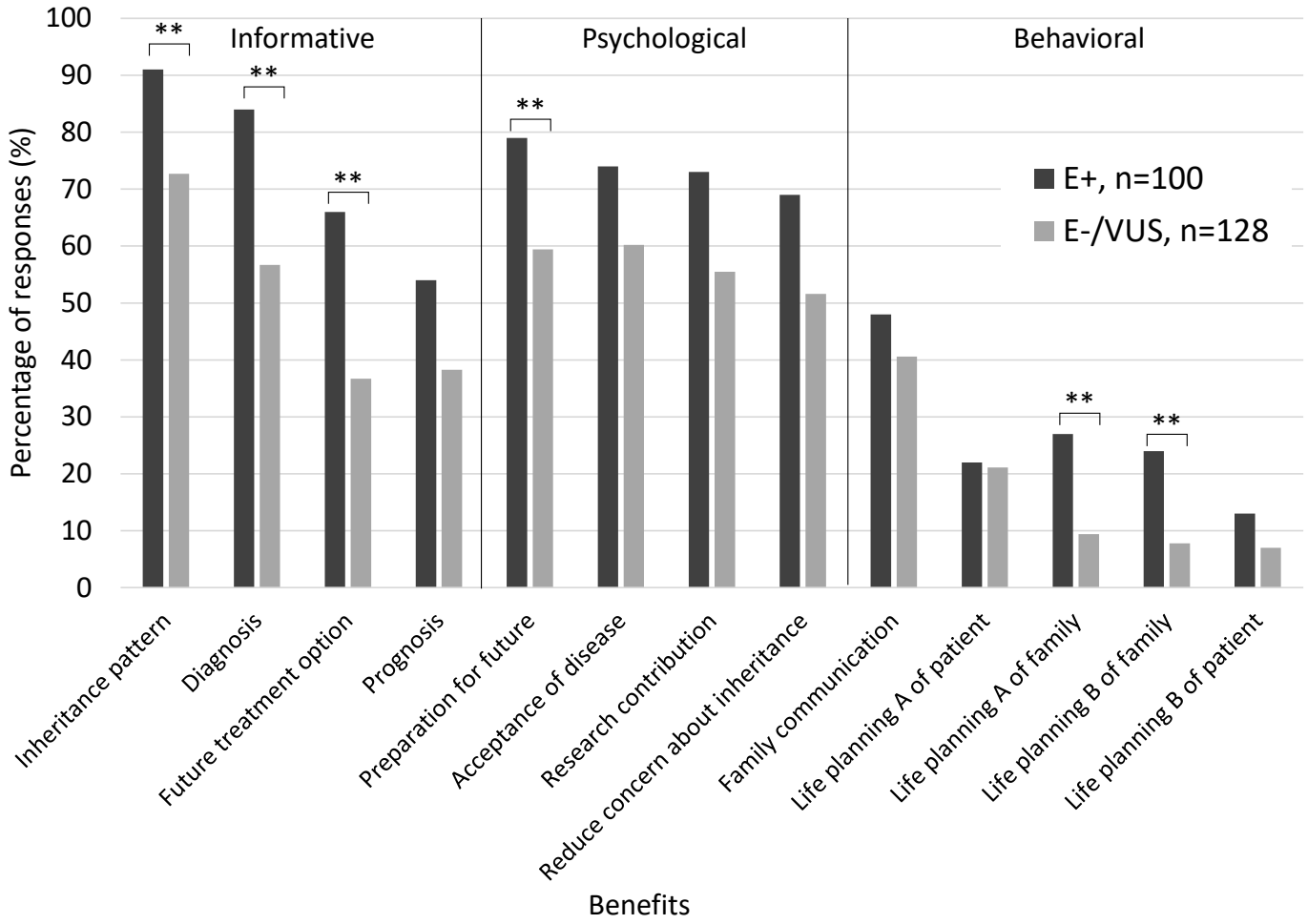
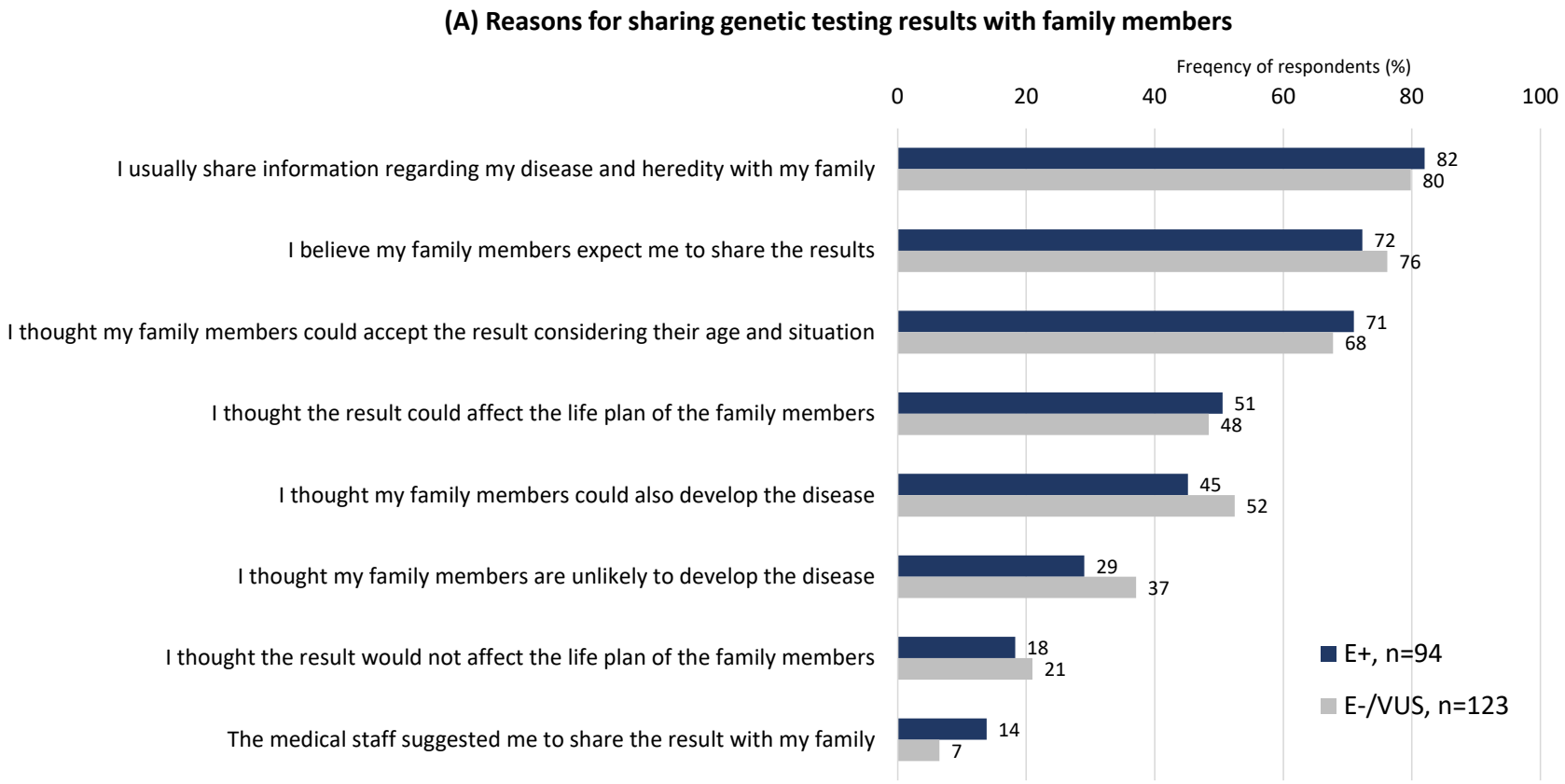
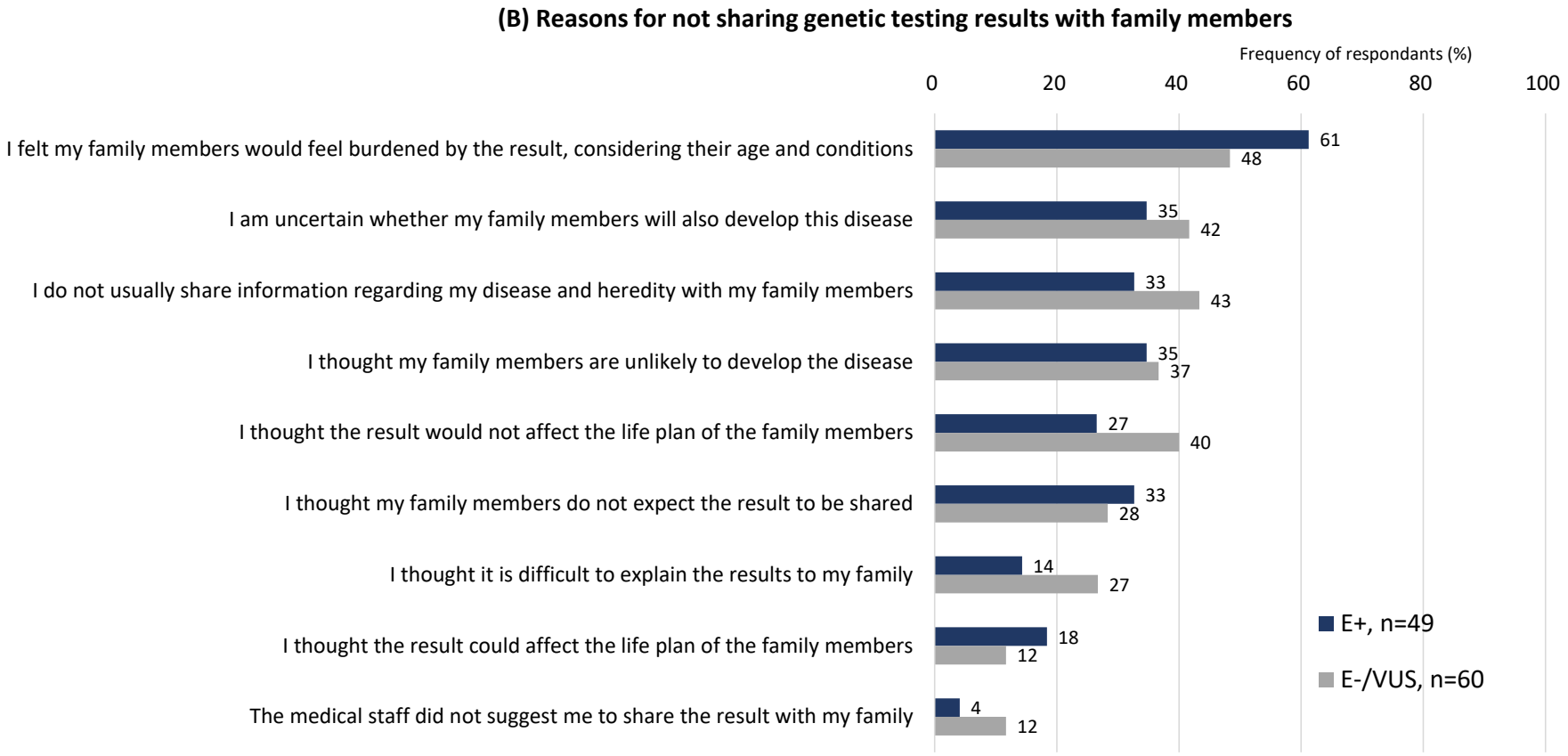

