Fast summary for curators- RPGR gene (V7)

Codes that are N/A for RPGR: BP1, BP2, BP5, BP6, BS3, PM1, PM3, PP2, and PP5. **Do not use these.**

RPGR-ORF15 transcript: NM_001034853.2 (ENST00000645032.1)- please use this transcript
RPGR(1-19) transcripts: NM_000328.3

Disease and inheritance:
In the VCI, under the header and summary on the right, please click on the Disease (enter MONDO:0100437 and then click on the "retrieve from OLS". The result should be "RPGR-related retinopathy" then click the "save") and then click on the inheritance (from the dropdown menu choose "X-linked").

Please use the standardized text file found on the BOX site to fill in the explanation for each code used. https://bcm.app.box.com/file/1203416537277

Search for the variant in gnomAD:
https://gnomad.broadinstitute.org/transcript/ENST00000645032?dataset=gnomad_r4

1. If it is not present or is not present in males, or has a frequency of <0.0000005, then use the PM2_supporting code. In the ORF15 region of low complexity (amino acids 725-1078), the population frequency data can be evaluated for PM2_Supporting / BS1 / BA1 but also needs to be reviewed by the VCEP by showing a screenshot of the gnomAD data during the presentation of the variant for VCEP approval.
2. If the variant is present in hemizygous and has a frequency >0.00005, use the BA1 (met) code.
3. If the variant is present in hemizygous and has a frequency >0.000005, use the BS1 (met) code.
4. Does the variant result in nonsense (stop codon) or frameshift (fs)?
    a. If so, is it in before amino acid 1132 of transcript NM_001034853.2 or 777 of transcript NM_000328.3? Use the PVS1 (met) code.
    b. In case it is after amino acid 1132 (NM_001034853.2) or 778 (NM_000328.3)- PVS1_strong code.
5. Does the variant result in exon deletion/ skipping?
    a. Does the variant result in exon deletion/skipping? Use the PVS1 code.
    (If the variant is an exon deletion/ skipping found in exon 3/4/5/6/7/8/9/10/0RF15- in the explanation please choose: "disrupt a critical functional domain".
    If your variant is an exon deletion/ skipping found in exon 1/11/12/14- in the explanation please choose: "introduce a premature stop codon".

If your variant is deleting/ skipping exon 2 or 13 please choose: "reviewed by the X-linked IRD VCEP and found to be deleterious").

6. Does the variant result in an exon skipping because of a variant in the splice site motif (3 last bases of exon/ +1 to +6 bases after an exon/ -1 to -20 bases before an exon)? Please check the RNA splice site table and decision tree to select the right PVS1 code and whether the PS1 code applies (next section #8).

Note this caveat- Please look at the PVS1 decision tree for more options regarding this code (splice site, exon deletions/duplication, and initiation codes): https://bcm.app.box.com/file/1423928402109.

Use the UCSC site to view the location. https://genome.ucsc.edu/index.html

1. Does the variant change the length of the protein due to in-frame insertion or deletion (indel) in a non-repetitive region? If so, use the PM4_moderate code.
2. Does the variant change the length of the protein due to a missense variant that results in a stop loss? If so, use the PM4_strong code.
3. Is the variant an in-frame insertion or deletion (indel) in a region with a low complexity? Use the BP3 (met) code.
4. Does the variant result in a synonymous (silent) variant or located in an intron? If so, check the SpliceAI score using this link: https://spliceailookup.broadinstitute.org/. If the spliceAI score is < 0.1, use the BP4 (met) code.
5. Does the variant result in a synonymous (silent) variant and has a spliceAI score <0.1? use the BP7_ supporting code.
6. Does the variant result in a missense? Is the location conserved? What is the REVEL score for this missense? Also, check the SpliceAI score using this link: https://spliceailookup.broadinstitute.org/.
    a. If the REVEL score is ≥0.932, use the PP3_strong code.
    b. If the REVEL score is 0.931 ≥ x ≥ 0.773, use the PP3_moderate code.
    c. If the REVEL score is 0.772 ≥ x ≥ 0.644, use the PP3 (met) code.
    d. If the REVEL score is between 0.291 and 0.644,
        i. If the SpliceAI score is ≥ 0.2, use the PP3 (met) code.

    Note this caveat- This code cannot be used with the PVS1 code.

    e. REVEL score is 0.290≤ x ≤0.184, and SpliceAI score is <0.1, use the BP4 (met) code.
    f. REVEL score is 0.183≤ x ≤ 0.017, and SpliceAI score is <0.1, use the BP4_moderate code.
    g. REVEL score is 0.016 ≤ x ≤0.04, and SpliceAI score is <0.1, use the BP4_strong code.
    h. REVEL score is ≤ 0.03, and SpliceAI score is <0.1, use the BP4_very strong code.

