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TITLE:

Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the Clinical Genome Resource

AUTHORS:

Natasha T. Strande[1,2], Erin Rooney Riggs[1,3], Adam H. Buchanan[4], Ozge Ceyhan-Birsoy[7-10], Marina DiStefano[7], Selina S. Dwight[5], Jenny Goldstein[2], Rajarshi Ghosh[6], Bryce A. Seifert[2], Tam P. Sneddon[5], Matt W. Wright[5], Laura V. Milko[2], J. Michael Cherry[5], Monica A. Giovanni[4], Michael F. Murray[4], Julianne M. O'Daniel[2], Erin M. Ramos[11], Avni B. Santani[12,13], Alan F. Scott[14], Sharon E. Plon[6], Heidi L. Rehm[7-10], Christa L. Martin*[3,4], Jonathan S. Berg*[2]

AFFILIATIONS:

[1] These authors contributed equally to this work

[2] Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

[3] Autism & Developmental Medicine Institute, Geisinger Health System, Danville, Pennsylvania, USA.

[4] Genomic Medicine Institute, Geisinger Health System, Danville, Pennsylvania, USA.

[5] Department of Genetics, Stanford University, Stanford, California 94305, USA.

[6] Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA.

[7] Laboratory for Molecular Medicine, Partners Personalized Medicine, Boston, Massachusetts, USA.

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[8] The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.

[9] Harvard Medical School, Boston, Massachusetts, USA.

[10] Department of Pathology, Brigham & Women's Hospital, Boston, Massachusetts.

[11]National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA.

[12]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.

[13]Division of Genomic Diagnostics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA.

[14]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

CORRESPONDING AUTHORS*: Jonathan S. Berg (jonathan_berg@med.unc.edu) and Christa L. Martin (clmartin1@geisinger.edu)

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ABSTRACT

With advances in genomic sequencing technology, the number of reported gene-disease relationships has rapidly expanded. However, the evidence supporting these claims varies widely, confounding accurate evaluation of genomic variation in a clinical setting. Despite the critical need to differentiate clinically valid relationships from less well-substantiated relationships, standard guidelines for such evaluation do not currently exist. The NIH-funded Clinical Genome Resource (ClinGen) has developed a framework to define and evaluate the clinical validity of gene-disease pairs across a variety of Mendelian disorders. In this manuscript we describe a proposed framework to evaluate relevant genetic and experimental evidence supporting or contradicting a gene-disease relationship, and the subsequent validation of this framework using a set of representative gene-disease pairs. The framework provides a semi-quantitative measurement for the strength of evidence of a gene-disease relationship which correlates to a qualitative classification: “Definitive”, “Strong”, “Moderate”, “Limited”, “No Reported Evidence” or “Conflicting Evidence.” Within the ClinGen structure, classifications derived using this framework are reviewed and confirmed or adjusted based on clinical expertise of appropriate disease experts. Detailed guidance for utilizing this framework and access to the curation interface is available on our website. This evidence-based, systematic method to assess the strength of gene-disease relationships will facilitate more knowledgeable utilization of genomic variants in clinical and research settings.

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INTRODUCTION

The human genome comprises approximately 20,000 protein-coding genes[1], of which about 3,000 have been reported in association with at least one Mendelian disease[2]. Roughly half[2] of these gene-disease relationships have been identified over the last decade, as technological advances have made it possible to use sequence information from small families or even single individuals to discover new candidate gene-disease relationships[3; 4]. However, there is substantial variability in the level of evidence supporting these claims, and a systematic method for curating and assessing evidence is needed.

Despite this variability, clinical laboratories may include genes with preliminary evidence of a gene-disease relationship on disease-targeted panels, or in results returned from exome/genome sequencing. Some of the gene-disease relationships are either unable to be confirmed for many years or are ultimately proven wrong[5]. Evaluating the clinical impact of variants identified in genes with an unclear role in disease is exceedingly difficult, and could lead to incorrect diagnoses, preventing further evaluations and/or resulting in errant management of the affected individual and their families. This scenario highlights the need for a standardized method to evaluate the evidence implicating a gene in disease and thereby determine the clinical validity[3] of a gene-disease relationship.

The NIH-funded Clinical Genome Resource (ClinGen)[6] is creating an open-access resource to better define clinically relevant genes and variants based on standardized, transparent evidence assessment for use in precision medicine and research. Our

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group has developed a method that 1) qualitatively defines gene-disease clinical validity using a classification scheme based on the strength of evidence supporting the relationship, and 2) provides a standardized semi-quantitative approach to evaluate available evidence and arrive at such a classification. Currently, this framework is optimized for genes associated with monogenic disorders following autosomal dominant, autosomal recessive, or X-linked inheritance. Future iterations will expand the framework to consider other modes of inheritance, such as mitochondrial, and diseases with more complex genomic etiologies, including oligogenic or multifactorial conditions. Our approach is neither intended to define multifactorial disease risk, nor to be a substitute for well-established statistical thresholds used for genome-wide association studies[7; 8].

This novel framework classifies gene-disease relationships by the quantity and quality of the evidence supporting such a relationship. It builds on efforts to catalog gene-disease associations, such as the Online Mendelian Inheritance in Man (OMIM)[1] and OrphaNet, by systematically organizing the supporting and refuting evidence, and categorizing the strength of evidence supporting these relationships. The resulting clinical validity classifications are valuable to both clinicians and clinical laboratories. First, they provide insight into the strength of clinical associations for clinicians interpreting genetic test results for clinical care. Second, they serve to guide clinical genetic testing laboratories as they develop disease-specific clinical genetic testing panels or interpret genome-scale sequencing tests. By including only those genes with established clinical validity, the possibility of returning ambiguous, incorrect, or uninformative results is reduced, improving the quality of interpretation of genomic data.