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of responses
26
2
36
23
3
52
3
78
4
92
27
3
48
43
7
46
20
4
45
24
3
65
E+
E- / VUS
E+
E- / VUS
E+
E- / VUS
E+
E- / VUS
Parent
Partner
Sibling
Child
Non-shared
Shared and Non-shared
Shared

Figure 3


Informative
Psychological
Behavioral
E+, n=100
E-/VUS, n=128
Percentage of responses (%)
100
90
80
70
60
50
40
30
20
10
0
**
Inheritance pattern
Diagnosis
Future treatment option
Prognosis
Preparation for future
Acceptance of disease
Research contribution
Reduce concern about inheritance
Family communication
Life planning A of patient
Life planning A of family
Life planning B of family
Life planning B of patient
Benefits


(A) Reasons for sharing genetic testing results with family members
Freqency of respondents (%)
0
20
40
60
80
100
I usually share information regarding my disease and heredity with my family
82
80
I believe my family members expect me to share the results
72
76
I thought my family members could accept the result considering their age and situation
71
68
I thought the result could affect the life plan of the family members
51
48
I thought my family members could also develop the disease
45
52
I thought my family members are unlikely to develop the disease
29
37
I thought the result would not affect the life plan of the family members
18
21
The medical staff suggested me to share the result with my family
14
7
E+, n=94
E-/VUS, n=123

1



Supplementary Figure S1: Distribution of responses based on the reasons for (A) sharing or (B) not sharing genetic testing results with family members from patients with inherited retinal disease who received positive (E+) or negative(E−)/variant of uncertain significance (VUS) results. The reasons are listed in the y-axis

and the percentage of responses for each response is indicated in the x-axis. The numeric values above each column indicate the specific percentage of responses in each group. Total number of responses collected from each group of patients is indicated in the graph legend.