    Note this caveat- If one part of the statement does not match, the code is not met.

7. Is the variant found where the XLIRD VCEP has already curated a missense variant in the same amino acid change?
    a. If it was curated as pathogenic, use the PS1_strong code.
    b. If it was curated as likely pathogenic, use the PS1_moderate code.
8. Is the variant found at the same position as a variant that was previously curated by the XLIRD VCEP, and the change resulted in a different amino acid? Check the "Grantham score" at https://en.wikipedia.org/wiki/Amino_acid_replacement#Grantham's_distance Is the "Grantham score" greater than the score for the variant that was curated before? If the score is lower one can note “the "Grantham score" for this variant results in a lower score and therefore, this code was not used.”
   In case the score is higher:
    a. If the VCEP curated the variant as a pathogenic missense (with more than one variant), use PM5_moderate code.
    b. If the VCEP had curated it as likely pathogenic, use the PM5_supportive code.
9. Is the variant found where the same/novel amino acid change variant has **yet** to be curated by the XLIRD VCEP? Please curate it as well. This will be a separate curation.
10. Is the variant found in a splice site motif and another variant at the same splice site motif previously classified by the X-linked IRD VCEP?
    a. If the variant is in the +/- 1,2 dinucleotide and meets the [PVS1_supp / PVS1_mod / PVS1_strong] and another variant in the same donor/acceptor +/-1,2 dinucleotide c.XXX was previously classified as pathogenic for RPGR-related retinopathy by the ClinGen X-linked IRD VCEP- use the PS1 (met) code.
    b. If the variant is outside the donor/acceptor +/- 1,2 dinucleotide and meets the PP3 code due to predicted splicing disruption and another predicted splicing variant in the same nucleotide, c.XXX, was previously classified as pathogenic for RPGR-related retinopathy by ClinGen X-linked IRD VCEP- use the PS1 (met) code.
    c. If the variant is in the +/- 1,2 dinucleotide and meets the [PVS1_Supp / PVS1_Mod / PVS1_Strong] and another variant in the same donor/acceptor motif but outside of the +-1,2 dinucleotide, c.XXX, was previously classified as pathogenic for the RPGR-related X-linked IRD VCEP- use the PS1_moderate code.
    d. If the variant is outside the +/- 1,2 dinucleotide and meets the PP3 code due to predicted splicing disruption and another predicted splicing variant, c.XXX, was previously classified as [likely pathogenic variant in the same nucleotide] / [a pathogenic variant in the same donor/acceptor motif] for the RPGR-related X-linked IRD VCEP- use the PS1_moderate code.
    e. If the variant is found in the +/- 1,2 dinucleotide and meets PVS1 (met) and another variant, c.XXX, was previously classified as pathogenic in the same donor/acceptor +/-1,2 dinucleotide for the RPGR-related X-linked IRD VCEP- use the PS1_supporting code.

f. If the variant is found in the +/- 1,2 dinucleotide and meets PVS1 (met) and another variant, c.XXX, was previously classified as [Likely pathogenic / pathogenic] in the same donor/acceptor motif outside of the +-1,2 dinucleotide] for the RPGR-related X-linked IRD VCEP- use the PS1_supporting code.
g. If the variant is found in the +/- 1,2 dinucleotide and meets the [PVS1_Supp / PVS1_Mod / PVS1_Strong] and another variant in the same donor/acceptor motif but outside of the +-1,2 dinucleotide, c.XXX, was previously classified as likely pathogenic for the RPGR-related X-linked IRD VCEP- use the PS1_supporting code.
h. If the variant is found outside the +/- 1,2 dinucleotide and meets PP3 code due to predicted splicing disruption and another predicted splicing variant in the same donor/acceptor motif, c.XXX, was previously classified as likely pathogenic for the RPGR-related X-linked IRD VCEP- use the PS1_supporting code.

Search literature on the variant (use Google, Google Scholar, PubMed, LOVD3, HGMD, and any other database).

1. Is the variant found in a healthy male >30 with a complete visual examination showing no evidence of retinopathy? If so, use the BS2_stand alone code.