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QUALITATIVE DESCRIPTION: CLINICAL VALIDITY CLASSIFICATIONS

The ClinGen Gene Curation Working Group (GCWG) is comprised of medical geneticists, clinical laboratory diagnosticians, genetic counselors, and biocurators with broad experience in both clinical and laboratory genetics. Over the course of three years, this group convened bi-monthly to develop the described framework for assessing gene-disease clinical validity through expert opinion and working group consensus (additional details provided on the ClinGen website). We first defined six classes to qualitatively describe the strength of evidence supporting a gene-disease association (Figure 1). The amount and type of evidence required for each clinical validity classification builds upon that of the previous classification level. Evidence used within this framework to assign a classification to a gene-disease pair is divided into two main types: genetic evidence and experimental evidence (described below). As evidence is likely to change over time, any given classification is only representative of the level of evidence at the time of curation.

The classification “No Reported Evidence” is used for genes that have not yet been asserted to have a causal relationship with a human monogenic disorder, but may have some experimental data (e.g., model system data) suggesting a potential role for that gene in disease. The “Limited” classification requires at least one variant, asserted to be disease-causing, to have plausible genetic evidence to support the association with human disease with or without gene-level experimental data. “Moderate” classification encompasses additional clinical evidence (e.g. multiple unrelated probands harboring variants with potential roles in disease) and supporting experimental evidence, all of which may be provided by multiple studies or a single robust study. Replication of the

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gene-disease association in subsequent independent publications and additional substantial genetic and experimental data are critical factors for the "Strong" classification. Finally, the hallmark of a "Definitive" gene-disease association is that, in addition to the accumulation of convincing genetic and experimental evidence, the relationship has been replicated, and ample time has passed since the initial publication (in general, greater than three years) for any conflicting evidence to emerge. It is important to highlight that these classifications do not reflect the effect size or relative risk attributable to variants in a particular gene, but instead the strength of the evidence. For example, a definitive gene-disease association does not imply that a pathogenic variant in that gene confers 100% penetrance of the phenotype. This metric is not intended to assess the penetrance or risk to develop a disease outcome.

A gene-disease relationship can be determined to have one of the above classifications provided no substantial relevant and valid contradictory evidence exists to call the gene-disease relationship into question. If such evidence emerges, then the relationship is described as "Conflicting Evidence Reported." Types of contradictory evidence may come from population studies (such as ExAC[9]), attempts to experimentally validate the gene-disease association, or re-analysis of the original family or cohort that was previously studied. Although the role of a specific *variant* in a given disease may be called into question by new evidence, this may not be sufficient to invalidate the role of the *gene* in that disease. Thorough evaluation by experts in the particular disease area is recommended to determine whether the contradictory evidence outweighs the existing supportive evidence to classify a gene into either a "Disputed" or "Refuted" category (see Figure 1 for additional details).

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METHODS: SEMI-QUANTITATIVE ASSESSMENT OF EVIDENCE

Assigning a clinical validity classification to a gene-disease pair requires assessment of the evidence supporting the association. We developed a semi-quantitative approach to evaluate both genetic (Figure 2) and experimental evidence (Figure 3) in a standardized manner that promotes consistent collection and weighting of evidence (a detailed standard operating procedure is available on the ClinGen website). Defined sub-categories of genetic and experimental evidence are given a suggested default "score." However, given that evidence of the same general type may vary in its strength (particularly when considering different diseases), the scoring system also allows these scores to be adjusted within a set range of points, with final approval by experts within the particular disease domain. Finally, the maximum number of points allowed for the various types of genetic and experimental evidence is capped to prevent a preponderance of weak evidence from inappropriately inflating the gene-disease classification. Similarly, certain evidence categories are provided higher maximum scores, allowing key pieces of stronger evidence to proportionately influence the classification of a gene-disease pair.

Genetic Evidence

For the purposes of scoring, genetic evidence is divided into two categories: case-level data and case-control data (Figure 2). Studies describing individuals or families with genetic variants are scored as case-level data, while studies using statistical analyses to compare variants in cases and controls are scored as case-control data. When case-level and case-control data are present in a single publication, points can be assigned in

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each category, but the same piece of evidence should not be counted more than once. For example, an individual case that is also included within a case-control cohort should not be given points in both the “case-level data” and “case-control data” categories. In this scenario, points should be assigned to the most compelling and informative evidence.

Assessing case-level data requires consideration of the inheritance pattern and evaluation of the individual variants identified in each case. Within this framework, a case should only be counted towards supporting evidence if the reported variant has some indication of a potential role in disease (e.g., impact on gene function, recurrence in affected individuals, etc.), does not have evidence that would contradict pathogenicity (e.g., population allele frequency), and is of the type consistent with the assumed disease mechanism (e.g. truncating variant for loss of function). Unless otherwise noted, the term “qualifying variant” implies that these criteria are met. In addition, points are assigned separately for segregation data to reflect the statistical probability that the locus is implicated in the disease. Figure 2 and Figure S1 provide guidance on the number of points that should be considered for segregation evidence by LOD score; if a LOD score is not provided within the publication being evaluated, an estimated LOD score may be calculated in certain scenarios, as described in the standard operating procedure document provided on the ClinGen website.

Each study categorized as “case-control data” should be independently assessed to evaluate the quality of the study design (see Figure 2). Consultation with a clinical domain expert group (such as those affiliated with ClinGen, https://www.clinicalgenome.org/working-groups/clinical-domain/) is recommended. For

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the purposes of this framework, studies are classified based on whether they include single variant analysis or aggregate variant analysis. Single variant analyses are those in which individual variants are evaluated for statistical enrichment in cases compared to controls. More than one variant may be analyzed, but the variants have been independently assessed with appropriate statistical correction for multiple testing. Aggregate variant analyses are those in which the total number of variants is assessed for enrichment in cases compared with controls. This comparison is typically accomplished by sequencing the entire gene in both cases and controls and demonstrating an increased "burden" of variants of one or more types.

