TITLE PAGE

Title:

Specification of Variant Interpretation Guidelines for Inherited Retinal Dystrophy in Japan

Running head:
Japanese Inherited Retinal Dystrophy Variant Interpretation Guidelines

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Type: Guideline

Word count: 2665 words

Abstract word count: 241 words

Number of references: 25

Number of figures: 1

Number of tables: 10

Funding Information

This study is funded by Research on rare and intractable diseases, Health and Labour Sciences Research Grants (Grant Number: JPMH23FC1043) from the Ministry of Health, Labour and Welfare of Japan, and Practical Research Project for Rare / Intractable Disease (Grant Number: JP23ek0109632) from the Japan Agency for Medical Research and Development, Japan.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Abstract

The accurate interpretation of sequence variants in inherited retinal dystrophy (IRD) is vital due to the significant genetic heterogeneity observed in this disorder. To achieve consistent and accurate diagnoses, it is essential to establish standardized guidelines for variant interpretation. The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines for variant interpretation serve as the global "cross-disease" standard for classifying variants in Mendelian hereditary disorders. These guidelines propose a systematic approach for categorizing variants into five classes based on various types of evidence, such as population data, computational data, functional data, and segregation data. However, for clinical genetic diagnosis and to ensure standardized diagnosis and treatment criteria, additional specifications based on features associated with each disorder are necessary. In this context, we present a comprehensive framework outlining the newly specified ACMG/AMP rules tailored explicitly for IRD in the Japanese population. These guidelines consider disease frequencies, allele frequencies, and both phenotypic and genotypic characteristics unique to IRD in the Japanese population. Adjustments and modifications have been incorporated to reflect the specific requirements of the population. By incorporating these IRD-specific factors and refining the existing ACMG/AMP guidelines, we aim to enhance the accuracy and consistency of variant interpretation in IRD cases, particularly in the Japanese population. These guidelines serve as a valuable resource for ophthalmologists and clinical geneticists involved in the diagnosis and treatment of IRD, providing them with a standardized framework to assess and classify genetic variants.

Keywords: inherited retinal dystrophy; ACMG/AMP guidelines; genetic diagnosis; variant interpretation; Japanese.

The advent of gene therapy as a potential treatment for inherited retinal dystrophy (IRD), aimed at targeting the underlying causative gene or disease variant, has sparked global initiatives to improve precise genetic testing and diagnosis. Accurate genetic testing and diagnosis have become critical components in improving patient care and making informed therapeutic decisions. Recognizing the importance of these advancements, Japan is actively working toward incorporating genetic testing for IRD patients into the national insurance covered investigation. As part of this broader effort, the "Research on rare and intractable diseases, Health and Labour Sciences Research Grants," funded by the Ministry of Health, Labour and Welfare, Japan has been ongoing.

In December 2022, the "Guidelines for Genetic Testing in Inherited Retinal Dystrophy" were issued by an IRD working group on behalf of the Japanese Retina and Vitreous Society (available at https://www.jrvs.jp/guideline/ird_rd_guideline.pdf). While these guidelines represent a significant step toward standardized genetic testing for IRD, discrepancies in result interpretation among physicians and institutions can still arise, leading to diagnostic confusion and uncertainty in determining treatment eligibility. Therefore, it is crucial to establish uniform criteria for identifying the pathological variants associated with IRD, to facilitate the consistent and accurate clinical application of genetic testing.

The current American College of Medical Genetics and Genomics (ACMG) guidelines serve as a commonly employed "cross-disease" standard for interpreting variant pathogenicity. [1] However, their lack of specificity has resulted in varying assessments of pathogenicity among different institutions. As a result, there is a need to develop unique

criteria that are tailored to the disease specificity and ethnic considerations of IRD. While the international group, Clinical Genome Resource (ClinGen: https://clinicalgenome.org/), which is responsible for formulating the ACMG guidelines and others, has categorized IRD into four groups according to disease categories and pathological variants, specific information pertaining to IRD is yet to be published.

To bridge these existing gaps, a task force for variant interpretation of IRD in Japan was formed within the IRD working group with the objective of developing detailed variant interpretation guidelines specifically tailored for Japanese IRD cases. This task force adopted the ACMG guidelines as the fundamental framework and incorporated additional guidelines recognized for their cross-disease specificity. [2] The comprehensive ACMG guidelines for inherited sensorineural hearing loss (SNHL), [3] which shares some similarities with IRD, were referenced during the guideline development process to ensure alignment with the established standards. In instances where certain aspects remained ambiguous, the task force formulated their own evaluation criteria, taking into consideration the unique characteristics of IRD int the Japanese population.

