Bi-allelic variants in the mitochondrial RNase P subunit PRORP cause mitochondrial tRNA processing defects and pleiotropic multisystem presentations

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Summary

Human mitochondrial RNase P (mt-RNase P) is responsible for the second step of processing mitochondrial tRNAs, a vital step in mitochondrial RNA maturation, and is comprised of three protein subunits: TRMT10C, SDR5C1 (HSD10), and PRORP. Pathogenic variants in TRMT10C and SDR5C1 are associated with distinct recessive or X-linked infantile onset disorders, resulting from defects in mitochondrial RNA processing. We report four unrelated families with multisystem disease associated with bi-allelic variants in PRORP, the metalloenzyme subunit of mt-RNase P. Affected individuals presented with variable phenotypes comprising sensorineural hearing loss, primary ovarian insufficiency, developmental delay, and brain white matter changes. Fibroblasts from affected individuals in two families demonstrated decreased steady state levels of PRORP, an accumulation of unprocessed mitochondrial transcripts, and decreased steady state levels of mitochondrial-encoded proteins, which were rescued by introduction of the wild-type PRORP cDNA. In mt-RNase P processing assays performed with recombinant mt-RNase P proteins, the disease-associated variants resulted in diminished mitochondrial tRNA processing. Identification of disease-causing variants in PRORP indicates that pathogenic variants in all three subunits of mt-RNase P can cause mitochondrial dysfunction, each with distinct pleiotropic clinical presentations.

Mitochondrial RNase P (mt-RNase P) is the endonuclease that processes the 5’ end of mitochondrial tRNAs and thereby also releases adjacent mRNAs and rRNAs from the polycistronic primary transcripts.1 In humans, the mt-RNase P complex is composed of three proteins, TRMT10C, SDR5C1 (HSD10), and PRORP (called MRPP1, MRPP2, and MRPP3, respectively), each encoded by the nuclear genome. Bi-allelic variants in TRMT10C (MIM: 615423) have been identified in two unrelated individuals with a lethal childhood multisystem disorder, characterized by muscle hypotonia, sensorineural hearing loss (SNHL), metabolic acidosis, and multiple oxidative phosphorylation (OXPHOS) deficiencies (MIM: 616974). SDR5C1 (also known as HSD10, HADH2, MRPP2, or ABAD [MIM: 300256]), encoded by the X chromosome gene HSD17B10, is a moonlighting protein with involvement in multiple biochemical pathways, including isoleucine metabolism.3,5 Pathogenic variants in HSD17B10

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https://doi.org/10.1016/j.ajhg.2021.10.002.
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cause HSD10 disease (MIM: 300438), manifesting in males as a severe, infantile-onset neurodegenerative condition with cardiomyopathy. Both disorders are characterized by defects of mitochondrial tRNA processing. PRORP (previously KIAA0391 [MIM: 609947]) encodes the endonuclease subunit of the mt-RNase P complex. Here, we describe four families with overlapping phenotypes resulting from bi-allelic variants in PRORP. All individuals or their guardians provided written informed consent to participate in the gene discovery study in accordance with local regulations (see supplemental information).

Affected individuals from two families, F1 and F2, presented with SNHL, which was accompanied in the affected females in F1 by primary ovarian insufficiency, consistent with a diagnosis of Perrault syndrome (MIM: 233400). In family F3, there was childhood onset of SNHL, lactic acidosis, and leukoencephalopathy, whereas affected individuals in family F4 presented with leukoencephalopathy. Recent reports of some individuals with variants in genes associated with Perrault syndrome have expanded the phenotypic spectrum to include presentations with childhood metabolic crises and leukoencephalopathy.

Family 1 (F1) is composed of three affected female siblings, two unaffected female siblings, two unaffected male siblings, and their unaffected parents (Figure 1A). At the last assessment the affected sisters were aged 30, 28, and 26 years of age. All three affected sisters presented in their late teenage years with primary
amenorrhea, consistent with a diagnosis of Perrault syndrome (see “GeneReviews” in web resources). Pelvic ultrasound noted the absence of ovarian tissue in all three sisters. Hormonal profiles indicated hypergonadotropic hypogonadism (Figure S1C) with otherwise normal endocrine and biochemical tests and a 46, XX karyotype. The affected sisters were prescribed estrogen to induce puberty and are currently maintained on hormone replacement therapy. Each affected sibling has mild non-progressive intellectual disability (brain MR imaging has not been performed). Echocardiography for each of the affected sisters was normal. All other physical and neurological examinations were normal.