Experimental Evidence

The experimental data scoring system is presented in Figure 3. The gene-level experimental data used in this framework to assess a gene-disease association are consistent with those proposed by MacArthur and colleagues to implicate a gene in disease[10]. The following experimental evidence types are used: biochemical function, experimental protein interactions, expression, functional alteration, phenotypic rescue and model systems (Figure 3 bottom panel). These categories capture the most relevant types of experimental information necessary to determine whether the function of the gene product is at least consistent with the disease with which it is associated, if not causally implicated.

Contradictory Evidence

While curators are encouraged to seek out and document (via qualitative description) conflicting evidence, no specific points are assigned to this category. The types of valid

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contradictory evidence and their relative weights will be unique to each gene-disease pair, and it would be misleading to attempt to uniformly quantify this type of negative evidence against the reported positive evidence. If there is substantial conflicting evidence, manual review and expert input is required to evaluate the strength of the contradictory evidence, determine whether it outweighs any available supporting evidence, and, if so, decide whether the gene-disease association should be classified as “Disputed” or “Refuted”.

Summary & Final Matrix

The scores assigned to both genetic and experimental evidence are tallied to generate a total score (ranging from 1-18) that corresponds to a preliminary clinical validity classification (Figure 4). The system provides a transparent method for summarizing and assessing all curated evidence for a gene-disease pair, encouraging consistency between curators. While the summary matrix facilitates a preliminary assessment of the gene-disease relationship, the initial curator or expert reviewer may adjust the classification, supplying a specific rationale for the change. Final classifications are determined in collaboration with disease experts, who review the preliminary classification and supporting evidence and work to come to a consensus with the preliminary curators. In the event that the disease experts and preliminary curators disagree on a final classification, a senior member of the ClinGen Gene Curation Working Group may be brought in to facilitate a final classification, erring towards the more conservative classification if consensus cannot be achieved. It should be noted that experimental data alone cannot justify a clinical validity classification beyond “No Reported Evidence,” and at least one human genetic variant with a plausible causal

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association must be present to attain "Limited" classification. The difference between "Limited," "Moderate," and "Strong" gene-disease classifications is justified by the quality and quantity of evidence; it is expected that valid gene-disease associations will gradually accumulate enough supporting evidence and be replicated over time to attain a "definitive" classification. This framework relies predominantly on evidence obtained from published primary literature, identified through resources such as PubMed and OMIM[1], and independently assessed by curators; however, if necessary, unpublished information available from publicly accessible resources, such as variant databases[11; 12], may be used as long as some supporting evidence is provided.

RESULTS: VALIDATION OF METHOD

Using this framework we evaluated 33 gene-disease pairs representing a variety of disease domains and spanning the spectrum of clinical validity classifications (see Table 1, Figure 5, and Supplemental Appendix). To assess the reproducibility of our scoring metric, each gene-disease pair was evaluated by two independent curators; paired curators reached concordant clinical validity classifications in 29 of the 31 (93.5%) gene-disease pairs with available published evidence (Figure 5; associations classified as "No Reported Evidence" were excluded). Each gene-disease pair was subsequently reviewed by clinical domain experts; experts agreed with the preliminary classifications for 87.1% (27/31) of the gene-disease pairs with published evidence (Figure 5). The four discrepancies between the expert and curator classifications were each different by only a single category (e.g. limited versus moderate). Of note, the original classifications for *HNRNPK* (MIM 600712) and *SMARCA1* (MIM 300012) were

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at the border between limited and moderate (6.5 points); in each case, the preliminary curators' lack of specific clinical expertise led to uncertainty regarding the scoring of evidence requiring such knowledge. Consulting with clinical experts in the disease resolved these issues resulting in both genes being upgraded to moderate. In the case of *WRAP53* (MIM 612661), the expert was aware of additional published experimental evidence that when included increased the classification from limited to moderate. Upon reviewing the curated evidence for *RAD51D* (MIM 602954) and breast cancer (MIM 614291), the domain expert upgraded the classification from disputed to limited (with the approval of the GCWG) due to the specificity of the experimental evidence and insufficient power of the current studies to rule out a role for *RAD51D* in breast cancer (Figure 5). Details and references for each curation are provided in Supplemental Appendix.

DISCUSSION

The evidence-based framework described here qualitatively defines clinical validity classifications for gene-disease associations in monogenic conditions and provides a systematic framework for evaluating key criteria required for these classifications. This method is intentionally flexible to accommodate curation of a wide spectrum of genes and conditions by curators with varying levels of expertise. The semi-quantitative scoring system combined with the qualitative classification scheme guides curators through the preliminary decision-making process, while the expert-level review provides disease-specific experience to weigh in on the final classification.

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This effort to create a generalized framework may result in some specific challenges due to the heterogeneity of genetic conditions, in both phenotype and prevalence. For example, conditions that span a large phenotypic spectrum may pose a challenge when defining what constitutes a condition and what is most relevant for curation purposes. In general, ClinGen encourages its expert curation groups to focus on disease associations that have been asserted in the literature or in other authoritative sources (e.g. OMIM, Orphanet Disease Ontology). Expert reviewers may find it useful in certain scenarios, to curate both a syndromic disease association as well as an isolated/non-syndromic disease association limited to a particular sub-phenotype. For example, when a disease entity encompasses sub-phenotypes that are caused by different mutational mechanisms. This is a topic of continued discourse within the ClinGen working groups and will be incorporated into future manuscripts that will focus on the curation approach for individual ClinGen disease-focused expert groups.