Description of the proband's phenotypes in a paper (PP4 code), choose the best-described proband (You can find a phenotype scoring list in Table 1):

1. Does the proband answer the required rules (<30 and has ERG/FAF)?
    a. If so, does it score 4-7.5 points? If so, use the PP4_supportive (met) code.
    b. Does the proband score 8-12 points, use the PP4_moderate code.
    c. Does the proband score <4? If so, do not use this code.
2. One can find the right HPO on this site https://hpo.jax.org/app/browse/gene/6103 Commonly used HPO terms and IDs are found at the end of this document.

Table 1. Proband phenotype scoring list:

Required for use of PP4 (0 points each)
• Males have functional vision impairment by age 30 (or by age 50 if harboring a truncating variant after c.2128) and diagnosis of cone or cone-rod dystrophy
• Decreased or absent cone and/or rod ERG/FAF responses
Specific RPGR Phenotype Findings List (2 points each)
• Family history consistent with X-linked inheritance, ○ no male-to-male transmission ★
• Previous exome, genome, or 100+ retinal dystrophy panel (that includes X-linked genes) testing that did not provide an alternative explanation for visual impairment
Consistent with RPGR Findings (0.5 or 1 point each)

• First/second-decade onset (1)
• Rod involvement is relatively greater than cone involvement (1) (or cone > rod (1) if harboring a truncating variant after c.2128 and diagnosis of cone or cone-rod dystrophy)
• Delayed or milder phenotype in females (1)   ○ Unlike carriers of other X-linked RP, female carriers show some retinal pathology by age 50 (usually by age 30), by abnormal fundus pigmentation, and more certainly by ERG response amplitude reduction
• Patient report of Night blindness/nyctalopia (0.5)
• Optic nerve pallor (0.5)
• Pigmentary retinopathy (0.5)
• Poor pupillary light response (0.5)
• Abnormal color vision (0.5)
• Decreased central vision acuity (0.5)
• Myopia (0.5) or high myopia (e.g., -6 Diopter and higher) (1.0)
• Visual field constrictions (0.5)
• Photo dysphoria/ Photophobia (0.5) (or 1 point if harboring a truncating variant after c.2128 and diagnosis of cone or cone-rod dystrophy)
• Macular atrophy (1) (only if harboring a truncating variant after c.2128 and diagnosis of cone or cone-rod dystrophy)

★ Family inheritance can be used with no clear genotyping in the following cases:

- Pedigree clearly shows inheritance from mother's side of family, with no male-to-male transmission.
- Patient comes from an X-linked cohort (not showing pedigree) assembled before genotyping, specifically indicating no male-to-male transmission.
- Patient was identified as an X-linked case within an IRD cohort that also included other sporadic cases (but no pedigree shown).

[The difference between the use of family inheritance points for the PP4 code and the PP1 code is the need for genetic testing of affected family members for the PP1 code].

3. Probands counting (PS4 code): Any affected individual who is related to the PP4 chosen proband cannot be counted. Count an affected individual who is unrelated to the PP4 chosen proband and who meets the required rule (<30 with visual impairment and/or has ERG/FAF). Affected female counting is eligible in cases where an affected male is described in a pedigree or the text [avoid double counting- one affected person per family can be counted].
    a. If you find one unrelated proband to a PP4 proband- use the PS4_supporting code. Caveat: To use this rule, you must have a proband eligible for the PP4 code.
    b. If you find **two** unrelated probands- use the PS4_supporting code.

c. If you find **3-5** unrelated probands- use the PS4_moderate code.
d. If you find **≥6** unrelated probands- use the PS4 (met) code.

Please note that you can count only one proband per family. Note that some groups might publish more than one paper on the same proband, so always check that the institution name and the group members for each report are different to be sure you are not double counting the same proband (in different papers).

4. Does the proband have a *de novo* (not inherited from either parent) variant? and who meets the required rule (<30 with visual impairment and/or has ERG/FAF).
   a. Were both his parents tested? If so, use the PS2 code (Score with Table 2 and give strength by Table 3). [RP can be caused by about 300 known genes and it has a wide range of phenotypes- therefore, genetic testing of the mother through a genotyping method that can confirm maternity is required and we do not use the PM6 code].

   Please note that these codes can be used in combination with PP4/PS4 code (on the same proband)

Table 2. "De novo" proband scoring table:

Points per de novo occurrence for X-linked IRD genes	Points per Proband Confirmed de novo with confirmed maternity
Phenotype highly specific for gene	N/A
Phenotype consistent with gene but not highly specific	1
Phenotype consistent with gene but not highly specific and high genetic heterogeneity**	0.5
Phenotype not consistent with gene	0
*Maximum allowable value of 1 may contribute to overall score ** The correct point values for each de novo proband.	