After conducting two rounds of pilot assessments and incorporating subsequent revisions, the finalized variant interpretation guidelines in Japanese, titled "Specification of Variant Interpretation Guidelines for Japanese Inherited Retinal Dystrophy-1st draft," were published (available at https://www.jrvs.jp/guideline/ird_acmg_guideline.pdf). These guidelines offer a comprehensive framework that integrates the fundamental principles of the ACMG guidelines, specific evaluations tailored to IRD, and considerations for the unique characteristics of the Japanese population. By combining these elements, the

guidelines aim to provide a standardized and comprehensive approach to variant interpretation in the context of IRD in Japan.

Outline

The Specification of Variant Interpretation Guidelines for Japanese Inherited Retinal Dystrophy was developed based on the established framework of the ACMG guidelines (**Table 1**, **Table 2**), [1] which serve as the global standard for interpreting variants across different diseases. In addition to the ACMG guidelines, specific considerations were made for IRD by incorporating disease frequencies, allele frequencies, and phenotypic and genotypic characteristics. Modifications were implemented to adapt the ACMG guideline design to the specific context of IRD (**Table 4**), as described below.

Summary of specifications

We have contributed the following recommended specifications for the ACMG/AMP rules in the Japanese IRD variant interpretation (J-IRD-VI) guidelines. **Tables 1** and **2** provide a concise summary of the categories and criteria outlined in the ACMG guidelines, along with the corresponding criteria for verdict assessment. [1] These tables serve as a comprehensive reference for guiding the interpretation of genetic variants associated with Mendelian inherited diseases, in accordance with the ACMG guidelines. Notably, the sensorineural hearing loss (SNHL) expert panel has also provided specifications for 21 ACMG/AMP rules (**Table 3**), aiming to establish standardized guidelines for the clinical application of variant interpretation. [3]

We recommended specifications for 18ACMG/AMP rules (**Table 4**). Five rules had general recommendations on the application of the rule (PM5, PP3, BS4, BP4, BP7). Five rules had gene or disease-based specifications (PS3, PM1, PM2, PP4, BA1). Six rules had strength-level specifications (PVS1, PS2, PM3, PM5, PM6, PP1). Two rules had both gene/disease-based specifications and strength-level specifications (BS1, BS2). No changes were recommended for seven rules (PS1, PS4, PM4, BS3, BP2, BP3, and BP5), and two rules were considered not applicable (PP2, BP1).

Detailed specifications

Population database (BA1, BS1, PM2)

The thresholds for estimated allele frequency vary depending on the summed prevalence of monogenic diseases and the inheritance pattern, including autosomal dominant (AD), autosomal recessive (AR), and X-linked inheritance. The estimated disease prevalence of IRD in Japan is approximately 1 in 4,000 to 8,000 live births. This prevalence is lower than that of SNHL, which is estimated to occur in 1 in 300-500 live births. [3]

For the pathogenicity classification criteria, the J-IRD-VI guidelines define allele frequencies of <0.00001 for AD-IRD and <0.00002 for AR-IRD as the thresholds for the PM2 criterion. For the BA1 criterion, the allele frequency threshold is >0.0003 for AD-IRD and >0.001 for AR-IRD.

Despite these thresholds, there have been reports of pathological variants with high allele frequencies (e.g. NM_001142800.2(EYS):c.2528G>A (p.Gly843Glu)). [4-7] Therefore, variants that have sufficient evidence established under other criteria may be

excluded from further consideration based on allele frequency alone. Additionally, the frequency of the underlying disease can vary significantly depending on the specific phenotype and gene involved.

The gnomAD database (https://gnomad.broadinstitute.org/) was utilized as a reference for global allele frequencies. Additionally, for calculating Japanese-specific allele frequencies, the Human Genetic Variation Database (HGVD, https://www.hgvd.genome.med.kyoto-u.ac.jp/) and TommoJPN database (Tohoku Medical Megabank Organization, https://www.megabank.tohoku.ac.jp/) were used.

The criteria for this item are considered met when the respective databases satisfy the specified threshold conditions.