Ultra-rare disorders may have a relatively small number of probands described in the medical literature, thus limiting their potential to achieve a high genetic evidence score within this matrix. This obstacle is mostly circumvented by allowing compelling pieces of genetic evidence to score the maximum number of points (for example, see *CD3E* (MIM 186830) and severe combined immunodeficiency (MIM 615615), detailed in the Supplemental Appendix). When substantial experimental evidence is also available, these conditions can attain a “Strong” or “Definitive” classification. On the opposite end of the spectrum are conditions that occur commonly in the general population, such as cancer, where the predominant etiology is multifactorial rather than monogenic. In the less common Mendelian cancer predisposition syndromes, incomplete penetrance is a

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typical feature that can lead to confounding factors in family genetic studies such as apparently non-penetrant family members who carry a disease-associated variant and phenocopies among family members without a disease-associated variant. For such conditions, case-control data may provide more compelling evidence to support the gene-disease association (see the curation of *PALB2* (MIM 610355) and hereditary breast cancer (MIM 114480) in the Supplemental Appendix as an example).

One limitation of any such system is the challenge of balancing thorough literature curation and practical time commitment. This system can accommodate an exhaustive literature review, but in most cases will only require curating the amount of information sufficient to reach the maximum number of points in the matrix. In some scenarios this method may fail to include pertinent information, which could impact the classification (e.g., omission of contradictory evidence). Another potential limitation is the subjective nature of certain evidence types (e.g., experimental), which may lead to variability between different groups assessing evidence. However, due to the transparency of the evidence base, the incorporation of expert review, and the ability to reassess classifications over time, such drawbacks are likely to be self-limiting.

ClinGen's ultimate goal is to enhance the incorporation of genomic information into clinical care, an important component of the Precision Medicine Initiative[13]. The implementation of this framework will be supported by an open-access ClinGen curation interface (under development) that will guide curators through the curation process and will serve as a platform for extension to the community. In essence, this framework aims to provide a systematic, transparent method to evaluate a gene-disease relationship in an efficient and consistent manner suitable for a diverse set of users. A detailed

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standard operating procedure for this framework is available on the ClinGen website. All curated evidence, including clinical validity assessments, will also be made readily accessible to clinical laboratories, clinicians, researchers, and the community via our website. Additionally, for community members that wish to contribute papers of interest and/or request curation of a gene-disease pair, a “reporter” form is available on the ClinGen website.

Carefully evaluated gene-disease clinical validity classifications, as provided by this framework, will be useful to clinical laboratories as they evaluate genes for inclusion on disease-targeted panels, or as they decide how to categorize, prioritize, and return results from exome/genome sequencing. Clinicians may choose to use these types of gene-disease classifications as they interpret laboratory results for the individuals they care for; for instance, they may choose not to adjust medical management based on variants in genes of limited clinical validity. Researchers could also utilize this framework to evaluate the clinical validity of their own newly discovered associations and identify promising target genes for future work in order to augment the currently available evidence and attain a “Strong” or “Definitive” classification. In addition, professional societies and regulatory bodies may utilize these clinical validity assessments when making recommendations or guidelines for clinical genetic testing. Ultimately, our systematic, evidence-based method for evaluating gene-disease associations will provide a strong foundation for genomic medicine.

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DESCRIPTION OF SUPPLEMENTAL DATA:

The Supplemental file includes one figure, an appendix with curated evidence for each example presented in Figure 5, and a list of references. A more comprehensive supplemental file is available on the BioRxIV preprint server (see doi: https://doi.org/10.1101/111039)

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like to thank Scott Goehringer for his invaluable help in preparing the curated examples for the ClinGen website and appendix.

WEB RESOURCES:

Clinical Genome Resource (ClinGen): www.clinicalgenome.org

ClinGen Gene Curation Working Group site: https://www.clinicalgenome.org/working-groups/gene-curation/

Standard operating procedure for the framework described in this manuscript: http://bit.ly/ClinGenGCSOP

Validation Method for the framework described in this manuscript: http://bit.ly/clingenGCValMethods

ClinGen “reporter” form: https://search.clinicalgenome.org/kb/agents/sign_up

OMIM: https://omim.org/

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FIGURE TITLES AND LEGENDS:

Figure 1: ClinGen clinical validity classifications and qualitative descriptions. The suggested minimum criteria needed to obtain a given classification are described for each clinical validity classification. The types of evidence comprising these criteria are described in the text. The default classification for genes without a convincing human disease-causing variant is "No Reported Evidence." The level of evidence needed for each supportive gene-disease association category builds upon the previous category (i.e. "Limited" builds upon "Moderate"). Gene-disease associations classified as "Contradictory" likely have supporting evidence as well as opposing evidence, but are described separately from the classifications for supportive gene-disease associations.

Figure 2: Classes of genetic evidence and their relative weights used in the ClinGen clinical validity framework. For additional points to consider when scoring genetic evidence, please see the standard operating procedure document available on our website. Genetic evidence is separated into two main categories: case-level data and case-control data. While a single publication may include both case-level and case-control data, individual cases should NOT be included in both categories. Each category is assigned a range of points with a maximum score that can be achieved. *Case-Level Data* is derived from studies describing individuals and/or families with qualifying variants in the gene of interest. Points should be assigned to each case based on the variant's inheritance pattern, molecular consequence and evidence of pathogenicity in disease. In addition to variant evidence points, a gene-disease pair may also receive points for compelling segregation analysis (see Figure S1). *Case-Control Data:* Studies utilizing statistical analysis to evaluate variants in cases compared to controls. Case-

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control studies can be classified as either single variant analysis or aggregate variant analysis, however the number of points allowable for either category is the same. Points should be assigned according to the overall quality of each study based on these criteria: variant detection methodology, power, bias and confounding factors, and statistical power. Note that the maximum total scores allowed for different types of Case-Level data are not intended to add up to the total points allowed for Genetic Evidence as a whole. This permits different combinations of evidence types to achieve the maximum total score.