A homozygous variant in PRORP, c.1454C>T (p.Ala485Val) (GenBank: NM_014672.3), was identified in the affected individuals of F1 via autozygosity mapping and whole-exome sequencing. The variant segregated with the phenotype in the family.

Family 2 (F2) comprises a male proband and his unaffected, unrelated parents (Figure 1B). The proband (F2, II-1) was born at 40 + 4 weeks by emergency caesarean section for fetal tachycardia and meconium stained liquor. Hearing loss in the proband was first noted at 3 years of age but formally diagnosed at 5 years, at which age his brain magnetic resonance imaging (MRI) was normal. He was 9 years at last assessment and had bilateral mild to moderate SNHL (Figure S1B). His speech and language skills are delayed as a result of the hearing loss and he wears bilateral hearing aids. No behavioral or neurological issues have been noted and cardiovascular, respiratory, and abdominal system examinations have been unremarkable. A maternally inherited c.1235A>G (p.Asn412Ser) variant and a paternally inherited c.1301C>A (p.Ala434Asp) variant in PRORP were identified in the proband from whole genome sequence data generated through the 100,000 Genomes Project (Figure 1B).12

In family 3 (F3), a male proband (F3, II-1) of non-consanguineous unaffected parents (Figure 1C) was born by emergency caesarean section for failure to progress at 41 + 2 weeks gestation. Examination shortly after birth found appendicular hypertonia, more pronounced on the left-hand side, and mild dysmorphism (mild hypertelorism, bilateral epicanthal folds, thin vermilion of the lips, and microretrognathia). At 7 months of age, plasma lactic acid levels were increased at 5.6 mmol/L (reference 0.5–2.5 mmol/L) despite normal levels of plasma amino acids, urine organic acids, acylcarnitines, and free and total carnitine. Urine analysis and serum creatinine were normal. Repeat testing at 24 months revealed plasma lactic acid levels still raised at 3.0 mmol/L. Severe feeding difficulties resulted in the insertion of a gastrostomy tube at 15 months. An electroencephalogram (photic stimulation included) at 19 months revealed no evidence of seizure activity. At 20 months, he had severe global developmental delay, diffuse asymmetric hypertonia, acquired microcephaly (head circumference: 45.7 cm, <2nd percentile), and a mild scoliosis. Brain MRI at 13 months revealed periventricular nodular heterotopias, a dysplastic corpus callosum, diffuse sub-cortical white matter loss, and bilateral connalatal cysts (Figure S2). Audiological tests were normal at 18 months, but at 3 years, an auditory brainstem response examination demonstrated evidence of auditory neuropathy spectrum disorder consistent with bilateral SNHL.

Microarray analysis performed on the proband (F3, II-1) detected no copy number variants. Whole-exome sequencing identified bi-allelic variants in PRORP, a maternally inherited missense variant c.1334G>A (p.Arg445Gln) (rs777185638) and a paternal frameshift variant c.1197dupA (p.Ser400Ilefs*6) (rs764714439), which was shown to result in nonsense-mediated decay of the transcript (figures 1E and 1F).

Family F4 is a family comprising three affected individuals, including a brother and sister and their affected mother (Figure 1D). The proband (F4, II-1) was 19 years of age at last assessment. He presented with a psychotic disorder, autistic traits, and learning disability at 7 years. At 8 years, he presented with brief generalized seizures consisting of loss of consciousness and generalized stiffening of the body and extremities. The EEG was normal, but he received treatment with levetiracetam with a good response. Recent physical examination showed obesity and genu and talus valgus. Fundoscopy displayed papillary pallor.

Brain MRI indicated bilateral multiple periventricular and subcortical T2 white matter hyperintense lesions with a posterior predominance that remain unchanged in successive controls (Figure S2). Spectroscopy was normal. Electromyogram and nerve conduction studies revealed no evidence of neuromuscular abnormalities. Ocular and auditory nerve response as assessed by visual evoked potential and auditory brainstem response were normal. Metabolic studies indicated increased lactate/pyruvate ratio with normal plasma lactate, plasma amino acids, and urine organic acids. The proband is currently treated with coenzyme Q10, vitamin B2, vitamin C, carnitine, and arginine.