Loss of function variants (PVS1, PVS1_Strong, PVS1_Moderate, PVS1_Supporting)

The determination of loss of function (LOF) variants in the J-IRD-VI guidelines is guided by a flowchart that is based on the recommendations for interpreting the LOF PVS1 ACMG/AMP variant criterion. (**Figure 1**).[2] The strength of evidence for LOF

variants may vary depending on the specific type of variant and the presence or absence of residual protein.

For splice site variants, the canonical splice site (+/- 2 bp) is primarily considered. However, other splice site variants can be evaluated if there is functional and other evidence supporting their impact on splicing. The inclusion of functional and other evidence allows for a comprehensive assessment of the variant's effect on splice site functionality. Detailed predicted and observed impact of variants on splicing and recommendations have been recently published by the ClinGen Sequence Variant Interpretation (SVI) Splicing Subgroup. [8]

Variants affecting the same amino acid residue (PS1, PM5)

The ACMG guidelines assign strong evidence (PS1) or moderate evidence (PM5) of pathogenicity when established pathological variants are found at the same amino acid residue. [1]

While the strength level for PS1 remains unchanged, the evaluation method of the J-IRD-VI guidelines for PM5 includes variants that were previously classified as "likely

pathogenic" or "variant of unknown significance (VUS)." To incorporate these variants, one VUS will be assigned 0.5 points, and one pathogenic/likely pathogenic variant will be assigned 1.0 points.

A total score of 0.5 points represents supporting evidence, while a score of 1.0 or more indicates moderate evidence.

Additionally, evolutionary conservation is a crucial factor in the evaluation process. Regions with a notably low evolutionary conservation score (below 0.3 as determined by UCSC, phyloP, phastCons scores, etc.: https://genome.ucsc.edu/) will not be considered for pathogenicity, while detailed calibration of the PhyloP for missense variant pathogenicity classification and ClinGen recommendations have been recently published. [9]

Computational predictive tools (PP3, BP4, BP7)

The ACMG guidelines include multiple prediction software for variant evaluation. [1] However, for the evaluation of missense variants in accordance with the guidelines for SNHL, the REVEL (Rare Exome Variant Ensemble Learner) tool, which provides

comprehensive evaluation, was adopted. [3]

The cutoff value for the REVEL score is taken from previous reports, with a score of 0.15 or less considered supporting evidence (BP4) and a score of 0.7 or higher considered strong evidence (PP3). [10] Detailed calibration of REVEL scores for missense variant pathogenicity classification and ClinGen recommendations for PP3/BP4 criteria have been recently published. [9]

For the prediction of splice site changes, a comprehensive assessment is made when any of the following three software criteria are met: MaxEntScan (https://www.ncbi.nlm.nih.gov/refseq/) a score (diff) greater than 3[11] or Human Splice Finder (http://umd.be/Redirect.html), or Splicing AI (https://asia.ensembl.org/index.html) scores (delta) greater than 0.8 (high precision). These criteria provide evidence to support the prediction of splice site changes. Detailed predicted and observed impacts of variants on splicing and recommendations have been recently published by the ClinGen SVI Splicing Subgroup. [8]

Functional studies (PS3, BS3)

Transgenic animal models that demonstrate the reproduction of the retinal phenotype (phenocopy) associated with a specific gene variant are considered strong evidence. Functional analysis using established experimental systems, such as mini gene assays or zebrafish models, can provide valuable insights into gene function. If such functional analysis demonstrates that a variant leads to a change in gene function, it is considered moderate evidence in support of its pathogenicity.

Mutational hot spots or functional domains (PM1)

In manifest AD retinitis pigmentosa (AD-RP), amino acids 650-780 of the RP1 gene have been identified as mutational hot spots. [12,13] Similarly, in manifest IRD (AD-IRD), amino acids 39-99 of the CRX gene are recognized as mutational hot spots. [14-16]

Furthermore, in AD-IRD, amino acids 123-265 of the PRPH2 gene, which constitute the D2 loop, are considered to be a functional domain within this category. [17,18]

Segregation data (PP1, PP1_moderate, PP1_strong, BS4)

Intrafamilial co-segregation data are evaluated according to the guidelines for SNHL, which differentiate between AD and AR inheritance (**Tables 5** and **6**). [19]

The evidence is weighted according to the guidelines, taking into account the phenotype (the observed clinical manifestation) within a given family. Three levels of evidence are established according to the number and likelihood of consanguineous matches between the phenotype and genotype.