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Article

Truncating Variants Contribute to Hearing Loss and Severe Retinopathy in *USH2A*-Associated Retinitis Pigmentosa in Japanese Patients

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Abstract: *USH2A* is a common causal gene of retinitis pigmentosa (RP), a progressive blinding disease due to retinal degeneration. Genetic alterations in *USH2A* can lead to two types of RP, non-syndromic and syndromic RP, which is called Usher syndrome, with impairments of vision and hearing. The complexity of the genotype–phenotype correlation in *USH2A*-associated RP (*USH2A*-RP) has been reported. Genetic and clinical characterization of *USH2A*-RP has not been performed in Japanese patients. In this study, genetic analyses were performed using targeted panel sequencing in 525 Japanese RP patients. Pathogenic variants of *USH2A* were identified in 36 of 525 (6.9%) patients and genetic features of *USH2A*-RP were characterized. Among 36 patients with *USH2A*-RP, 11 patients had syndromic RP with congenital hearing problems. Amino acid changes due to *USH2A* alterations were similarly located throughout entire regions of the *USH2A* protein structure in non-syndromic and syndromic RP cases. Notably, truncating variants were detected in all syndromic patients with a more severe retinal phenotype as compared to non-syndromic RP cases. Taken together, truncating variants could contribute to more serious functional and tissue damages in Japanese patients, suggesting important roles for truncating mutations in the pathogenesis of syndromic *USH2A*-RP.

Keywords: retinitis pigmentosa; Usher syndrome; *USH2A*; inherited retinal degeneration; clinical sequence

1. Introduction

Retinitis pigmentosa (RP) is the most common type of inherited retinal degenerative disease (IRD) and is clinically and genetically heterogeneous. Symptoms of this disease include night blindness, visual field constriction and a decline in vision. More than 70 and 270 causal genes in RP and in IRD, respectively, have been reported by the University of Texas Houston Health Science Center, Houston, TX, USA., (https://sph.uth.edu/retnet/). Although there are racial differences in causal genes, *USH2A* is one of the most frequent genes in Caucasian, Japanese and other populations [1,2]. Alterations in *USH2A* are responsible for Usher syndrome, which is the most common syndromic RP with sensorineural hearing loss [3]. Usher syndrome is classified into three types, type I, II and III, and causal genes of type II include *USH2A*, *ADGRV1*, and *WHRN* (*DFNB31*) [4]. *USH2A* is responsible

for about 80–90% of Usher syndrome type II and *USH2A*-causing Usher syndrome is called Usher syndrome type IIa, which is an autosomal recessive disease [5–7]. In addition, non-syndromic RP and non-syndromic hearing loss can be also caused by *USH2A* variants [8–10]. Alterations in *USH2A* lead to a wide range of phenotypes and severity of the diseases.

USH2A is located on chromosome 1q41 in the human genome and is approximately 790 kb of genomic DNA containing 72 exons [11]. This gene codes for the transmembrane protein USH2A (Usherin) which is expressed in the junction between the inner and outer segments, the cilia region in the photoreceptor cells [12–14]. USH2A plays important roles in the development and homeostasis of the inner ear and the retina [15,16]. Because this protein belongs to cilial proteins, *USH2A*-associated diseases are categorized to ciliopathies [17,18]. In previous studies, *USH2A* is a frequent causal gene in Japanese RP patients as well as Caucasian RP patients, yet the mutation spectrum is different among different ethnic groups [19,20]. Genotype–phenotype correlation in *USH2A* has been reported where truncating variants are associated with more severe visual and hearing impairments [9,21,22], and similar trends have also been reported in Asians [23,24]. In contrast, there are studies reporting that the differences in phenotypes are not clear in syndromic and non-syndromic or truncating variants and non-truncating variants [25,26]. Furthermore, there are few reports in Japanese patients examining the genotype–phenotype correlation in *USH2A*-RP.

In this study, we investigated the genetic and clinical characteristics focusing on the relationship between gene alterations and syndromic or non-syndromic *USH2A*-RP in Japanese patients. This study could provide additional evidence of race-specific genetic features in IRD and emphasize important roles of truncating variants, which lead to remnant protein functions for the pathogenesis of *USH2A*-RP.

2. Results

2.1. Syndromic and Non-Syndromic USH2A-RP

A total of 525 RP patients underwent genetic analysis and pathogenic variants were identified in 287 (54.7%) RP patients in this cohort. In these 525 RP patients, 36 (6.9%) cases carried *USH2A* variants which could explain their symptoms (Figure 1a). Among 36 patients with *USH2A*-RP, 11 (30.6%) patients were syndromic RP with congenital hearing loss (Figure 1b).

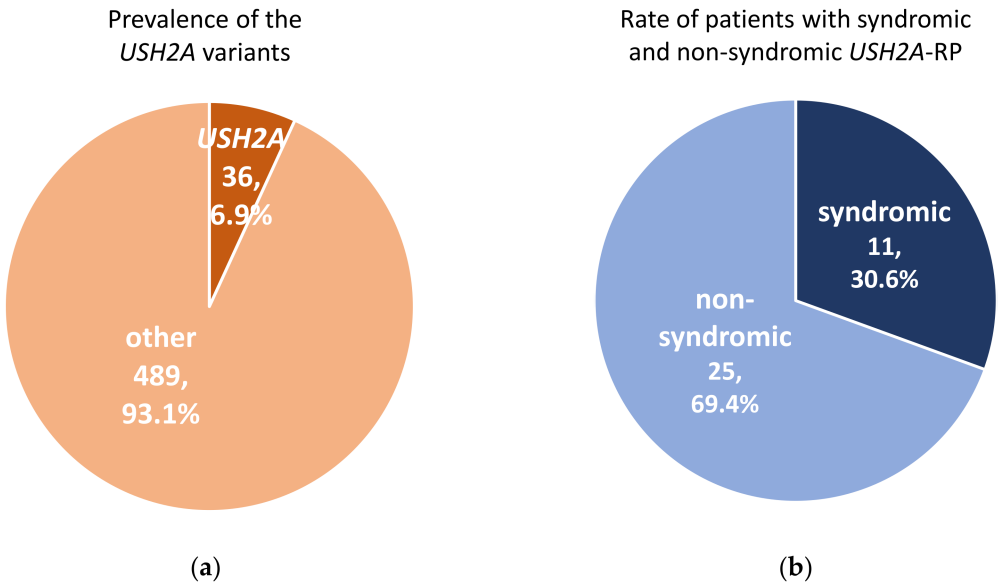


Figure 1. Prevalence of pathogenic variants in *USH2A* and rate of syndromic and non-syndromic *USH2A*-retinisis pigmentosa (RP) patients. (**a**) Pathogenic *USH2A* variants were detected in 36 of 525 RP cases. (**b**) There were 11 syndromic and 25 non-syndromic *USH2A*-RP patients in this study.

The genetic characteristics of syndromic and non-syndromic RP patients are presented in Tables 1 and 2. Among both groups, no significant differences were observed regarding age, gender, and consanguineous marriage. All 36 *USH2A*-RP cases showed an autosomal recessive pattern of inheritance, but affected family members were found at a higher rate in syndromic *USH2A*-RP cases than non-syndromic *USH2A*-RP cases, although statistical differences were not detected ($p = 0.25$).

Table 1. *USH2A* variants detected in RP patients in this study.