The affected sister of the proband (F4, II-2) was aged 17 years at last assessment. F4, II-2 presented with intrauterine growth retardation, global developmental delay, and seizures in the first years of life. At the age of 15 years, she presented with tremor in her legs, migraines, and hyperglycemia. Lactate levels were normal. Her hearing is normal as are her electromyogram (EMG) and nerve conduction studies. Brain MRI displayed bilateral multiple periventricular and subcortical T2 white matter hyperintense lesions with a posterior predominance that remain unchanged in successive controls (Figure S2). Spectroscopy was normal. Like her brother, she is treated with coenzyme Q10, vitamin B2, vitamin C, and carnitine.

The mother (F4, I-2) of the proband presented with retrolubar optic neuritis and tonic pupil (a dilated pupil that responded slowly to light) at 39 years of age. Subsequently, she presented with asthenia, myalgias, memory...
## Table 1. Analysis of variants in PRORP in four families with distinct clinical presentations

| Family | F1 | F2 | F3 | F4 |
|--------|----|----|----|----|
| **Family details** | | | | |
| Family details | three affected female siblings | one affected male | one affected male | two affected siblings and their affected mother |
| Phenotype | Perrault syndrome | SNHL | developmental delay, SNHL, lactic acidosis, and white matter changes | white matter changes |

| Variant details | | | | |
| Variant | PRORP: c.1454C>T (p.Ala485Val) | PRORP: c.1235A>G (p.Asn412Ser) | PRORP: c.1301C>A (p.Ala434Asp) | PRORP: c.1334G>A (p.Arg445Gln) | PRORP: c.1197dupA (p.Ser400Ief56X6) | PRORP: c.1261C>T (p.Arg421Cys) |
| Location | Chr14(GRCh37): g.35739636C>T | Chr14(GRCh37): g.35649943A>G | Chr14(GRCh37): g.35735958C>A | Chr14(GRCh37): g.35735991G>A | Chr14(GRCh37): g.35649905dup | Chr14(GRCh37): g.35649969C>T |
| dbsNP | not present | rs148259590 | rs144536804 | rs777185638 | rs764714439 | rs147065101 |
| Zygosity | homozygous | heterozygous | heterozygous | heterozygous | heterozygous | homozygous |
| Inheritance | N/A | maternal | paternal | maternal | paternal | N/A |
| gnomAD MAF (count) | not present | 0.0001382 (39 heterozygotes, 0 homozygotes) | 0.0008385 (237 heterozygotes, 0 homozygotes) | 0.00002475 (7 heterozygotes, 0 homozygotes) | 0.00003593 (9 heterozygotes, 0 homozygotes) | 0.0001279 (36 heterozygotes, 0 homozygotes) |

| Prediction tools | | | | |
| SIFT | deleterious (0.0) | deleterious (0.0) | deleterious (0.03) | deleterious (0.0) | N/A | deleterious (0.03) |
| PolyPhen | probably damaging (1.0) | probably damaging (1.0) | benign (0.443) | probably damaging (1.0) | N/A | probably damaging (1.0) |
| MutationTaster | disease causing (1.0) | disease causing (1.0) | disease causing (0.811) | disease causing (1.0) | N/A | disease causing (1.0) |
| VarCards | 0.91 (extreme) | 0.74(extreme) | 0.39 | 0.83 (extreme) | N/A | 0.57 (extreme) |
| CADD | 34 | 26.4 | 28.2 | 34 | N/A | 35 |
| Conservation | highly conserved | highly conserved | moderately conserved | highly conserved | N/A | highly conserved |

(Continued on next page)
loss, and frequent headaches. She also had two episodes of left hemiparesis and hypoesthesia that resolved in 15 days without specific treatment and repetitive episodes of lower limb thrombophlebitis. Examination revealed afferent pupillary defect, nasal hemianopsia of the right visual field, and abnormal color perception. She also had distal weakness of the lower limbs, areflexia, and vibratory hypoesthesia of the left side of her body. Her hearing is normal, and she has no evidence of ovarian insufficiency. Routine blood tests and metabolic and thrombophilia studies were all normal. Specific genetic testing for metachromatic leukodystrophy, Krabbe disease, CADASIL, and Leber optic neuropathy were negative. Cerebrospinal fluid (CSF) oligoclonal bands and anti-MOG and anti-NMO antibodies were also negative. Visual evoked potentials displayed abnormal conduction in the right eye. EMG and nerve conduction studies were normal. Brain MRI indicated bilateral multiple periventricular and subcortical T2 white matter hyperintense lesions affecting both hemispheres, corpus callosum, pons, and cerebellum with no contrast enhancement and no changes in successive controls (Figure S2).