Figure 3: Types of gene-level experimental evidence and their relative weights used in the ClinGen clinical validity framework. Experimental evidence types used in the ClinGen gene curation framework are modified from MacArthur, et al. 2014. Evidence types are divided into three categories based on their relative contribution to the overall clinical validity of a gene-disease pair giving more weight to *in vivo* data. Each category is assigned a range of points with a maximum score that can be achieved, allowing more weight to be given to *in vivo* data (e.g. Models & Rescue) over *in vitro* experimental data. Evidence within the function category is given the least weight and is comprised of the following types of evidence: biochemical function, interactions, and expression. Functional alteration experiments in cells from affected individuals carrying candidate pathogenic variants are given more weight than the function category. Finally, model systems and phenotypic rescue experiments are given the most weight in our framework. Note that the maximum total scores allowed for different categories of Experimental Evidence are not intended to add up to the total

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allowable points. This permits different combinations of evidence types to achieve the maximum total score.

Figure 4. Final summary matrix used to provisionally classify gene-disease associations. A summary matrix was designed to generate a "provisional" clinical validity assessment using a point system consistent with the qualitative descriptions of each classification. *Genetic Evidence:* total number of points (not exceeding 12) obtained using the scoring metric in Fig. 2. If no human variants associated with disease have been reported in the literature, then the default classification is "No Reported Evidence." *Experimental Evidence:* total number of points (not exceeding 6) derived from each of the experimental categories in Fig. 3. *Replication Over Time* – Yes, if more than three years has passed since the publication of the first paper reporting the gene-disease relationship AND more than two publications with human mutations exist. *Contradictory Evidence* – No points are assigned to this category. Instead, the curator should provide a summary of contradictory information. *Scoring* - The sum of the quantified evidence from each category can be used to determine a "provisional" classification using the scale at the bottom of the figure. If a curator does not agree with this classification, he/she may provide a different suggested classification along with appropriate justification.

Figure 5. Comparison of provisional clinical validity classifications and associated matrix scores for selected gene-disease pairs evaluated by multiple curators. Of the 33 gene-disease pairs (y-axis) curated to validate the clinical validity curation framework, 31 were classified using the summary matrix (2 gene-disease pairs, *PMS2*:pancreatic cancer and *ARSD*:chondrodysplasia punctata, were classified as "No

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evidence reported" and are not shown). Genetic evidence (grey bars) and experimental evidence (black bars) were evaluated by two independent curators (C1-C9) to arrive at a provisional classification (x-axis). Gene-disease relationships scoring between 12-18 points can be "Strong" or "Definitive," depending on whether the association has been replicated over time (indicated by the squared "r/t"), in which case the preliminary classification is "Definitive". Clinical validity classifications that were discordant between preliminary curators are represented with a dashed background. Gene-disease pairs in which conflicting evidence was reported are represented by diagonal lines through the evidence bars and a grey background. The letter "C" in a triangle indicates that the curators classified the gene-disease pair as "Conflicting Evidence Reported". Each gene-disease pair was ultimately evaluated by an expert in the field for a final classification (far right column). Final expert classifications that differed from the preliminary classification are indicated by italics and asterisks.

Table 1: Categorization of gene-disease pairs used to validate the gene-validity framework

Disease category	HGNC Gene Symbol	Gene MIM ID	Disease curated	Inheritance Pattern	Orphanet ID/ Phenotype MIM ID	Expert Reviewed Classification[a]
Bone Marrow Failure	*NHP2*	606470	Dyskeratosis congenita	Recessive	ORPHA1775/MIM 613987	Limited
	RAD51C	602774	Fanconi anemia	Recessive	ORPHA84/MIM 613390	Moderate
	RPS10	603632	Diamond-Blackfan anemia	Dominant	ORPHA124/MIM 613308	Definitive
	RPS24	602412	Diamond-Blackfan anemia	Dominant	ORPHA124/MIM 610629	Definitive
	TSR2	300945	Diamond-Blackfan anemia with mandibulofacial dysostosis	X-Linked	ORPHA124/MIM 300946	Limited
	WRAP53	612661	Dyskeratosis congenita	Recessive	ORPHA1775/MIM 613988	Moderate
Cardiovascular Disorders	*AKAP9*	604001	Romano-Ward syndrome	Dominant	ORPHA101016/MIM 611820	Limited
	SCN4B	608256	Long QT syndrome	Dominant	ORPHA768/MIM 611819	Limited
	SMAD3	603109	Loeys-Dietz type 3	Dominant	ORPHA284984/MIM 613795	Definitive
	TMPO	188380	Familial or idiopathic dilated cardiomyopathy	Dominant	ORPHA154/MIM 613740[b]	Contradictory (refuted)
Hereditary Cancer	*DICER1*	606241	Pleuropulmonary blastoma	Dominant	ORPHA64742/MIM 601200	Definitive
	PALB2	610355	Hereditary breast cancer	Dominant	ORPHA227535/MIM 114480	Definitive
	PMS2	600259	Hereditary pancreatic cancer	N/A	N/A	No Reported Evidence
	RAD51D	602954	Hereditary breast cancer	Dominant	ORPHA227535/MIM 614291	Limited
Immune Disorders	*C1QB*	120570	Immunodeficiency due to C1Q deficiency	Recessive	ORPHA169147/MIM 613652	Definitive
	CD3E	186830	Severe combined immunodeficiency	Recessive	ORPHA183660/MIM 615615	Definitive
Skeletal Dysplasia	*ARSD*	300002	Chondrodysplasia punctata	N/A	N/A	No Reported Evidence
	COL2A1	120140	Spondyloepiphyseal dysplasia (Stanescu type)	Dominant	ORPHA94068/MIM 616583	Moderate
	FGFR3	134934	Achondroplasia	Dominant	ORPHA15/MIM 100800	Definitive
	LBR	600024	Anadysplasia-like, spontaneously remitting spondylometaphyseal dysplasia	Recessive	ORPHA448267/None	Moderate