ID	Nucleotide Change	Protein Change	Zygosity
P1	c.490G>T;c.13631dupG	p.(Val164Phe);p.(Pro4545Serfs*17)	Het;Het
P2	c.1923T>A;c.3958C>T;c.5396delA	p.(Cys641*);p.(Pro1320Ser) [1];p.(Lys1799Serfs*18)	Het;Het;Het
P3	c.13576C>T;c.13847G>T	p.(Arg4526*);p.(Gly4616Val)	Homo;Homo
P4	c.2653C>T;c.9751T>C; c.13576C>T;c.13847G>T	p.(His885Tyr) [2]; p.(Cys3251Arg) [2];p.(Arg4526*) [2];p.(Gly4616Val) [2]	Het;Het; Het;Het
P5	c.8559-2A>G;c.14133+2T>A	p.(?);p.(?)	Het;Het
P6	c.8396delG	p.(Gly2799Valfs*31)	Homo
P7	c.10353_10356delTCAT;c.13010C>T	p.(His3452Glnfs*4);p.(Thr4337Met)	Het;Het
P8	c.2802T>G;c.5158delC	p.(Cys934Trp);p.(Leu1720*)	Het;Het
P9	c.8559-2A>G;c.10712C>T	p.(?);p.(Thr3571Met)	Het;Het
P10	c.8559-2A>G;c.14004delG	p.(?);p.(Leu4668Phefs*10)	Het;Het
P11	c.8559-2A>G	p.(?)	Homo
P12	c.10859T>C;c.11328T>G	p.(Ile3620Thr);p.(Tyr3776*)	Het;Het
P13	c.14243C>T	p.(Ser4748Phe)	Homo
P14	c.3596_3598delAAG;c.8254G>A	p.(Glu1199del);p.(Gly2752Arg)	Het;Het
P15	c.662C>A;c.7068T>G;c.7234G>A	p.(Thr221Lys) [1];p.(Asn2356Lys);p.(Val2412Met) [1]	Het;Het;Het
P16	c.490G>T;c.3595_3597delGAA	p.(Val164Phe);p.(Glu1199del)	Het;Het
P17	c.10859T>C;c.14766G>A	p.(Ile3620Thr);p.(Trp4922*)	Het;Het
P18	c.8559-2A>G;c.14243C>T	p.(?);p.(Ser4748Phe)	Het;Het
P19	c.11156G>A;c.13010C>T	p.(Arg3719His);p.(Thr4337Met)	Het;Het
P20	c.2802T>G;c.13847G>T	p.(Cys934Trp);p.(Gly4616Val)	Het;Het
P21	c.(11712_12066)del;c.15233C>G	p.(?);p.(Pro5078Arg)	Het;Het
P22	c.8254G>A	p.(Gly2752Arg)	Homo
P23	c.850G>A;c.2802T>G	p.(Glu284Lys);p.(Cys934Trp)	Het;Het
P24	c.4310_4312dupATA;c.8254G>A	p.(Tyr1437_Arg1438insAsn);p.(Gly2752Arg)	Het;Het
P25	c.6399G>A;c.13887G>T	p.(Trp2133*);p.(Glu4629Asp)	Het;Het
P26	c.2802T>G;c.9815C>T	p.(Cys934Trp);p.(Pro3272Leu)	Het;Het
P27	c.14243C>T;c.15233C>G	p.(Ser4748Phe);p.(Pro5078Arg)	Het;Het
P28	c.9371+1G>T;c.12094G>A	p.(?);p.(Gly4032Arg)	Het;Het
P29	c.3596_3598delAAG;c.8254G>A;c.13894C>G	p.(Glu1199del);p.(Gly2752Arg);p.(Pro4632Ala) [1]	Het;Het;Het
P30	c.685G>C;c.13708C>T	p.(Gly229Arg);p.(Arg4570Cys)	Het;Het
P31	c.8254G>A;c.8396delG	p.(Gly2752Arg);p.(Gly2799Valfs*31)	Het;Het
P32	c.2802T>G;c.11811_11812delCT	p.(Cys934Trp);p.(Tyr3938Argfs*8)	Het;Het
P33	c.490G>T;c.12383A>G	p.(Val164Phe);p.(Tyr4128Cys)	Het;Het
P34	c.2609G>T;c.5608C>T; c.12305T>A;c.15355C>T	p.(Cys870Phe) [1];p.(Arg1870Trp); p.(Ile4102Asn) [1];p.(Arg5119Trp) [1]	Het;Het; Het;Het
P35	c.10495C>T;c.10712C>T	p.(Pro3499Ser);p.(Thr3571Met)	Het;Het
P36	c.8339T>A;c.12407C>T	p.(Val2780Asp);p.(Thr4136Ile)	Het;Het

P1–P11 are syndromic RP patients and they are indicated in gray. Pathogenicity of each variant needs further evaluation in the patients with more than three variants detected. [1] Novel variants those pathogenicity are suggested by in silico analysis. [2] These 4 variants were confirmed by segregation analysis.

Table 2. Characteristics of syndromic and non-syndromic RP patients.

Characteristics		Syndromic RP [1]	Non-Syndromic RP [2]
Age (years, mean ± SD, range)		48.5 ± 12.9, 27–69	50.9 ± 15.7, 26–82
Gender (*n*, %)	Male	6 (54.5)	13 (52.0)
Family History (*n*, %)	Yes	5 (45.5)	6 (24.0)
Consanguineous Marriage (*n*, %)	Yes	1 (9.1)	2 (8.0)

[1] syndromic *USH2A*-RP patients ($n = 11$), [2] non-syndromic *USH2A*-RP patients ($n = 25$).

2.2. Truncating USH2A Variants were More Frequently Detected in Syndromic than Non-Syndromic USH2A-RP Patients

Twenty-seven variants of *USH2A* were detected in 11 syndromic *USH2A*-RP patients and 54 variants in 25 non-syndromic *USH2A*-RP patients, which can explain their symptoms. Ten missense variants (37.0%), 6 frameshift variants (22.2%), 5 nonsense variants (18.5%), and 6 splicing variants (22.2%) were detected in syndromic *USH2A*-RP patients (Figure 2a). Forty-two missense variants

(77.8%), 4 inframe variants (7.4%), 2 frameshift variants (3.7%), 3 nonsense variant (5.6%), 2 splicing variants (3.7%), and 1 large deletion variant (1.9%) were detected in non-syndromic *USH2A*-RP patients (Figure 2b).

