Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource

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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 semiquantitative measurement for the strength of evidence of a gene-disease relationship that correlates to a qualitative classification: "Definitive," "Strong," "Moderate," "Limited," "No Reported Evidence," or "Conflicting Evidence." Within the ClinGen structure, classifications derived with 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.

Introduction

The human genome comprises approximately 20,000 protein-coding genes (see OMIM website in Web Resources), of which about 3,000 have been reported in association with at least one Mendelian disease.[1] Roughly half[1] 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.[2,3] 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 or genome sequencing. Some of the gene-disease relationships are either unable to be confirmed for many years or are ultimately proven wrong.[4] 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[2] of a gene-disease relationship.

The NIH-funded Clinical Genome Resource (ClinGen)[5] 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 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 semiquantitative 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

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inheritance, such as mitochondrial, and diseases with more complex genomic etiologies, including oligogenic or multifactorial conditions. Our approach is intended to neither define multifactorial disease risk nor to be a substitute for well-established statistical thresholds used for genome-wide association studies.[6,7]

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) and OrphaNet (see Web Resources), by systematically organizing the supporting and refuting evidence and then 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.

Material and Methods

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 3 years, this group convened bi-monthly to develop the described framework for assessing gene-disease clinical validity through expert opinion and working group consensus. 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 representative only 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 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 3 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[8]), 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).

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 semiquantitative approach to evaluate both genetic (Figure 2) and experimental (Figure 3) evidence in a standardized manner that promotes consistent collection and weighting of evidence (a detailed standard operating procedure is available on the ClinGen website; see Web Resources). Development of the quantitative aspect of this framework was based on the qualitative descriptions outlined in Figure 1. Both the qualitative classifications and their quantitative counterparts were determined by consensus of the ClinGen Gene Curation Working Group members comprised of a diverse group of genetics experts and professionals with additional input from experts in multiple clinical domains. Throughout development of the framework, several gene-disease pairs (see Table 1) were iteratively curated as benchmarks with a known "anticipated classification" to determine appropriate scores and assigned ranges (e.g., *FGFR3* [MIM: 134934]:achondroplasia [MIM: 100800]).

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.

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.		

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 (e.g., "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.

	Evidence Type		Case Information			Suggested Points/Case		Points Given	Max Score
						Default	Range		
Case-Level Data[A]	Variant Evidence	Autosomal Dominant OR X-Linked Disorder[B]	Variant is *de novo*[C]			2	0-3		12
			Proband with predicted or proven null variant[D]			1.5	0-2		10
			Proband with other variant type with some evidence of gene impact[E]			0.5	0-1.5		7
		Autosomal Recessive	Two variants in *trans* and at least one *de novo*[C] or a predicted/proven null variant[D]			2	0-3		12
			Two variants (not predicted/proven null) with some evidence of gene impact[E] in *trans*			1	0-1.5		
	Segregation[F] Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7		7
					2	4			
					1.5	3			
					1	1.5			
Case-Control Data[G]	Case-Control Study Type[H]		Case-Control Quality Criteria[I]			Suggested Points/Study		Points Given	Max Score
	Single Variant Analysis[Ha]		• Variant Detection Methodology[Ia] • Power[Ib]			0-6			12
	Aggregate Variant Analysis[Hb]		• Bias and Confounding Factors[Ic] • Statistical Significance[Id]			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.
- Footnotes A-I are explained in the legend for Figure S2.

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 are 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 case subjects compared to control subjects. Case-control studies can be classified as either single-variant analysis or aggregate variant analysis, but 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.

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 case and control subjects are scored as case-control data. When case-level and case-control data are present in a single publication, points can be assigned in 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 be counted toward supporting evidence only if the reported variant has some

Evidence Category	Evidence Type	Suggested Points		Points Given	Max Score
		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