Table 3. Code strength table:

Supporting	Moderate	Strong	Very Strong
PS2_Supporting	PS2_Moderate	PS2	PS2_VeryStrong
0.5	1	2	4

5. Family genotype code (PP1 code): This code can be used in the following scenarios:
   - Pedigree clearly shows affected genotyped-positive individuals on the mother's side of the family with no male-to-male transmission.
   - Pedigree clearly shows affected individuals on the mother's side of the family with the mother and affected son genotyped.

- Patient comes from a family with only the number of affected and genotyped family members listed / the number of segregations, but no pedigree.

This PP1 code must meet the required rule (proband is <30 with visual impairment and/or has ERG/FAF, female proband must have an affected male relative). Affected female counting is eligible in cases where an affected male is described in a pedigree or the text.

a. For a mother and son pedigree where they are **both** affected/ symptomatic or a pedigree with two segregations, use the PP1_supporting code.
b. For one pedigree with >3 segregations, use the PP1_moderate code.
c. If you find two family pedigrees with >4 segregations total, use the PP1_strong code.

6. A healthy >30-year-old male, who is a member of a family with a diagnosed RP proband (who had a complete visual examination and carries the same variant)- use the BS4_stand alone code.
7. Functional studies evidence code (PS3/BS4 code):
    a. For any paper showing a damaging effect caused by the variant- use the PS3_supporting code.
    b. Is the variant located in the intron after the splice site and PP3 code was used (after nucleotide ±1/2) and a functional paper on this variant was published? If so, use the PVS1 code as applied for the exons themselves (see **Search for the variant in gnomAD** section).

We have an approved functional evidence table on the BOX site.
https://bcm.app.box.com/file/1202129484472

Use the point system to resolve the ambiguities if possible. (An earlier system used to combine rules to reach a final variant classification.)

1. Use Table 4 to score the codes used, and Table 5 to interpret the result. These tables were taken from PMID 32720330.

Table 4. Point values for ACMG/AMP strength of evidence categories

Evidence Strength	Point Scale	
	Pathogenic	Benign
Indeterminate	0	0 §
Supporting	1	−1
Moderate	2	−2 †
Strong	4	−4
Very Strong	8	−8 †

Table 5. Point-based variant classification categories

Category	Point ranges
Pathogenic	≥ 10
Likely Pathogenic	6 – 9 ¥
Uncertain	0 – 5
Likely Benign	−1 – −6 ¥
Benign	≤ −7

2. When a variant has conflicting evidence, the VCI will give it a VUS classification. This system weighs the evidence by strength and any result that differs from the VCI default must be modified manually in the VCI.
    a. Go to summary (top of the page to the right).
    b. To the left, you will find a drop-down menu with different classifications (Modify Classification). Choose the revised classification category from the list.
    c. Explain the reason(s) for the change in the window below. You can use the following text: "The ClinGen XLIRD VCEP recommends calculation of the final variant classification using a points system rather than the standard auto-calculation (VCEP specifications version PILOT; date of approval 09/05/2023)."

Final step:

At the top of the page click on "Summary". A different page will appear (other than the interpretation).

1. Please give some info about the variant (please choose and fill in the red parts):

"The NM_001034853.2(RPGR):XXX variant is a **[**choose one:

- missense variant encoding the substitution of XXX with XXX at position number.
- nonsense variant in amino acid number, which results in a premature stop codon / nonsense mediated decay and causes a truncated protein.
- frameshift variant due to number nucleotide deletion/insertion/indel/duplication introducing a premature stop codon after number amino acid(s) and causing a truncated protein / nonsense mediated decay.
- synonymous variant at amino acid number.
- canonical splice site variant in intron number, located ??? nucleotide(s) before/after exon number.
- intronic variant at intron ???, located number nucleotides before/after exon ???.
- splice variant motif located number nucleotides before/after exon [#].
- in-frame deletion/insertion/duplication variant in amino acid number not predicted to result in premature protein termination.**]**"

2. Next, please fill in the codes used in the window designated for summary (you can copy & paste from the information below).
3. At the end of this summary please add:

"In summary, this variant is classified as (choose one: benign / likely benign / a variant of uncertain significance / likely pathogenic / pathogenic) for RPGR-related retinopathy based on the ACMG/AMP criteria applied, as specified by the ClinGen X-linked IRD VCEP: (please enter the codes, and strengths for each code, used for this classification)."

4. Please make sure to save the summary and then scroll down and save it again as a provisional.

HPO terms(s):

[At the end of this list, there are some ERG test results terminologies that could help you.]

