Appendix: Examples of How to Describe Evidence for Variant Classification on Clinical Reports

A. Example of a table to report the structured elements of a variant

Gene Transcript	**Location**	**Variant**	**Zygosity**	**Classification**	**Disease**	**Inheritance**	**Parental Origin**
YY NM_12345.6	Exon X/ Intron X/ Promoter/ UTR	c.XXX (p.XXX)	(Apparently) homozygous/ Heterozygous/ Hemizygous/ Heteroplasmic/ Homoplasmic/ Mosaic/ Somatic	Pathogenic / Likely pathogenic / Uncertain significance	Disease Z	Autosomal recessive/ Autosomal dominant/ X-linked/ Mitochondrial	Paternal/ Maternal/ De novo/ Unknown

B. Example of a Pathogenic Variant

The p.XXX pathogenic variant in the *YY* gene has been reported previously in patients with Disease Z (ref1, ref2, ref3). Eight affected individuals (between the 3 publications) were homozygous for the variant in two families. In one family, this variant was found in *trans* with another previously established pathogenic variant in this gene. A well-established enzyme assay performed on the patient's blood sample showed decreased enzyme activity. The NHLBI Exome Sequencing Project and the 1000 Genomes Project each report that p.XXX was observed in 5/8598 and 3/2100 alleles, respectively, a carrier frequency consistent with the frequency of Disease Z . Multiple lines of computational evidence predict this variant is probably damaging to the protein structure, function, or protein-protein interaction. In particular, the p.XXX variant is a non-conservative amino acid substitution at a position that is conserved in all members of the YY protein family and conserved down to lower species (zebrafish) and is located near the hinge region between the YY N-terminal domain and the catalytic domain. In addition, this individual has a collection of clinical features that are highly specific for Disease Z and for which pathogenic variants in the *YY* gene are the only known cause, providing a higher probability that any variant identified would be pathogenic. In summary, this collective evidence supports p.XXX as a recessive pathogenic variant for Disease Z.

C. Example of a Likely Pathogenic Variant

The p.XXX variant in the *YY* gene has not been published to our knowledge but was observed as a *de novo* occurrence in this individual whose phenotype matches Disease Z described for this gene. p.XXX was not observed in approximately 6,500 individuals of European and African American ancestry in the NHLBI Exome Sequencing Project . The p.XXX variant is a conservative amino acid substitution, which occurs at a position that is conserved across species, though in silico analysis tools are inconsistent in predicting whether or not the variant may damage protein function. Many missense pathogenic variants in nearby residues in the *YY* gene have been reported in association with disease Z (ref1), supporting the

functional importance of this region of the protein. p.XXX is therefore interpreted to be likely pathogenic for disease Z and act in a dominant manner.

D. Example of a Variant of Uncertain Significance

The maternally-inherited p.XXX variant in the YY gene has not been published, to our knowledge. p.XXX is a conservative amino acid substitution that occurs at a residue that is conserved in mammalian species. In silico analysis predicts this variant is probably damaging to the protein structure/function. The p.XXX variant was not observed in approximately 6,500 individuals of European and African American ancestry in the NHLBI Exome Sequencing Project. We interpret p.XXX in *YY* gene as a variant of uncertain significance with respect to disease Z.

E. Example of a Likely Benign Variant

The p.XXX variant in the *YY* gene has been observed in 9/2100 alleles in the 1000 Genomes database. This allele frequency would be higher than that expected, based on the prevalence of disease Z in the population. In silico analysis predicts a benign impact on the resultant protein. Missense changes are relatively rare in gene *YY*, as most variants result in loss of function. In addition, an alternate cause of disease was identified that is likely the cause of disease in this individual. Based on this information, the variant is likely benign with respect to disease Z.

F. Example of a Benign Variant

The p.XXX variant found in the proband with disease Z has been observed in the 1000 Genomes database at a frequency of 2.3%. The similarly-affected brother of the proband was found to not have the p.XXX variant. In silico analysis predicts a benign impact on the resultant protein. The p.XXX is interpreted to be a benign variant with respect the disease Z.