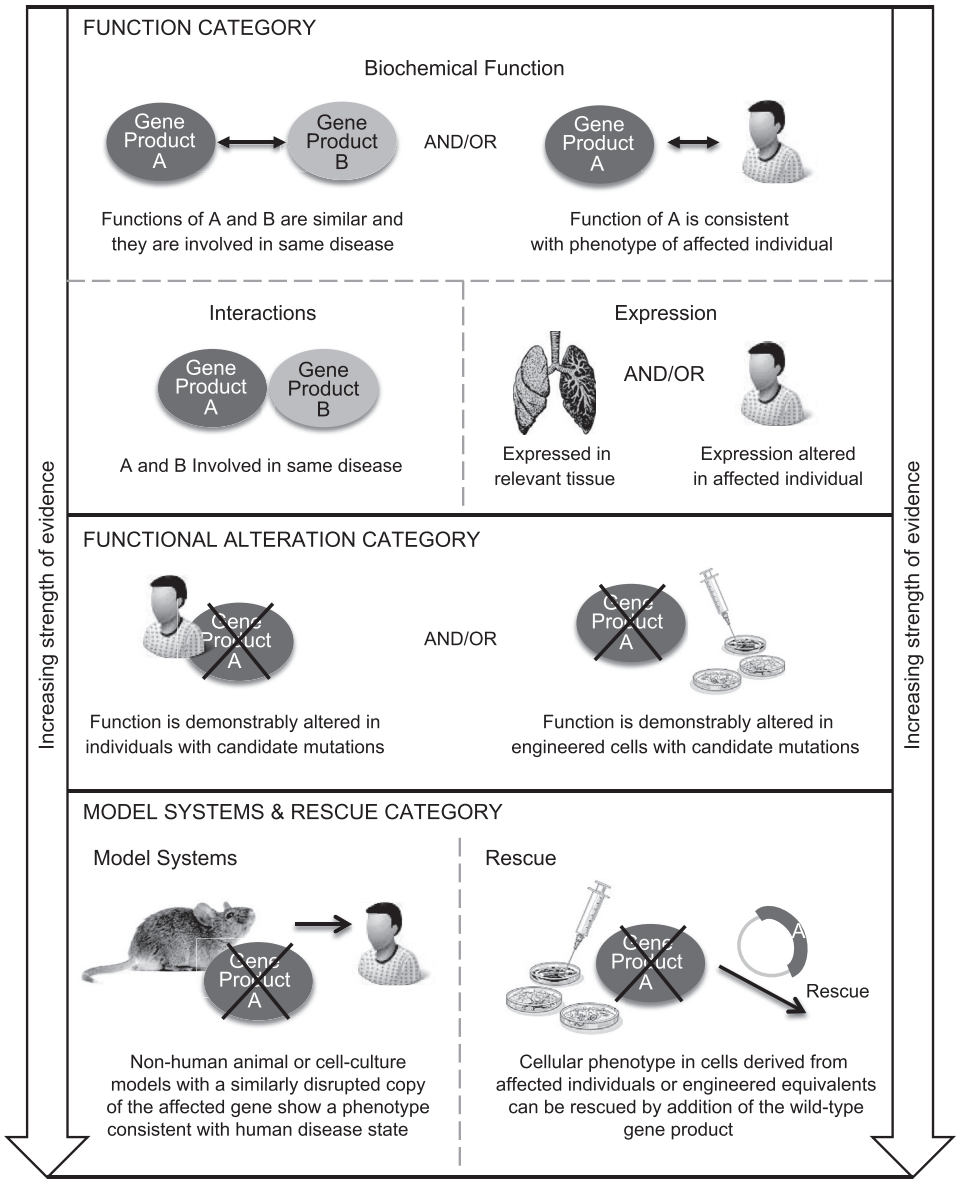

Figure 3. Types of Gene-Level Experimental Evidence and Their Relative Weights Used in the ClinGen Clinical Validity Framework
Experimental evidence types used in the ClinGen gene curation framework are modified from MacArthur et al.[9] Evidence types are divided into three categories based on their 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

(legend continued on next page)

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. Figures 2 and 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) is recommended. For 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 case subject compared to control subjects. 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 case subjects compared with control subjects. This comparison is typically accomplished by sequencing the entire gene in both case and control subjects 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.[9] The following experimental evidence types are used: biochemical function, experimental protein interactions, expression, functional alteration, phenotypic rescue, and model systems (Figure 3, bottom). 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 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 and Final Matrix

The scores assigned to both genetic and experimental evidence are tallied to generate a total score (ranging from 1 to 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 toward 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 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 (see Web Resources), and independently assessed by curators; however, if necessary, unpublished information available from publicly accessible resources, such as variant databases,[10,11] may be used as long as some supporting evidence is provided.

Results

With 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). These pairs were intentionally chosen to be representative of the diversity in monogenic disorders with regards to inheritance patterns, disease prevalence, and levels of evidence to support a relationship. 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). All major discrepancies between curators were discussed and resolved when possible prior to review by clinical domain experts (either ClinGen Clinical Domain Working Group [CDWG] members or ad hoc disease experts mentioned

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

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
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

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Table 1. ***Continued***

Disease Category	HGNC Gene Symbol	Gene MIM ID	Disease Curated	Inheritance Pattern	Orphanet ID, Phenotype MIM ID	Expert Reviewed Classification[a]
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	x	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

Abbreviations: N/A, not applicable.
[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.

in the Acknowledgments); 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 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 figures (Figures S2–S65).

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 semiquantitative 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.

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.

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 publications with 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 references and describe evidence:**			
CURATOR CLASSIFICATION				
FINAL CLASSIFICATION				

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 Figure 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 Figure 3. Replication over time: yes, if more than 3 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.