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Neuromuscular Disorders	*BAG3*	603883	Myofibrillar myopathy	Dominant	ORPHA593/MIM 612954	Definitive
	MYO9A	604875	Arthrogryposis	Recessive	ORPHA109007/None	Limited
	PSD3	614440	Antecubital pterygium syndrome	Dominant	ORPHA2987/None	Limited
	VPS8	N/A	Arthrogryposis	Recessive	ORPHA109007/None	Limited
Miscellaneous	*AGTR2*	300034	X-linked non-syndromic intellectual disability	X-Linked	ORPHA777/None	Contradictory (Disputed)
	ATF6	605537	Achromatopsia	Recessive	ORPHA49382/MIM 616517	Strong
	CHD1L	613039	Renal or urinary tract malformation	Dominant	ORPHA93545/None	Limited
	HNRNPK	600712	Au-Kline syndrome	Dominant	ORPHA453504 /MIM 616580	Moderate
	LAMB1	150240	Lissencephaly 5	Recessive	ORPHA352682/MIM 615191	Moderate
	NGLY1	610661	Congenital disorder of deglycosylation	Recessive	ORPHA404454/MIM 615273	Definitive
	SMARCA1	300012	Syndromic intellectual disability with Coffin-Syris-like features	Dominant	None/None	Moderate
	SKI	164780	Shprintzen-Goldberg	Dominant	ORPHA311140/MIM 182212	Definitive
	SOS2	601247	Noonan syndrome	Dominant	ORPHA648/MIM 616559	Moderate

[a] All gene-disease classifications are accurate as of January 2017.

[b] Phenotype MIM was associated with *TMPO* at the time of curation, but has since been removed due to updated information.

Abbreviations: N/A, not applicable.

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Evidence Level		Evidence Description
Supportive Evidence	**DEFINITIVE**	The role of this gene in this particular disease has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time (in general, at least 3 years). No convincing evidence has emerged that contradicts the role of the gene in the specified disease.
	STRONG	The role of this gene in disease has been independently demonstrated typically in at least two separate studies providing **strong** supporting evidence for this gene's role in disease, usually including both of the following types of evidence: • Strong variant-level evidence demonstrating numerous unrelated probands with variants that provide convincing evidence for disease causality[1] **as well as** • Compelling gene-level evidence from different types of supporting experimental data[2]. In addition, no convincing evidence has emerged that contradicts the role of the gene in the noted disease.
	MODERATE	There is **moderate** evidence to support a causal role for this gene in this disease, typically including both of the following types of evidence: • Several probands with variants that provide convincing evidence for disease causality[1] • Moderate experimental data[2] supporting the gene-disease association The role of this gene in disease may not have been independently reported, but no convincing evidence has emerged that contradicts the role of the gene in the noted disease.
	LIMITED	There is **limited** evidence to support a causal role for this gene in this disease, such as: • Fewer than three observations of variants that provide convincing evidence for disease causality[1] OR • Variants have been observed in probands, but none have sufficient evidence for disease causality. • Limited experimental data[2] supporting the gene-disease association The role of this gene in disease may not have been independently reported, but no convincing evidence has emerged that contradicts the role of the gene in the noted disease.
NO REPORTED EVIDENCE		Evidence for a causal role in disease has not been reported. These genes might be "candidate" genes based on linkage intervals, animal models, implication in pathways known to be involved in human diseases, etc., but no reports have directly implicated the gene in human disease cases.
Contradictory Evidence	**CONFLICTING EVIDENCE REPORTED**	Although there has been an assertion of a gene-disease association, conflicting evidence for the role of this gene in disease has arisen since the time of the initial report indicating a disease association. Depending on the quantity and quality of evidence disputing the association, the association may be further defined by the following two sub-categories: 1. **Disputed** a. Convincing evidence *disputing* a role for this gene in this disease has arisen since the initial report identifying an association between the gene and disease. b. Refuting evidence need not outweigh existing evidence supporting the gene:disease association. 2. **Refuted** a. Evidence refuting the role of the gene in the specified disease has been reported and significantly outweighs any evidence supporting the role. b. This designation is to be applied at the discretion of clinical domain experts after thorough review of available evidence

NOTES

[1]Variants that disrupt function and/or have other strong genetic and population data (e.g. *de novo* occurrence, absence in controls, strong linkage to a small genomic interval, etc.) are considered convincing of disease causality in this framework.

[2]Examples of appropriate types of supporting experimental data based on those outlined in MacArthur et al. 2014.

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Figure 2

	Evidence Type		Case Information			Suggested Points/Case		Points Given	Max Score
						Default	Range		
Case-Level Data	Variant Evidence	Autosomal Dominant OR X-Linked Disorder[1]	Variant is *de novo*[2]			2	0-3		**12**
			Proband with predicted or proven null variant[3]			1.5	0-2		**10**
			Proband with other variant type with some evidence of gene impact[4]			0.5	0-1.5		**7**
		Autosomal Recessive	Two variants in *trans* and at least one *de novo*[2] or a predicted/proven null variant[3]			2	0-3		**12**
			Two variants (not predicted/proven null) with some evidence of gene impact[4] in *trans*			1	0-1.5		
	Segregation Evidence		Evidence of segregation in one or more families[5]	LOD Score Examples	3	5	0-7		**7**
					2	4			
					1.5	3			
					1	1.5			
Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria[6]			Suggested Points/Study		Points Given	Max Score
	Single Variant Analysis		• Variant Detection Methodology[6a] • Power[6b] • Bias and Confounding Factors[6c] • Statistical Significance[6d]			0-6			**12**
	Aggregate Variant Analysis					0-6			
							TOTAL ALLOWABLE POINTS for Genetic Evidence		**12**

General Notes

- **Detailed guidance for utilizing this scoring matrix is available on the ClinGen website in the standard operating procedure.**
- All variants under consideration should be rare enough in the general population to be consistent with disease.
- Cohorts/cases should not be double counted. For example, individual cases included as part of case-control studies should not be given points from both the "Case Level Data" and "Case-Control Data" categories.
- Case-Level Data includes studies describing individuals or families with variation in the gene of interest
- Case-Control studies are those in which statistical analysis is used to evaluate variation in cases compared to controls.