- X-liked inheritance (HP:0001417)
- Childhood-onset (HP:0011463)
- Juvenile onset (HP:0003621)
- Late young adult onset (HP:0025710)
- Middle-age onset (HP:0003596)
- Adult onset (HP:0003581)
- Rod-cone dystrophy (HP:0000510)
- Cone dystrophy (HP:0008020)
- Reduced electroretinogram (HP:0000654)
- Undetectable/Abolished electroretinogram (HP:0000550)
- Undetectable light-adapted electroretinogram (HP:0030465)
- Abnormal light-adapted electroretinogram (HP:0008275)
- Undetectable dark-adapted electroretinogram (HP:0030474)
- Electronegative electroretinogram (HP:0007984)
- Nyctalopia (HP:0000662)
- Myopia (HP:0000545)
- High myopia (HP:0011003)
- Mild myopia (HP:0025573)
- Moderate myopia (HP:0031624)
- Retinal pigment epithelial atrophy (HP:0007722)
- Retinal pigment epithelial mottling (HP:0007814)
- Pigmentary retinopathy (HP:0000580)
- Bone spicule pigmentation of the retina (HP:0007737)
- Peripheral retinal atrophy (HP:0200070)
- Peripapillary atrophy (HP:0500087)
- Macular dystrophy (HP:0007754)
- Macular degeneration (HP:0000608)
- Macular atrophy (HP:0007401)

- Retinal atrophy (HP:0001105)
- Bull's eye maculopathy (HP:0011504)
- Abnormal macular morphology (HP:0001103)
- Abnormal fundus autofluorescence imaging (HP:0030602)
- Abnormal best corrected visual acuity test (HP:0030534)
- Reduced visual acuity (HP:0007663)
- Progressive visual loss (HP:0000529)
- Poor vision (HP:0000505)
- Blindness (HP:0000618)
- Peripheral visual field loss (HP:0007994)
- Visual field defect (HP:0001123)
- Constriction of peripheral visual field (HP:0001133)
- Color vision defect (HP:0000551)
- Cataract (HP:0000518)
- Optic disc pallor (HP:0000543)
- Retinal arteriolar constriction (HP:0008043)
- Photophobia (HP:0000613)
- Attenuation of retinal blood vessels (HP:0007843)

Other HPOs term that can be found for RPGR:

- Rhegmatogenous retinal detachment (HP:0012230)
- Exotropia (HP:0000577)
- Retinal hemorrhage (HP:0000573)
- Chorioretinal atrophy (HP:0000533)
- Abnormal central microtubular pair morphology of respiratory motile cilia (HP:0012260)
- Abnormal respiratory motile cilium physiology (HP:0012261)
- Abnormal sperm morphology (HP:0012864)
- Dynein arm defect of respiratory motile cilia (HP:0012255)
- Immotile cilia (HP:0012263)
- Recurrent respiratory infections (HP:0002205)
- Central scotoma (HP:0000603)
- Hearing impairment (HP:0000365)
- Nasal polyposis (HP:0100582)
- Recurrent bronchitis (HP:0002837)
- Recurrent ear infections (HP:0410018)
- Recurrent lower respiratory tract infections (HP:0002783)
- Recurrent sinusitis (HP:0011108)
- Renal insufficiency (HP:0000083)
- Astigmatism (HP:0000483)
- Decreased light- and dark-adapted electroretinogram amplitude (HP:0000654)
- Hypoautofluorescent retinal lesion (HP:0025159)
- Monochromacy (HP:0007803)
- Undetectable pattern electroretinogram (HP:0030844)

- Abnormal axonemal organization of respiratory motile cilia (HP:0012258)
- Absent central microtubular pair morphology of respiratory motile cilia (HP:0012264)
- Absent inner dynein arms (HP:0012257)
- Absent outer dynein arms (HP:0012256)
- Airway obstruction (HP:0006536)
- Atelectasis (HP:0100750)
- Bronchiectasis (HP:0002110)
- Chronic sinusitis (HP:0011109)
- High-frequency hearing impairment (HP:0005101)
- Otitis media with effusion (HP:0031353)

ERG Scotopic-rod, photopic-cone

ERG undetectable dark-adapted and abnormal light-adapted- rod-cone dystrophy.

ERG light-adapted is reduced or delayed- cone/cone-rod dystrophy.

ERG light-adapted is reduced or delayed and Dark-adapted involvement- cone-rod dystrophy.

European (non-Finnish)

European (Finnish)

Ashkenazi Jewish

Admixed American

South Asian

African/African American

East Asian

Middle Eastern

Amish

Remaining