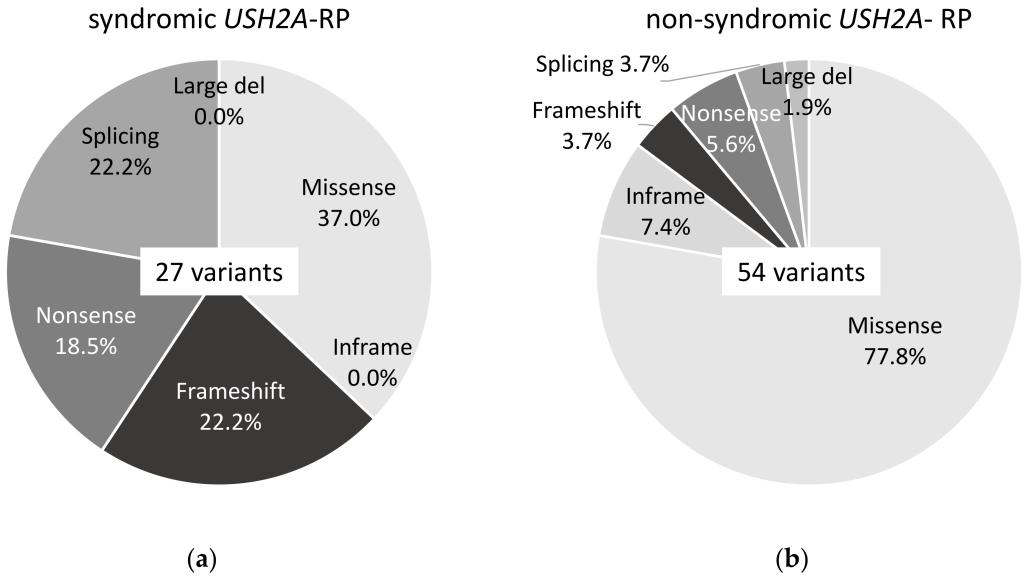


Figure 2. Frequency of each type of *USH2A* variant in this study. (**a**) Frequency of 6 types of 27 *USH2A* variants in syndromic *USH2A*-RP patients (n = 11). (**b**) Frequency of 6 types of 54 *USH2A* variants in non-syndromic *USH2A*-RP patients (n = 25).

Notably, a pattern of missense/missense or truncating/truncating was not observed in syndromic or in non-syndromic *USH2A*-RP patients, respectively (Table 3). In contrast to the observation that 32.0% (8 of 25) non-syndromic *USH2A*-RP patients had truncating variants such as nonsense, frameshift, or out of frame exon deletion, all syndromic RP patients (100.0%; 11 of 11) carried at least one truncating variant, which was significantly higher in syndromic RP ($p < 0.01$) (Table 3). All *USH2A*-RP patients with two truncating variants (n = 6) had both RP and early onset hearing loss.

Table 3. Three types of combinations of *USH2A* variants in this study.

Type of Variant	Syndromic RP [1]	Non-Syndromic RP [2]
Truncating / Truncating (n, %)	6 (54.5)	0 (0.0)
Truncating / Missense (n, %)	5 (45.5)	8 (32.0)
Missense / Missense (n, %)	0 (0.0)	17 (68.0)

[1] syndromic *USH2A*-RP patients (n = 11), [2] non-syndromic *USH2A*-RP patients (n = 25).

Frequently detected *USH2A* variants were p.(Cys934Trp) (one syndromic patient and four non-syndromic patients), p.(Gly2752Arg) (five non-syndromic patients), and c.8559-2A>G (four syndromic patients and one non-syndromic patient) (Table 4).

Locations of each variant in the *USH2A* protein structure were schematically presented (Figure 3). Notably, *USH2A* variants were similarly found throughout entire regions of the *USH2A* protein structure in non-syndromic and syndromic *USH2A*-RP cases, suggesting that localization of variants does not correlate with hearing loss in *USH2A*-RP.

Table 4. *USH2A* variants detected in this study.

USH2A Variants	Syndromic RP [1]	Non-Syndromic RP [2]
p.(Val164Phe)	1	2
p.(Cys934Trp)	1	4
p.(Glu1199del)	0	3
p.(Gly2752Arg)	0	5
p.(Gly2799Valfs*31)	1	1
c.8559-2A>G	4	1
p.(Thr3571Met)	1	1
p.(Ile3620Thr)	0	2
p.(Ser4748Phe)	0	3
p.(Thr4337Met)	1	1
p.(Arg4526*)	2	0
p.(Gly4616Val)	2	1
p.(Pro5078Arg)	0	2

[1] syndromic *USH2A*-RP patients (n = 11), [2] non-syndromic *USH2A*-RP patients (n = 25).

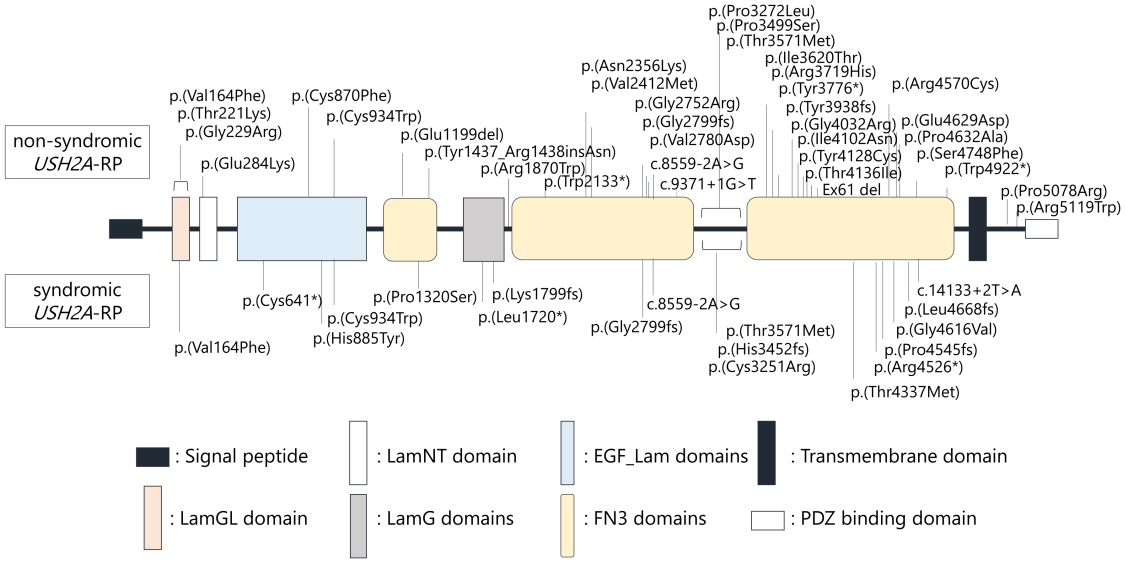
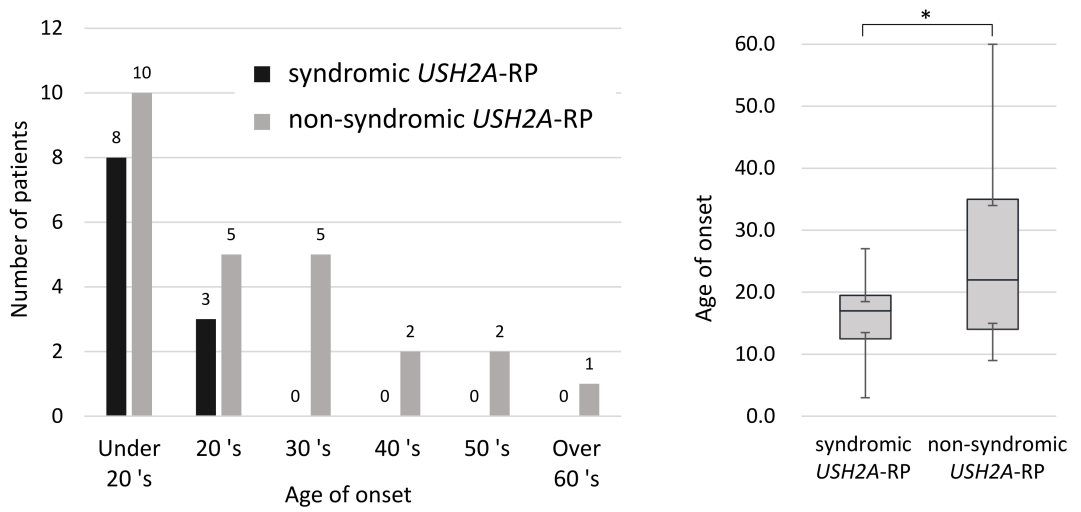