However, it is important to note that the BS4 criterion does not apply to phenotypes, genes, or variants that are expected to manifest in adulthood. The focus is on the evaluation of co-segregation data for early-onset or pediatric-onset diseases rather than adult-onset conditions.

De novo occurrence (PS2, PS2_very strong, PS2_moderate, PS2_supporting, PM6)

In the ACMG guidelines, when the maternity and paternity of a de novo variant are unconfirmed, the PM6 criterion is applied.[1] However, if paternity and maternity have been confirmed, the PS2 criterion is applicable for the J-IRD-VI guidelines (**Table 7**).

In the SNHL guidelines, a weighted point system is employed to account for phenotypic/genotypic specificity. Furthermore, additional points can be added based on

the number of originators involved in the inheritance pattern (**Table 8**).

Allelic data (PM3, BS2)

In the ACMG guidelines, a moderate evidence is defined as an allelic variant of the variant under evaluation in the case of a latent genetic disease. [1] For AR-IRD, the identification of a pathogenic variant at the allele of the variant under evaluation (i.e., compound heterozygosity) is considered moderate evidence.

The SNHL guidelines provide specific criteria for variant evaluation. These guidelines consider the presence or absence of allele information (phase information) for the identified pathological variant and the number of originators (i.e., family members) in whom the pathological variant has been identified. Points are assigned based on this information. If the variant under evaluation is homozygous, points are assigned according to family history. The strength of the evidence is determined by the cumulative points (**Tables 9** and **10**).

However, in the BS2 criterion, if the phenotype, gene, or variant is expected to manifest in adulthood or cause adult-onset disease, the item is not applicable for evaluation

purposes.

Phenotypic data (PP4, BP5)

The ACMG guidelines define a gene as providing supporting evidence when a specific phenotype is associated with a disease caused by a single responsible gene, and a variant is identified in that gene that matches the phenotype. [1] These J-IRD-VI guidelines do not limit the specific phenotype to a single responsible gene but consider specific genes to provide evidence of a phenotypic association.

Examples of gene-phenotype associations considered supporting evidence include the following:

(1) SAG/GRK1 and Oguchi disease: the presence of a prominent golden reflex seen circumferentially and an electronegative waveform with a severely reduced b-wave and milder reduction of the a-wave in dark-adapted bright flash electroretinogram. [20,21]

(2) CYP4V2 and Bietti crystalline corneoretinal dystrophy: the presence of diffuse crystalline deposits scattered throughout the retina, followed by progressive atrophy of the retinal pigment epithelium (RPE), choriocapillaris, and neuroretina. [22]

(3) NR2E3 and Enhanced S-cone syndrome: pathognomonic electrophysiological features,

such as a slow rod-like response that appears to have a similar waveform under both scotopic and photopic conditions. [23,24]

These associations between specific genes and phenotypes are considered supporting evidence in the evaluation process.

Reputable source (PP5, BP6)

In the ACMG guidelines, if a variant under evaluation has been previously reported as pathogenic by a reputable source, it is considered supporting evidence. [1] Specifically, the J-IRD-VI guidelines define a pathological variant as one that has been reported by a reliable source and meets the evaluation criteria provided in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

However, variants that are reported in sources such as HMGD (http://www.hgmd.cf.ac.uk/) where the criteria for evaluating pathogenicity are not specified are not applicable for the J-IRD-VI guidelines.

Discussion

The "Specification of Variant Interpretation Guidelines for Japanese Inherited Retinal Dystrophy" provides detailed specifications for interpreting genetic variants in Japanese patients with IRD. These guidelines play a critical role in ensuring accurate diagnosis and treatment decisions by providing standardized criteria. By integrating the ACMG framework and incorporating disease-specific considerations and ethnic factors unique to the Japanese population, these guidelines address the specific challenge associated with IRD in Japan.

The guidelines cover multiple aspects of variant interpretation, including the utilization of population databases, assessment of LOF variants, analysis of amino acid residue impact, application of computational predictive tools, consideration of functional studies, identification of mutational hotspots, evaluation of segregation data, assessment of de novo occurrences, analysis of allelic data, utilization of phenotypic information, and reliance on reputable sources. This comprehensive approach ensures that all relevant factors are considered during the variant interpretation process, leading to consistent and accurate results.

However, the guidelines also have certain limitations that should be addressed. One limitation is the lack of gene/disease-specific information, such as prevalence, allele frequency, functional assessment, mutational hotspots, allelic data, and phenotypic data. These missing data points could significantly enhance the quality and specificity of the guidelines. To overcome this limitation, efforts should be made to gather and store detailed data for specific genes and diseases, allowing for more precise variant interpretation and better-informed treatment decisions.

