Supplementary Table SVIII **Recommendations for providing evidence for gene–disease relationships.**

Topic	Recommendation
Population frequency	Public databases can be used with care. Please note that public databases contain female samples and infertile men. Local databases and/or healthy controls are preferred if available. Examples of public databases: • GnomAD: http://gnomad.broadinstitute.org/ • Exome Variant Server: http://evs.gs.washington.edu/EVS/ • dbSNP: https://www.ncbi.nlm.nih.gov/SNP/
Segregation analysis	Testing of parents and other family members preferred to establish if identified variant segregates with disease or occurred *de novo*
Recurrence in affected individuals	Other patients with similar phenotypes should be tested for variants in same gene. If no patients are available, collaborative efforts and matchmaking services are recommended. Example of matchmaking service: Matchmaker Exchange: https://www.matchmakerexchange.org/
Statistical evidence	Gene burden analysis can be used to proof that a gene shows a statistical excess of rare variants in a patient cohort compared to control cohorts.
In silico prediction	Prediction models may be used to filter variants that are likely to be deleterious.Examples for missense variants: • SIFT: http://sift.jcvi.org/ • MutationTaster: http://www.mutationtaster.org/ • PolyPhen2: http://genetics.bwh.harvard.edu/pph2/ Examples for splice variants: • GeneSplicer: http://ccb.jhu.edu/software/genesplicer/ • Human Splicing Finder: http://www.umd.be/HSF3/ • MaxEntScan:http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
Gene expression	Report if the newly identified gene is expressed in relevant tissue(s). Expression can be studied in patient/healthy control material or through public databases. Examples of public databases: Protein Atlas: https://www.proteinatlas.org/ Expression Atlas: https://www.ebi.ac.uk/gxa/home
Protein interaction	Report if newly identified gene interacts with known disease genes. Interaction can be studied *in vitro* or through public databases. Example of public database: STRING: https://string-db.org/
Functional validation	Prove disruptive nature of identified sequencing variant in patient cells or in an *in vitro* model
In vivo evidence	The phenotype of an animal model recapitulates the human phenotype. Preferably rescue experiments are performed.