Figure 3. Schematic distribution of the *USH2A* variants identified in this study. Upper variants were detected in non-syndromic patients (n = 25), lower variants were detected in syndromic patients (n = 11).

2.3. Earlier Onset of RP in Syndromic Patients than Non-Syndromic USH2A-RP Patients

In order to understand clinical features between syndromic and non-syndromic *USH2A*-RP, age of disease onset was examined. All syndromic RP patients were aware of symptoms related to RP such as night blindness and constriction of visual field by their third decade of life (Figure 4a). Comparing the onset age of RP in 11 syndromic and 25 non-syndromic *USH2A*-RP patients, syndromic RP patients were aware of symptoms significantly earlier than non-syndromic patients ($p < 0.05$) (Figure 4b). The mean age of onset was 16.1 and 26.5 years old in syndromic and non- syndromic *USH2A*-RP patients, respectively.

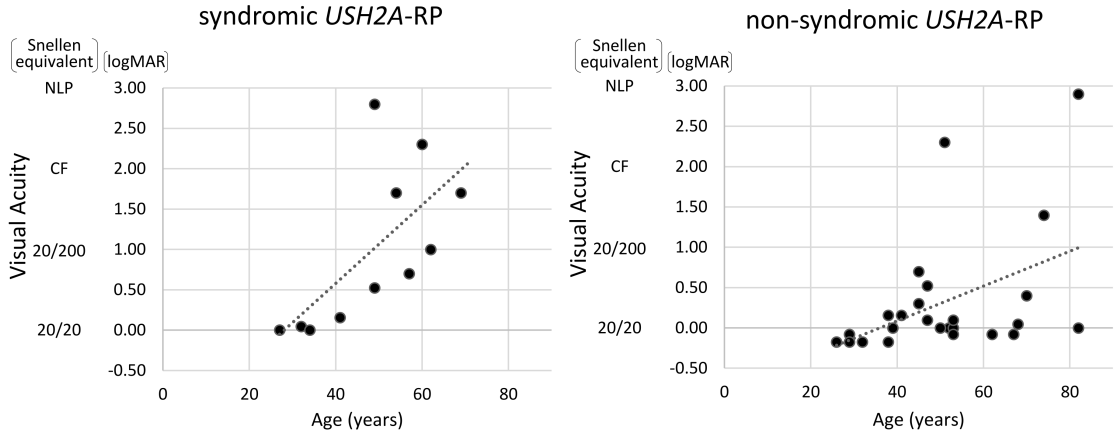


(**a**) (**b**)

Figure 4. Onset age of RP in 11 syndromic and 25 non-syndromic *USH2A*-RP patients. (**a**) Distribution of onset age in syndromic and non-syndromic *USH2A*-RP patients. The vertical line shows the number of patients and the horizontal one shows the onset age of RP. Numeric numbers above each column indicate patients' numbers. (**b**) Box plot of onset age in syndromic and non-syndromic *USH2A*-RP patients. The bottom and top of each box represent the lower and upper quartiles, respectively, and the line inside each box represents the median. The bottom and top bars represent the minimum and maximum value, respectively. $p < 0.05$ (*).

2.4. Visual Acuity and Visual Field Constriction in Syndromic and Non-Syndromic USH2A-RP Patients

Next, visual acuity and degrees of visual field constriction were compared between syndromic and non-syndromic *USH2A*-RP cases. As shown in Figure 5, the correlation between age and visual acuity was more pronounced in syndromic *USH2A*-RP patients than in non-syndromic *USH2A*-RP patients. These data also indicate that a decline in visual acuity is more rapid in syndromic *USH2A*-RP cases than non-syndromic *USH2A*-RP cases.



(**a**) (**b**)

Figure 5. Scatter distribution of visual acuity and age in *USH2A*-RP cases. The vertical line shows visual acuity (Snellen equivalent and logMAR) and the horizontal one shows age of patient. One plot shows one patient and dash lines represent approximate straight lines. Results of 11 syndromic (**a**) and 25 non-syndromic *USH2A*-RP patients (**b**) are respectively presented. NLP: no light perception, CF: counting fingers.

To evaluate the visual field changes, HFA data were obtained from five syndromic and 10 non-syndromic *USH2A*-RP patients (Figure 6). Lower MD values and a decline in these values were revealed in syndromic *USH2A*-RP patients, indicating more severe visual field problems. Non-syndromic *USH2A*-RP cases were divided into two groups, a low MD (lower than −30) group aged in their 40s versus a moderate MD group in their 50s–70s. Because patients with truncating *USH2A*-variants belong to both groups, one of four in the low-MD group and two of six in the moderate-MD group, a clear contribution of truncating *USH2A* variants was not observed.

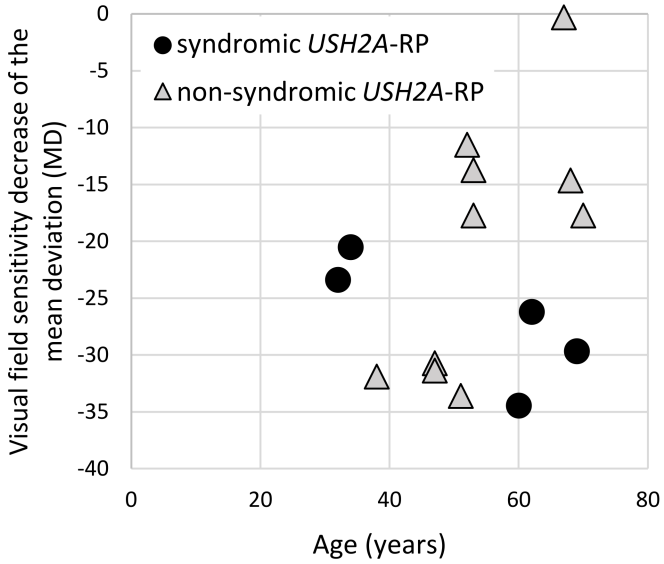


Figure 6. Correlation of the visual field changes and age of syndromic and non-syndromic *USH2A*-RP patients. The vertical line shows the mean deviation value (MD) which was obtained from HFA and the horizontal one shows age of patient. One dot represents each patient and round dots and triangle dots indicate syndromic (n = 5) and non-syndromic (n = 10) *USH2A*-RP patients, respectively.