Periodic revisions of the guidelines will be necessary to keep pace with the rapid advancements in genome analysis technology and data science. The field of genomic diagnosis and treatment is continually evolving, and as new knowledge and technologies emerge, the guidelines must be updated to reflect the latest standards and practices. In fact, the ACMG guidelines themselves are currently undergoing a revision process, highlighting the need for ongoing refinement and improvement.

Another important consideration is the availability of experimental data to support variant interpretation. While the guidelines emphasize the utilization of reputable sources and databases, there is a need for robust experimental studies and in silico

molecular genetic analyses to improve the accuracy of clinical effect and pathogenicity assessment for each variant. Access to comprehensive experimental data would strengthen the guidelines and enhance their clinical utility.

The role of genomic diagnosis and treatment is expected to expand as genome analysis technology and data science continue to advance. These guidelines aim to optimize the efficiency and uniformity of variant evaluation in IRD, with the ultimate goal of widespread genetic diagnosis for IRD patients in Japan.

Acknowledgments:

We express our gratitude to the working group for inherited retinal dystrophy, representing the Japanese Retina and Vitreous Society, for their valuable contributions to the development of the Japanese inherited retinal dystrophy variant interpretation guidelines. Their dedication and expertise have played a significant role in advancing the field of genetic testing and diagnosis for inherited retinal dystrophy in Japan.

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Figure Legends

Figure 1. Evaluation of loss of function variants (PVS1 workflow)

NMD, nonsense-mediated decay; LOF, loss of function.

This diagram has been modified for the specific purpose of the Japanese inherited retinal dystrophy variant interpretation (J-IRD-VI) guidelines, using the oliginal publication as a foundation.

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Initiation Codon

Different functional transcript uses alternative start codon

No known alternative start codon in other transcripts

No pathogenic variant(s) upstream of closest potential in-frame start codon

≥1 pathogenic variant(s) upstream of closest potential in-frame start codon

Duplication (≥1 exon in size and must be completely contained within gene)

Proven not in tandem

Presumed in tandem

Proven in tandem

Reading frame presumed disrupted and NMD predicted to occur

No or unknown impact on reading frame and NMD

Reading frame disrupted and NMD predicted to occur

Deletion (Single exon to full gene)

Single to multi exon deletion-Preserves reading frame

Single to multi exon deletion-Disrupts reading frame and is NOT predicted to undergo NMD

Single to multi exon deletion-Disrupts reading frame and is predicted to undergo NMD

Full gene deletion

Truncated/altered region is critical to protein function

Role of region in protein function is unknown

LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)

Lof variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s)

Variant removes <10% of protein

Variant removes >10% of protein

GT--AG 1,2 splice sites

Exon skipping or use of a cryptic splice site preserves reading frame

Exon skipping or use of a cryptic splice site disrupts reading frame and is NOT predicted to undergo NMD

Exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD

Exon is absent from biologically-relevant transcript(s)

Exon is present in biologically-relevant transcript(s)

Nonsense or Frameshift

Not predicted to undergo NMD

Predicted to undergo NMD

N/A

PVS1 Supp

PVS1 Moderate

PVS1 Strong

PVS1

Table 1. Summary of categories and criteria

	Categories	Criteria
1	PVS1	Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.
2	PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
3	PS2	De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.
4	PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
5	PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.
6	PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.
7	PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.
8	PM3	For recessive disorders, detected in trans with a pathogenic variant
9	PM4	Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants.
10	PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

11	PM6	Assumed de novo, but without confirmation of paternity and maternity.
12	PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
13	PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missens variant is a common mechanism of disease.
14	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
15	PP4	Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.
16	PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.
17	BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.
18	BS1	Allele frequency is greater than expected for disorder.
19	BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.
20	BS3	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.
21	BS4	Lack of segregation in affected members of a family.
22	BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease.

23	BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.
24	BP3	In-frame deletions/insertions in a repetitive region without a known function.
25	BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)
26	BP5	Variant found in a case with an alternate molecular basis for disease.
27	BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.
28	BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. May 2015;17(5):405-24. Modified for the purpose.

Each criterion is weighted as very strong (PVS1), strong (PS1-4), moderate (PM1-6), or supporting (PP1-5), and each benign criterion is weighted as stand-alone (BA1), strong (BS1-4), or supporting (BP1-6).