Numbered Footnotes

1. In X-linked disorders, affected probands will often be hemizygous males and/or heterozygous females. Recognizing that there can be rare cases of females affected by X-linked recessive disorders (due to chromosomal aneuploidy, skewed X inactivation, or homozygosity for a sequence variant) evaluators must interpret individual cases and X-linked pedigrees with caution.
2. Points should be adjusted depending on statistical expectation of *de novo* variation in the gene in question for variants.
3. Null variants (typically nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletions) are considered "very strong evidence for pathogenicity" in genes for which loss of function is a known disease mechanism. Disease mechanism can be assumed loss of function (LOF) if the gene is LOF constrained. LOF constraint scores must be interpreted in the context of the disease in question.
4. For variants that are NOT "null" (typically missense), at least some impact to gene function must be demonstrated for the case to count.
5. LOD scores reported by the authors of a peer-reviewed journal article may be used to assign segregation points as outlined in the scoring matrix above. If a LOD score is not provided by the authors, one may be estimated for informative families with rare, highly penetrant disorders in which phenocopies are expected to be rare or absent. Guidelines for calculating estimated LOD scores are included in the Figure S1.
6. Points for case-control studies may be assigned at the discretion of expert opinion based on the overall quality of each study. The following should be considered when evaluating case-control study quality:
   a. *Variant Detection Methodology*: Cases and controls should ideally be analyzed using methods with equivalent analytical performance (e.g. equivalent genotype methods, sufficient and equivalent depth and quality of sequencing coverage, correction for batch effects).
   b. *Power*: The study should analyze a sufficient number of cases and controls given the prevalence of the disease, the allele frequency, and the expected effect size in question to provide appropriate statistical power to detect an association.
   c. *Bias and Confounding factors:* The manner in which cases and controls were selected for participation and the degree of case-control matching may impact the outcome of the study.
   d. *Statistical Significance* – The level of statistical significance should be weighed carefully. When an odds ratio is presented, its magnitude should be consistent with a monogenic disease etiology. When p-values or 95% confidence intervals (CI) are presented, the strength of the statistical association can be weighed in the final points assigned. Factors, such as multiple testing, that might impact that interpretation of uncorrected p-values and CIs should be considered when assigning points

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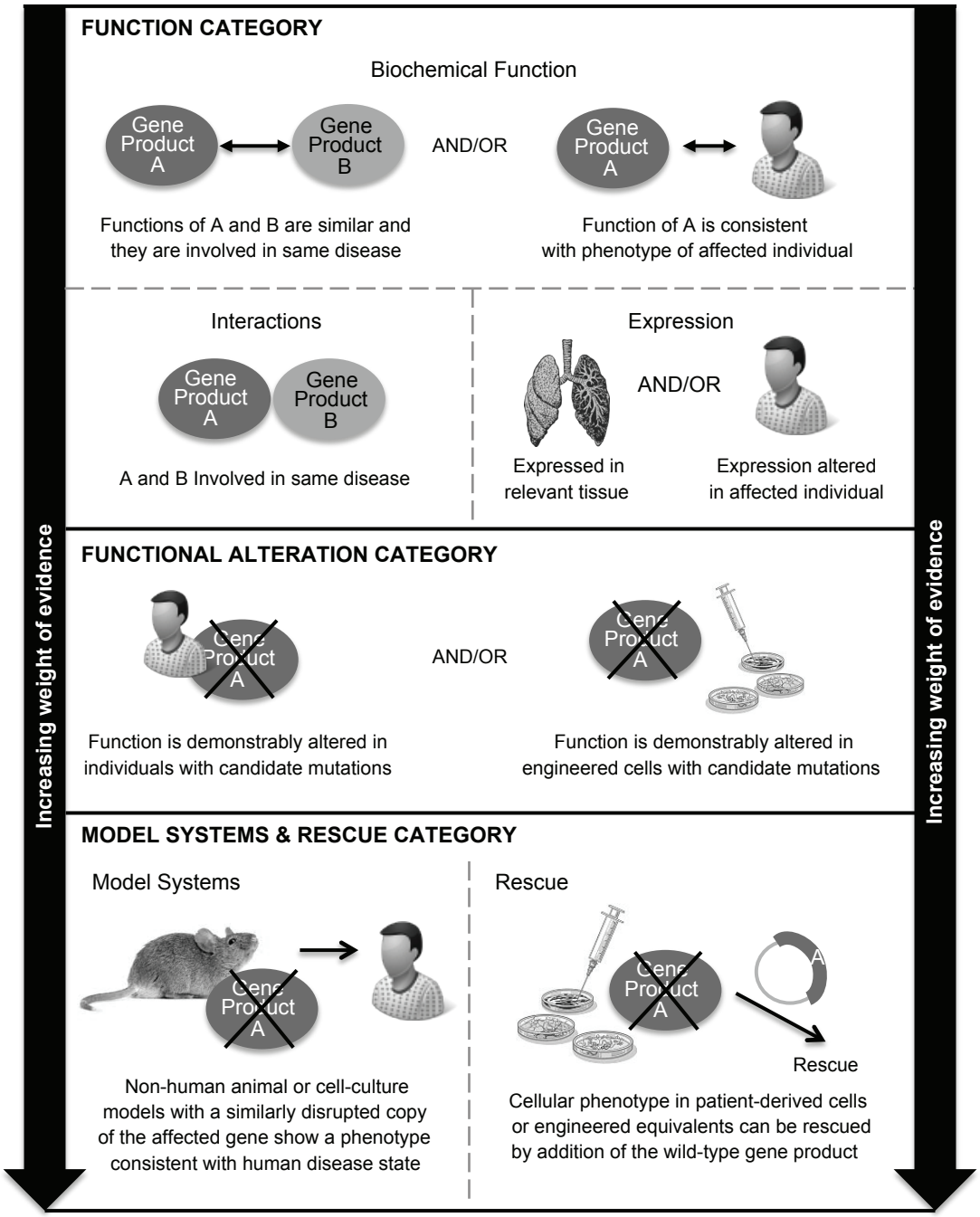
Figure 3