Additionally, time-dependent changes in the same patients were investigated. One syndromic and five non-syndromic *USH2A*-RP patients had performed HFA evaluations more than twice (Table 5). Rate of changes in MD values were −1.54 (dB/Y) in one syndromic *USH2A*-RP patient. On the other hand, the MD changes of −0.67 (dB/Y), −0.88 (dB/Y), −0.65 (dB/Y), −0.48 (dB/Y), and −0.34 (dB/Y) were observed in five non-syndromic *USH2A*-RP patients. These rates of MD changes in the syndromic *USH2A*-RP patient were higher than the average of the MD changes in non-syndromic *USH2A*-RP patients (−0.60 (dB/Y)).

Table 5. Rate of changes in MD values of *USH2A*-RP patients.

Phenotype	Patient	Rate of Changes in MD Values	Observation Period (Years)
syndromic RP	P8	−1.54	1
non-syndromic RP	P12	−0.67	8
	P25	−0.88	1
	P27	−0.65	6
	P33	−0.48	2
	P36	−0.34 *	6

* The value of P36 was calculated with the left eye. P36 was not able to undergo HFA of the right eye due to low vision (hand motion) in the latest exam.

Taken together, these results suggest that 1. truncating *USH2A* variants contribute to hearing loss in *USH2A*-RP cases and 2. syndromic *USH2A*-RP patients had a more severe retinal disease with earlier onset and a more rapid decline in visual function than non-syndromic *USH2A*-RP patients.

3. Discussion

In this study, genetic analyses of 525 RP patients were conducted using the targeted panel sequencing, and genetic and clinical features were characterized in 36 patients who were found to carry *USH2A* variants. Molecular diagnosis was made in 287 of 525 RP cases (54.7%) and *USH2A* disease-causing variants were identified in 6.9% of RP patients (36 of 525 patients) in this cohort. The frequency is similar with previous investigations in Japanese [2], reporting that *USH2A* is one of the major causal genes in Japanese RP patients.

All syndromic *USH2A*-RP patients in this cohort noticed their symptoms by their age of 30. The mean age at which our patients noticed symptoms, such as night blindness and visual constriction, was 16.1 years old in syndromic *USH2A*-RP patients and 26.5 years old in non-syndromic *USH2A*-RP patients, revealing that the onset of RP was significantly earlier in syndromic patients than in non-syndromic patients ($p < 0.05$). This trend has also been reported in previous reports of other ethnic groups [23]. In addition, not only age of onset but also RP symptoms were more severe in syndromic patients than in non-syndromic patients. A rapid decline in visual acuity was observed in syndromic *USH2A*-RP patients as compared to non-syndromic *USH2A*-RP patients whose acuity was reasonably well-maintained by their 70s. Although the data did not track changes in each patient over a long time, poorer visual acuity prognosis in syndromic *USH2A*-RP than non-syndromic *USH2A*-RP cases agreed with a trend in previous studies [22,23]. Similarly, as for visual acuity, syndromic *USH2A*-RP patients suffered more severe visual field constriction than non-syndromic *USH2A*-RP patients. In non-syndromic *USH2A*-RP patients, the rate of MD changes was approximately −0.5 (dB/year), which is the average reported progression rate of RP [27]. In contrast with non-syndromic *USH2A*-RP patients, one of our syndromic *USH2A*-RP patients showed the value of −1.5 (dB/year). In our clinic, the Goldmann perimetry is the first-choice visual field test for patients with low vision (lower than 1.0 of logMAR visual acuity), which was more preferably conducted in our syndromic *USH2A*-RP patients. Indeed 5 of 11 syndromic *USH2A*-RP patients had difficulty performing visual field tests with HFA due to their severely affected vision. These observations in visual acuity and visual field suggest that eye symptoms of *USH2A*-RP are more severe in patients with syndromic *USH2A*-RP than those with non-syndromic *USH2A*-RP.

Notably, the detection rate of truncating variants is significantly higher in syndromic *USH2A*-RP patients in this study. Considering characteristics of clinical features and genotype in *USH2A*-RP cases, truncating variants, which can largely affect protein functions and expression, possibly lead to severe symptoms related to RP and hearing loss. This genotype–phenotype correlation of variants which could contribute to remnant protein functions was reported in *CDHR1* [28], *ABCA4* [29], and *CDH23* [30,31]. Although syndromic RP cases without truncating variants in *USH2A* have been reported [23], truncating variants were detected in all syndromic *USH2A*-RP patients in this study, emphasizing the important roles of truncating variants for more severe phenotypes including hearing problems.

The variants frequently detected in this study were p.(Cys934Trp), p.(Gly2752Arg), and c.8559-2A>G. The last variant c.8559-2A>G is reported as a specific variant in the Japanese population [19]. On the contrary, *USH2A* variants often reported in the Caucasian population, such as p.(Cys759Phe) (pathogenicity of this variant is being reviewed elsewhere [32,33]) and p.(Glu767Serfs*21), were not identified in our Japanese cohort. Interestingly, the most frequent variant p.(Cys934Trp) was common in Chinese and Japanese populations, but other frequent variants differed. The variants often reported in the Chinese population, such as p.(Tyr2854_Arg2894del) and p.(Ser5060Pro), were not identified in our cohort. These observations suggest that there are unique variants in Japanese *USH2A*-RP patients as ethnic features.

It is not fully understood why *USH2A* variants lead to a wide range of phenotypes and severity of the diseases. *Ush2a*-knockout in mice and in zebrafish recapitulated a phenotype of human Usher syndrome with retinal degeneration and hearing problems [16,34]. These models could support our observation that all *USH2A*-RP patients with two truncating variants (n = 6) developed RP

and early-onset hearing loss. Two protein isoforms, a long isoform and a short N-terminal isoform, are spliced from the *USH2A* gene [11]. Expression of the N-terminal isoform was only detected in the inner ear; in contrast, the long isoform was localized in photoreceptors and ears [16]. Interestingly, supplementation of a shortened form of *Ush2a* that lacks exon 12 rescued hearing loss in *Ush2a*-knockout mice [35]. Additionally, the roles of a partner protein, PDZD7, could be different in photoreceptors and inner ears [36]. Remarkably, *PDZD7* variants are only responsible for congenital hearing loss, but not for RP [36]. These facts and the accumulation of additional evidence could contribute to better understanding the pathogenesis of *USH2A*-associated diseases.