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] in Figures S14 and S15). 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 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 Figures S36 and S37 for *PALB2* [MIM: 610355] and hereditary breast cancer [MIM: 114480] 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 require curating only 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.

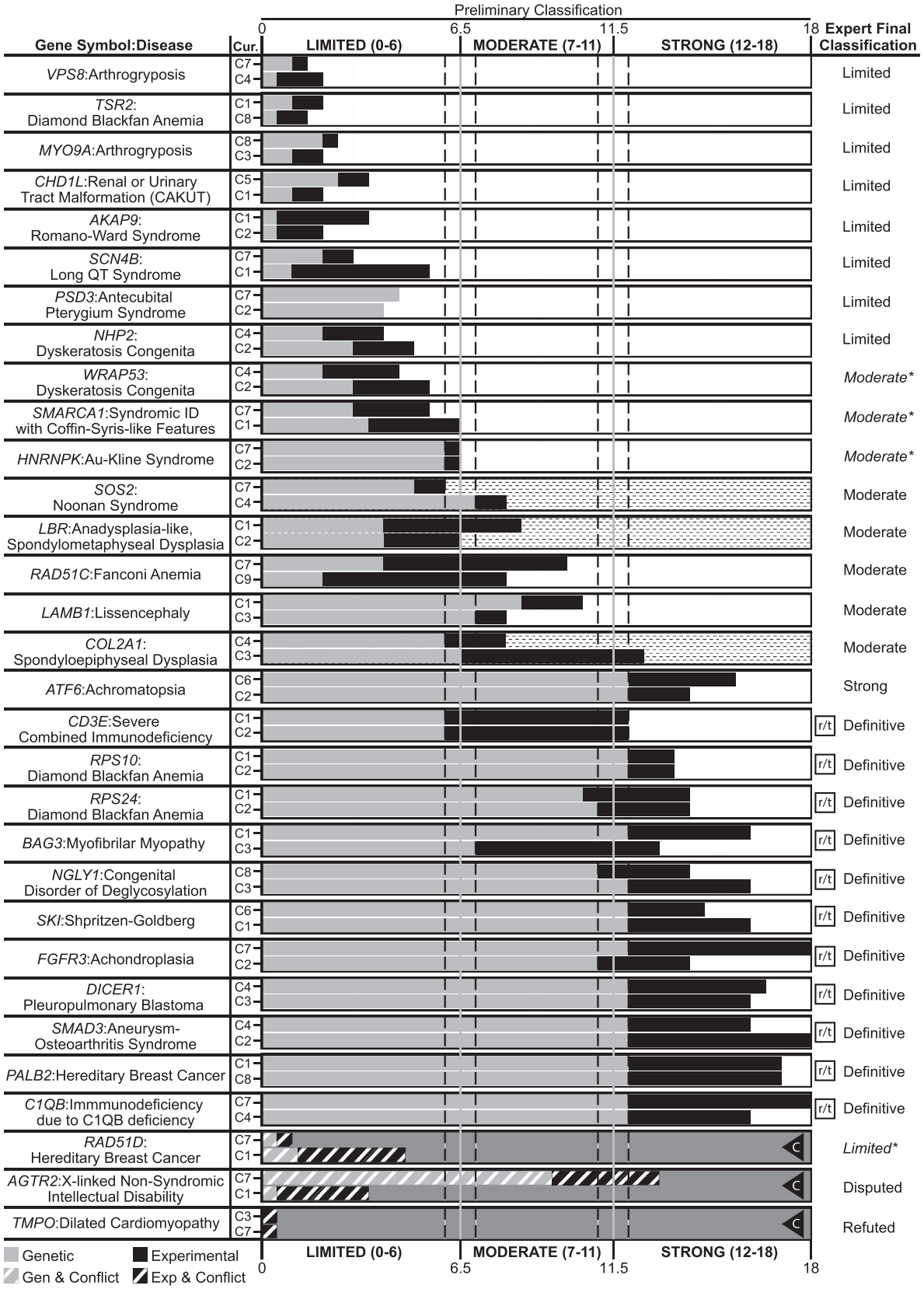

Figure 5. Comparison of Provisional Clinical Validity Classifications and Associated Matrix Scores for Selected Gene-Disease Pairs Evaluated by Multiple Curators

Of the 33 gene-disease pairs (y axis) curated to validate the clinical validity curation framework, 31 were classified using the summary matrix (two gene-disease pairs, *PMS2*:pancreatic cancer and *ARSD*:chondrodysplasia punctata, were classified as "No evidence reported"

(legend continued on next page)

ClinGen's ultimate goal is to enhance the incorporation of genomic information into clinical care, an important component of the Precision Medicine Initiative.[12] 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 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.

Supplemental Data

Supplemental Data include 65 figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2017.04.015.