Whole-genome sequencing identified a homozygous c.1261C>T (p.Arg421Cys) PRORP variant in all three affected individuals. The father (F4, I-1) of the two affected children was a carrier for the variant, and subsequently, the family was confirmed to be consanguineous, consistent with the pseudo-dominant inheritance pattern. There was no evidence of any other putative disease-associated variants in the genome and exome datasets in the four families when the filtering steps were applied (supplementary information).

The altered residues in PRORP identified in the affected individuals from the four families are all highly conserved from vertebrates to fly (Figure S3). All of the variants are either absent from gnomAD or have a very low minor allele frequency and are not present as homozygous variants. All missense variants were predicted to be deleterious by multiple prediction software (Table 1). Of note, homozygous loss-of-function variants in PRORP are absent from publicly available databases and from a consanguineous cohort of >3,200 British Pakistani individuals.14

Mitochondrial tRNAs (mt-tRNAs) are processed at the 5’ end by mt-RNase P and at the 3’ end by mt-RNase Z (encoded by ELAC2 [MIM: 605367]).15,16 This tRNA cleavage also releases most of the RNA species from the polycistronic mitochondrial precursor transcripts according to the mitochondrial tRNA punctuation model.17,18 PRORP, as a subunit of mt-RNase P, catalyzes the Mg2+-dependent phosphodiester-bond cleavage of 5’ extensions of mitochondrial tRNAs.2,19 The processing of mitochondrial tRNAs proceeds in a stepwise manner and 5’ cleavage by mt-RNase P precedes tRNA 3’ end processing.1,18

We investigated the steady-state levels of the mt-RNase P subunits TRMT10C, SDR5C1, and PRORP in dermal fibroblasts available from affected individuals in families F1 and F3 by immunoblotting and detected a decrease in
PRORP levels in both affected individuals compared to controls (Figure 2A). The decrease in PRORP suggests that the variant p.Ala485Val is either less stable than the wild-type protein or downregulated in affected individuals from family F1. The decreased PRORP levels in F3, II-1 may partially result from absence of protein due to the allele that is subject to nonsense-mediated decay (Figures 1E and 1F). We also detected decreased steady-state levels of respiratory chain complex I (NDUFB8) and complex IV (COXI) subunits in fibroblasts from affected individuals compared to controls—both complexes contain mitochondrial DNA-encoded subunits. There was no change of other OXPHOS components, most notably complex II, which is entirely nuclear encoded (Figure 2B). This profile is consistent with a generalized defect in mitochondrial translation. The decreased stability of complex I and IV subunits was more severe in subject F3, II-1, consistent with his more severe clinical phenotype. There was no noticeable difference in levels of several mitoribosomal proteins (MRPs) between the fibroblasts from affected individuals and controls (Figure 2C), indicating that any effect on translation most likely reflects a defect of transcript processing rather than a defect in the stability or assembly of the mitoribosome itself.

To determine the status of mitochondrial-encoded RNA transcripts in subject dermal fibroblasts, Northern blots were performed. We designed biotinylated strand-specific probes to detect transcripts from four different regions of the mitochondrial genome. An MT-ND1 probe revealed the accumulation of a precursor RNA of approximately 2.5 kb in the samples from F1, II-4 and F3, II-1 (C1 and C2) (Figure 3), corresponding to unprocessed 16S rRNA-tRNALeu(UUR)-ND1 mRNA and apparently resulting from impaired 5' end processing of mt-tRNA^{Leu(UUR)}^{16S}. This RNA species was previously termed RNA 19 and observed to be upregulated by the 3243A>G MELAS and other variants in mt-tRNA^{Leu(UUR)}^{16S}. A larger RNA species was detected on a longer exposure for the MT-ND1 probe, indicating that mt-tRNA{Val} processing is also decreased. The MT-ND2 and MT-CO2 probes both detected multiple RNA species seen in affected individuals but not control samples. A MT-ND6 probe detected a mitochondrial light strand transcript of approximately 2.3 kb in the F1, II-4 and F3, II-1 samples, which can be explained by impaired 5' processing of mt-tRNA^{Glu} (Figure 3). The presence of multiple large transcripts in the samples from the affected individuals, not seen in the control samples at the same intensity, indicates a deficiency in 5' processing across multiple mt-tRNA sites.