In our clinic, we recommend otorhinolaryngologic consultation for both syndromic and non-syndromic *USH2A*-RP patients to check their hearing ability. The previous study reported that some *USH2A*-RP patients are not aware of their hearing problems, suggesting non-syndromic *USH2A*-RP could have mild hearing deterioration [9]. Because we categorized groups of syndromic and non-syndromic *USH2A*-RP depending on the patients' interview, possibilities of mis-grouping cannot be ruled out if such patients participated in this study. An additional concern could be that this study did not include patients who have only hearing loss due to *USH2A* variants. Pathogenic variants of *USH2A* in hearing loss have been reported in a previous meta-analysis [37]. Accordingly, further evaluations of *USH2A*-associated diseases from the standpoints both of ophthalmology and otolaryngology will be necessary.

In conclusion, truncating *USH2A* variants were more frequently identified in syndromic *USH2A*-RP patients who have congenital hearing loss than in non-syndromic *USH2A*-RP patients without hearing loss. Syndromic *USH2A*-RP patients have a more severe retinal disease with earlier-onset and a more rapid decline in visual function than non-syndromic *USH2A*-RP patients. Truncating variants could contribute to serious functional and tissue damages, suggesting important roles of truncating mutations for the pathogenesis of *USH2A*-RP, especially syndromic *USH2A*-RP.

4. Materials and Methods

4.1. Ethical Statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the institutional Review Board of Kobe City Eye Hospital (Protocol no. E19002 and Permit no. ezh200901, 04.09.2020).

4.2. Patients Recruitment or Inclusion Criteria

A total of 525 IRD patients were included in this study. All the patients visited the IRD and Genetic Counseling Clinic in Kobe Eye Center Hospital from June 2015 to April 2020 (except four patients in 2013 and 2015) and gene alterations were identified in the genetic analysis studying IRD. In this cohort, patients with *USH2A* changes were further evaluated regarding their genetic and phenotypic characteristics.

4.3. Genetic Analysis

All patients underwent DNA sequencing using either a panel of 39 (238 patients) or 50 (287 patients) genes (Table 6) causing inherited retinal diseases which were selected based on previous reports [38–40]. *USH2A* (NM_206933.2) was included in both gene panels. The target capture panel covers entire coding exons and exon–intron boundaries of these genes, except *RPGR(ORF15)*. Targeted libraries were sequenced on an illumina NextSeq500 (NextSeq 500 System, illumina, San Diego, CA, USA).

Table 6. The list of genes in our target capture panel.

ABCA4	*BEST1*	*BBS1* *	*C2orf71*	*CEP290* *
CDH23 *	*CDHR1* *	*CHM* *	*CNGA1*	*CNGB1*
CNGB3	*CRB1*	*CRX*	*CYP4V2*	*EYS*
FAM161A *	*GPR98* *	*GUCA1A* *	*GUCY2D*	*IMPDH1*
IMPG2	*KLHL7* *	*LRAT*	*MAK*	*MERTK*
MYO7A *	*NR2E3*	*NRL*	*PCDH15* *	*PDE6B*
PRCD	*PROM1*	*PRPF31*	*PRPF6*	*PRPH2*
RDH5	*RDH12*	*RHO*	*ROM1*	*RP1*
RP1L1	*RP2*	*RP9*	*RPE65*	*RPGR*
RS1 *	*SNRNP200*	*TOPORS*	*TULP1*	*USH2A*

* indicates genes involved only in the 50 genes panel.

The interpretation of sequence variants was performed based on the criteria and guidelines recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [41]. Briefly, the variants shown below were classified as pathogenic variants: 1. null variants, which include nonsense, frameshift, start loss and out-of-frame exon deletion, and splice site (+/- 1,2); 2. variants with an allele frequency less than 5% in Exome Aggregation Consortium (ExAC), 1000 Genomes database, and Human Genetic Variation Databases (HGVD); 3. missense variants which were reported as disease-causing variants in previous reports or predicted a pathogenic effect in silico analysis (SIFT (https://sift.bii.a-star.edu.sg/) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml)). Clinvar information (https://www.ncbi.nlm.nih.gov/clinvar/) and previous reports have also been used for the interpretation of variants.

Supplemental sanger sequencing of *RPGR(ORF15)* was performed in male patients whose pathogenic variants were not detected in panel analysis. Segregation analysis using sanger sequencing was also performed in family members. After a data filtering and interpretation process for the detected variants, the variants were checked against clinical conditions and family history of each patient to determine molecular diagnosis in an expert meeting. More details on the analysis can be found in our previous reports [40].

Sequence variants are described in accordance with recommendations from the Human Genome Variation Society [42].

4.4. Clinical Evaluations

Symptoms and other clinical information such as age, gender, age of onset, family history, and the presence of hearing loss, were obtained from their medical and genetic counseling records. Ophthalmological evaluations were performed in the IRD clinic. Evaluations include best corrected visual acuity (BCVA) with the Snellen chart, slit-lamp biomicroscopy, dilated indirect ophthalmoscopy, ophthalmic imaging including fundus autofluorescence and retinal cross-section with OPTOS 200Tx and a SPECTRALIS_Spectral (Heidelberg Engineering, Heidelberg, Germany) domain optical coherence tomography (OCT) scanner, full-field electroretinogram (ERG), visual fields with Goldmann perimetry (GP) and Humphrey field analyzer (HFA).

4.5. Statistical Analysis

Statistical analysis was performed with R version 3.1.3 (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/). The Welch Two Sample *t*-test was used to compare age of onset in each group of syndromic and non-syndromic RP, and Fisher's exact test was used to compare the characteristics of the genetic mutations in each group. p-values < 0.05 were considered statistically significant in a two-sided test. The value a of visual acuity (a) was converted into the value of Logarithm of the Minimum Angle of Resolution (logMAR) for statistical analysis (=log(1/a)). The hand motion value has a logMAR conversion of 2.30, the light sense value has a log MAR conversion of 2.80, and the

no light sense value has a logMAR conversion of 2.90 [43,44]. Regarding the visual field condition, Mean Deviation (MD) values which obtain from HFA test were used for statistical analysis.

Author Contributions: Conceptualization, A.M. and A.I.; methodology, A.M. and A.I.; validation, A.M., A.Y. and K.K.; formal analysis, A.M. and A.I.; investigation, A.M., A.Y., K.K., Y.H., M.T. and A.I.; data curation, A.M., A.Y., K.K. and A.I.; writing—original draft preparation, A.M. and A.I.; writing—review and editing, A.Y., K.K., Y.H., Y.K., S.K. and M.T.; visualization, A.M., A.Y., K.K. and A.I.; supervision, M.T. and S.K.; project administration, Y.K. and M.T.; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

RP	Retinitis Pigmentosa
IRD	Inherited Retinal Degenerative Disease
USH2A-RP	*USH2A*-associated RP
HFA	Humphrey field analyzer
MD	Mean Deviation

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