Evidence Category	Evidence Type	Suggested Points		Points Given	Max
		Default	Range		
Function	Biochemical Function	0.5	0-2		2
	Protein Interaction		0-2		
	Expression		0-2		
Functional Alteration	Cells from affected individual	1	0-2		2
	Engineered cells	0.5	0-1		
Models & Rescue	Animal model	2	0-4		4
	Cell culture model system	1	0-2		
	Rescue in animal model	2	0-4		
	Rescue in engineered equivalent	1	0-2		
Total Allowable Points for Experimental Evidence					6



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Clinical Validity Summary Matrix

GENE/DISEASE PAIR:				
Assertion criteria	**Genetic Evidence (0-12 points)**	**Experimental Evidence (0-6 points)**	**Total Points (0-18)**	**Replication Over Time (Y/N)**
Description	Case-level, family segregation, or case-control data that support the gene-disease association	Gene-level experimental evidence that support the gene-disease association	Sum of Genetic & Experimental Evidence	> 2 pubs w/ convincing evidence over time (>3 yrs)
Assigned Points				
CALCULATED CLASSIFICATION		**LIMITED**	**1-6**	
		MODERATE	**7-11**	
		STRONG	**12-18**	
		DEFINITIVE	**12-18 & Replicated Over Time**	
Valid contradictory evidence (Y/N)*	**List PMIDs and describe evidence:**			
CURATOR CLASSIFICATION				
FINAL CLASSIFICATION				

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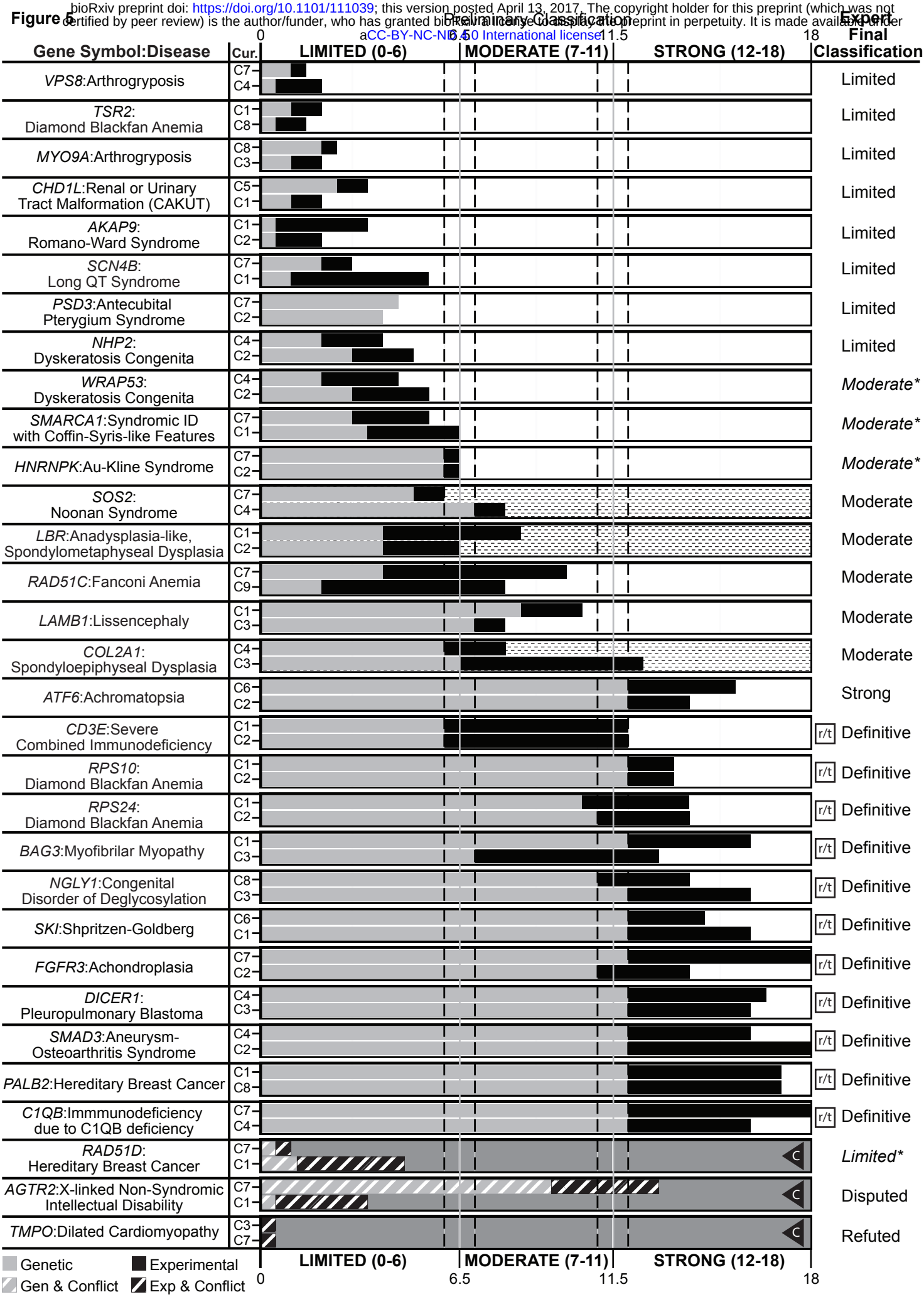
Figure 5