All disease-associated variants are located in the metallo-nuclease domain of PRORP (Figure S4A), as revealed by its crystal structure. Residue Ala485 is situated close to four conserved aspartate residues implicated in metal-ion binding (Figure S4B). Replacing the conserved alanine at residue 485 with the bulkier valine (F1) could distort the active site and impair catalysis by interfering with proper coordination of the metal ions, thereby reducing the endonucleolytic activity of PRORP. Residue Ala434 is typically expressed and the substitution Ala434Asp (F2) is predicted to slightly reduce the stability of PRORP but no structural change to the protein (Figure S4C). Residue Asn412 is located in the active site next to catalytic residue Asp409 (Figure S4D). Substitution Asn412Ser (also F2) results in no drastic structural changes but may interfere with the shape of the active site, thereby reducing the endonucleolytic activity of PRORP. Residue Arg445 forms stabilizing interactions with essential catalytic residues (e.g., Asp479, Asp478) (Figure S4E). It is likely that Arg445Gln (F3) would directly impact nuclease activity. In 150
orthologs, the residue equivalent to Arg445 is invariably Arg (data not shown). The amino acid at residue 421 is highly variable and is disordered in the structure. During the review of this manuscript, the cryo-EM structure of PRORP in complex with tRNA, TRMT10C, and SDR5C1 was determined. In addition to supporting the above interpretations of disease-associated variants, this structure reveals that PRORP residue Arg445 forms an interaction with the 5’ end of the tRNA substrate, which will be broken by Arg445Gln (F3). Additionally, Arg421 becomes ordered in the context of the complex and forms stabilizing interactions with residue Glu429, which will be disrupted by Arg421Cys (F4). We investigated whether the disease-associated variants in PRORP affected the catalytic activity of the mt-RNase P complex. The three mt-RNase P complex wild-type proteins (TRMT10C, SDR5C1, and PRORP), and the PRORP variant proteins, were individually produced by recombinant expression in bacteria and purified. Recombinant mt-RNase P was reconstituted in vitro and 5’ leader processing monitored with fluorescent labeled mt-pre-tRNA. All the amino acid variants in PRORP led to a reduction in mt-tRNA cleavage product compared to wild-type PRORP (Figure 4A). The fluorescence intensity of the mt-tRNA cleavage product was quantified. Upon 15 min from the start of the reaction, the mt-RNase P complexes with variants PRORP p.Arg445Gln (F3) and p.Asn412Ser (F2) displayed the most dramatic decreases in mt-tRNA cleavage products compared to wild-type of approximately 76% and 87%, respectively. The mt-RNase P complexes with variants PRORP p.Ala485Val (F1), p.Ala434Asp (F2), and p.Arg421Cys (F4) reduced 5’ leader processing by approximately 19%, 15%, and 10%, respectively. The reductions in processing persisted after 60 min (Table 1). These data indicate that disease-associated PRORP variants reduce the RNase P activity of the complex in vitro. Of note, the greatest reduction of activity is seen in the mitochondria of efferent synapses but possibly also in mitochondria of efferent synapses of the inner hair cells, indicating possible importance for synapatic functions after the onset of hearing.