Table 2: Criteria for verdict assessment

Pathogenic		Criteria
1	Very Strong (PVS1) AND	
	a	≥1 Strong (PS1–PS4) OR
	b	≥2 Moderate (PM1–PM6)
	c	1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5)
	d	≥2 Supporting (PP1–PP5)
2	≥2 Strong (PS1–PS4)	
3	1 Strong (PS1–PS4) AND	
	a	≥3 Moderate (PM1–PM6)
	b	2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5)
	c	1 Moderate (PM1–PM6) AND ≥4 Supporting (PP1–PP5)

Likely Pathogenic	Criteria
	1 Very Strong (PVS1) AND 1 Moderate (PM1–PM6)
	1 Strong (PS1–PS4) AND 1–2 Moderate (PM1–PM6)
	1 Strong (PS1–PS4) AND ≥2 Supporting (PP1–PP5)
	≥3 Moderate (PM1–PM6) OR
	2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5)
	1 Moderate (PM1–PM6) AND ≥4 Supporting (PP1–PP5)

Benign	Criteria

	1 Stand-Alone (BA1) ≥2 Strong (BS1–BS4)
Likely Benign	
	1 Strong (BS1–BS4) and 1 Supporting (BP1–BP7) ≥2 Supporting (BP1–BP7)
If the other criteria are not met, or if the criteria for pathological and benign are conflicting, the variant is classified as Uncertain Significance (VUS).	
Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. May 2015;17(5):405-24. Modified for the purpose.	

Table 3. Specifications for genetic sensorineural hearing loss (SNHL)	
Categories	Contents
PS1, PP3, BS4, BP4, BP5	Establishment of general recommendation rules
PS3, PM1, PM2, PP4, BA1, BS4, BP2	Detailed settings for gene and disease entity
PVS1, PS2, PM3, PM5, PM6, PP1, BS3	Detailed strength level settings
PS4, BS1, BS2	Detailed settings for genes, disease entity, and strength levels
PM4, BP3, BP7,	No changes
PP2, PP5, BP1, BP6	Removed from the criteria
Verdict assessment	Modification
Likely pathogenic	PVS1 and PM2_Supporting = likely pathogenic
Likely benign	BS1 without valid conflicting evidence
Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat. Nov 2018;39(11):1593-1613.	

Table 4. Summary of specifications for inherited retinal dystrophy in Japan

	Categories	Specifications/applied rules	Comments
1	PVS1	ClinGen SVI Recommendation (PVS1, PVS1 strong/moderate/supporting)	For splice site alteration, canonical splice site (+/-2bp) is the main indication.
2	PS1	No change	No change
3	PS2	SVI Recommendation for De Novo Criteria (PS2 & PM6) - Version 1.1 (Very strong/strong/moderate/supporting)	Indicated when the phenotype and genotype of the parents are identified
4	PS3	Added some identified functional changes as PS3_moderate.	Mini gene assay and zebrafish model studies showing phenocopy (strong) or functional characteristics (moderate) are applicable.
5	PS4	No change	Ancestry matched cohorts (N>20000) are preferred.
6	PM1	No change	RP1 (amino acid 650-780)、CRX cone-rod homeobox protein (39-99) 、PRPH2 D2 Loop (123-265)
7	PM2	Allele frequency for recessive diseases<0.00002. Allele frequency for dominant diseases<0.00001.	gnomAD, HGVD, Tommo8.3k JPN
8	PM3	SVI Recommendation for (PM3) - Version 1.1 (moderate)	Pathogenic and likely pathogenic are considered according to the SNHL specification.

9	PM4	No change	Exon that deletes the entire Exon falls under the PVS1 category and is therefore not applicable.
10	PM5	Likely pathogenic variants or VUS are also included in the analysis, using a point system of 0.5 supporting 1.0 moderate, assuming 0.5 for 1 VUS and 1.0 for 1 pathogenic variant.	Partially modified. Higher conservation should be considered.
11	PM6	SVI Recommendation for De Novo Criteria (PS2 & PM6) - Version 1.1 (Very strong/strong/moderate/supporting)	Indicated when the phenotype and genotype of the parents are identified.
12	PP1	Application of SNHL point system (Strong/moderate/supporting)	Indicated when phenotype and genotype are identified
13	PP2	Removal	Removed due to variable gene size, etc.
14	PP3	REVEL scores ≥ 0.7 or damaging splice sight alteration predicted by software.	REVEL, MaxEntScan, Human Splice Finder, Splicing AI.
15	PP4	Modified (definitions for particular genes)	SAG/GRK1, CYP4V2, NR2E3/NRL.
16	PP5	Modified (suggestions for reliable resources)	Peer-reviewed publications, ClinVAR minor (criterion provided).
17	BA1	Allele frequency for recessive diseases>0.01 Allele frequency for dominant diseases>0.0003.	gnomAD, HGVD, Tommo8.3k JPN. Variants for which sufficient evidence has been established for other items may be excluded