We performed rescue experiments to establish whether expression of wild-type PRORP could reduce the accumulation of unprocessed transcripts in the fibroblasts from an affected individual. Fibroblasts from individual F3, II-1 were transduced with a retroviral vector containing the wild-type PRORP cDNA or wild-type TRMT10C cDNA as a control. Expression of wild-type PRORP restored both the amount of PRORP and MT-CO1 protein, whereas TRMT10C and SDHA levels were unaffected (Figure 4B). This result indicates that increased levels of wild-type PRORP in the cells from the affected individual enhances the steady state levels of mitochondrial-encoded MT-CO1 but does not affect levels of nuclear-encoded SDHA. This effect is not seen with the empty vector or TRMT10C (Figure 4C). Transducing fibroblasts from the affected individual with PRORP also decreased the levels of unprocessed mitochondrial transcripts in the cells to near wild-type levels (Figure 4B). Again, this effect is not seen with the empty vector or the vector containing the coding sequence for TRMT10C. Taken together, these data indicate that the expression of wild-type PRORP in cells from an affected individual can rescue the molecular defects.

We undertook localization studies of PRORP in the mouse organ of Corti to understand why variants in PRORP may be associated with hearing loss (Figure S5). After the onset of hearing, which occurs in mice at postnatal day 12, PRORP is detected around the afferent and efferent synapses of the inner hair cells and the efferent synapses of the outer hair cells, indicating possible importance for synaptic functions after the onset of hearing.

PRORP partially co-localizes with a synaptic marker (SNAP25), indicating that it may not only be present in the mitochondria of efferent synapses but possibly also...
mitochondria of afferent synapses and nerve fibers around the inner hair cells. The pattern of PRORP staining does not entirely co-localize with mitochondrial marker TOM20. This lack of complete co-localization with TOM20 suggests that the high levels of PRORP found in a subset of mitochondria associated with the synapses and neurons of the organ of Corti hair cells reflect the increased demand for mitochondrial tRNA processing and translation in these cells, which may be a characteristic of a particular type of mitochondria at these locations.25–27

In summary, we present genetic and functional evidence that bi-allelic variants in PRORP are associated with pleiotropic clinical presentations and that PRORP should be considered another gene associated with the Perrault syndrome clinical spectrum. Such variability in clinical
presentation is not uncommon for mitochondrial disorders and is increasingly being shown for genes associated with Perrault syndrome.9 Bi-allelic hypomorphic variants in CLPP, for example, are associated with Perrault syndrome,28 whereas more deleterious variants result in a more severe phenotype associated with SNHL, seizures, and brain white matter changes.11 The clinical spectrum observed in individuals with different bi-allelic PRORP variants is consistent with this phenotypic range and most likely reflects altered mitochondrial dysfunction in different tissues at different time points. It is important to note that PRORP is ubiquitously expressed in the GTEx dataset. This is consistent with many disorders of mitochondrial function, which have specific clinical phenotypes despite these expression profiles. Notably in the families with multiple affected individuals (F1 and F4), the phenotypes were consistent, indicating that certain PRORP variants may result in specific phenotypes.

Similar OXPHOS defects to those seen in individuals with PRORP variants have been observed in individuals with pathogenic variants in the mt-RNase P genes TRMT10C and HSD17B10,29 suggesting a common pathogenic mechanism in these disorders. Despite the similarities in defective mitochondrial tRNA processing, variants in the three subunits of mt-RNase P result in different clinical phenotypes. With our work, we demonstrate that bi-allelic variants in PRORP result in mitochondrial dysfunction and that all three subunits of mitochondrial RNase P have now been associated with mitochondrial disease, each with distinct pleiotropic clinical presentations.

Data and code availability

The PRORP variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (GenBank: NM_014672.4; accession numbers SCV001943322–SCV001943327). The exome and genome datasets supporting this study have not been deposited in a public repository because of ethical restriction but are available from the corresponding author on request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2021.10.002.

Acknowledgments

We would like to thank the families for their participation. Family F2 was ascertained via the 100,000 Genomes Project.12 Families F3 and F4 were identified via GeneMatcher.20 Further funding details are available in the supplemental information.

Declaration of interests

The authors declare no competing interests.

Received: August 24, 2021
Accepted: October 7, 2021
Published: October 28, 2021

Web resources

dbSNP, https://www.ncbi.nlm.nih.gov/projects/SNP/
Exome Variant Server, https://evs.gs.washington.edu/EVS/
FoldX, http://foldxsuite.crg.eu/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
GeneMatcher, https://genematcher.org/
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LOVD, https://www.lovd.nl/
MutationTaster, http://www.mutationtaster.org/
OMIM, https://www.omim.org/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, https://sift.bii.a-star.edu.sg/

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