18	BS1	Allele frequency for recessive diseases 0.001< <0.01 Allele frequency for dominant diseases 0.0006< <0.0003	gnomAD, HGVD, Tommo8.3k JPN. Variants for which sufficient evidence has been established for other items may be excluded
19	BS2	SVI Recommendation for (PM3) - Version 1.1 (moderate)	Pathogenic and likely pathogenic are considered according to the SNHL specification. Not indicated for adult-onset retinal dystrophies such as RP.
20	BS3	No change	Indicated when phenotype and genotype are identified. Not indicated for adult-onset retinal dystrophies such as RP.
21	BS4	Application of SNHL point system (Strong/moderate/supporting)	
22	BP1	Removal	
23	BP2	No change	
24	BP3	No change	
25	BP4	REVEL scores ≤ 0.15 or no damaging splice sight alteration predicted by software	REVEL, MaxEntScan, Human Splice Finder, Splicing AI.
26	BP5	No change	
27	BP6	No change	Peer-reviewed publications, ClinVAR minor (criterion provided).

28	BP7	No splice sight alteration	Mainly for variants +/- 10bp from exon edge, MaxEntScan, Human Splice Finder, and Splicing AI are applied.

Sequence Variant Interpretation General Recommendations for Using ACMG/AMP Criteria are provided by Clinical Genome Resource (ClinGen): https://clinicalgenome.org/working-groups/sequence-variant-interpretation/.

Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat. Nov 2018;39(11):1593-1613.

Specifications for SNHL was applied/modified for PS2, PM6, PP1, PP2, PP3, BP1, and BP4.

Table 5. General recommendations for segregation scoring

	Supporting	Moderate	Strong
Likelihood	4:1	16:1	32:1
LOD Score	0.6	1.2	1.5
Autosomal dominant threshold	2 affected segregations	4 affected segregations	5 affected segregations
Autosomal recessive threshold	See Table 6	See Table 6	See Table 6

Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.

Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. *Am J Hum Genet*. Jun 1 2017;100(6):895-906.

Table 6. Segregation scoring for autosomal recessive diseases

		Unaffected recessive segregations					
		0	1	2	3	4	5
Affected segregations	0	<0.5	<0.5	<0.5	<0.5	<0.5	0.62
	1	0.6	0.73	0.85	0.98	1.1	1.23
	2	1.2	1.33	1.45	1.58	1.7	1.83
	3	1.81	1.93	2.06	2.18	2.31	2.43
	4	2.41	2.53	2.66	2.78	2.91	3.03
	5	3.01	3.14	3.26	3.39	3.51	3.63

Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.

Table 7. General recommendations for de novo scoring

Supporting (PS2_Supporting or PM6_Supporting)	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5 points	1.0 points	2.0 points	4.0 points

Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.

Table 8. Phenotypic consistency for de novo scoring		
Phenotypic consistency	Points per proband	
	Confirmed de novo	Assumed de novo
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5
Phenotype consistent with gene but not highly specific and high genetic heterogeneity	0.5	0.25
Phenotype not consistent with gene	0	0
Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.		

Table 9. General recommendations for classification/zygosity of other variant			
Supporting (PM3_Supporting)	Moderate (PM3)	Strong (PM3_Strong)	Very Strong (PM3_VeryStrong)
0.5 points	1.0 points	2.0 points	4.0 points
Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.			

Table 10. Classification/zygosity of other variant for scoring		
Classification/zygosity of other variant	Points per proband	
	Known in trans	Phase unknown
Pathogenic/Likely pathogenic	1	0.5
Homozygous occurrence (Max points from homozygotes=1.0)	0.5	NA
Rare uncertain significance variant on other allele, or homozygous occurrence due to consanguinity, (max point= 0.5)	0.25	NA
Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. Hum Mutat 2018;39:1593-1613.		