Acknowledgments

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Web Resources

ClinGen, https://www.clinicalgenome.org/
ClinGen Gene Curation, https://www.clinicalgenome.org/working-groups/gene-curation/
ClinGen Gene Curation SOP, https://www.clinicalgenome.org/working-groups/gene-curation/projects-initiatives/gene-disease-clinical-validity-sop/
ClinGen Knowledge Base, https://search.clinicalgenome.org/kb/agents/sign_up
OMIM, http://www.omim.org/
Orphanet, http://www.orpha.net/consor/cgi-bin/index.php

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and are not shown). Genetic evidence (gray 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 and 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 gray 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.

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Supplemental Data

Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource

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A. Dominant/X-linked

$$Z = \log_{10}[1/(0.5)^n]$$

n = dominant segregations

n	LOD	Points
15	4.5	6.5
14	4.2	6.0
13	3.9	5.5
12	3.6	5.5
11	3.3	5.0
10	3.0	5.0
9	2.7	4.5
8	2.4	4.0
7	2.1	4.0
6	1.8	3.5
5	1.5	3.0
4	1.2	1.5

B. Recessive

$$Z = \log_{10}\{1/[(0.25)^{x-1}(0.75)^y]\}$$

x = affected individuals
y = unaffected individuals

x/*y*	LOD	Points
7 / 4	4.11	6.0
7 / 1	3.73	5.5
6 / 1	3.14	5.0
5 / 1	2.53	4.5
4 / 3	2.18	4.0
4 / 1	1.90	3.5
3 / 3	1.50	3.0
3 / 2	1.45	2.5
3 / 1	1.30	2.5
2 / 3	1.00	1.5
2 / 2	0.85	1.0
2 / 1	0.72	1.0

C. Proposed Matrix Scoring for LOD Ranges

LOD Range	Points (Max = 7)
≥ 5.00	7.0
4.50 – 4.99	6.5
4.00 – 4.49	6.0
3.50 - 3.99	5.5
3.00 – 3.49	5.0 (1000:1)
2.50 – 2.99	4.5
2.00 – 2.49	4.0 (100:1)
1.75 – 1.99	3.5
1.50 – 1.74	3.0
1.25 – 1.49	2.5 (10:1)
1.00 – 1.24	1.5
0.72 – 0.99	1.0

Figure S1: Guidelines for approximating LOD scores within the ClinGen clinical validity framework. (A, B) LOD score (Z) estimates are given for multiple segregation scenarios with a suggested number of points to be assigned in the genetic evidence category (Figure 3). **A.** LOD scores for disorders inherited in a dominant or X-linked manner should calculated using the same equation, where *n* equals the number of dominant segregations. **B.** For autosomal recessive disorders, both unaffected carriers (*y*) and affected genotype positive individuals (*x*) should be included in the calculation of the LOD score. In general, the number of affected individuals (*x*) - 1 is equal to the number of affected segregations and can be used interchangeably in this equation. **C.** A suggested number of points is provided for multiple ranges of LOD scores to facilitate consistent scoring in the summary matrix (Figure 4).

AGTR2 and X-linked intellectual disability											
Genetic Evidence	Case-Level Data[A]	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder[B]	Variant is de novo[C]			2	0-3	12			
				Proband with predicted or proven null variant[D]			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact[E]			0.5	0-1.5	7	0.5	0.5	Takeshita E et al. 2012 Oct (22269148)[1]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo[C] or a predicted/proven null variant[D]			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact[E] in trans			1	0-1.5				
		Segregation[F] Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7			
						2	4					
						1.5	3					
						1	1.5					
	Case-Control Data[G]	Case-Control Study Type[H]		Case-Control Quality Criteria[I]			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis[Ha]		1. Variant Detection Methodology[Ia] 2. Power[Ib] 3. Bias and confounding[Ic] 4. Statistical Significance[Id]			0-6		12			
		Aggregate Variant Analysis[Hb]					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									0.5	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	0.5	0.5	Vervoort VS et al. 2002 Jun 28 (12089445)[2]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	0 - 2				
		Functional Alteration		Cells from affected individual			1	0 - 2	2	0.5	0.5	Pawlowski TL et al. 2009 Sep (19501643)[3]
				Engineered cells			0.5	0 - 1				
		Models & Rescue		Animal model			2	0 - 4	4	2.0	2	Maul B et al. 2008 May (18335189)[4]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									3	

Figure S2: Summary of evidence for a relationship between *AGTR2* and X-linked intellectual disability.

Evidence for the examples presented in Table 1 and Figure 5 is summarized in Figures S2-S65. The number of points awarded for each type of evidence and their corresponding references are provided. Footnotes A-I are the guidelines used to assess genetic evidence within this framework and apply to all of the examples presented in the following figures.

A. Each case may be given points for A) variant evidence (in the context of the appropriate mode of inheritance) and B) segregation evidence, if applicable (see footnote F and Figure S1 for more details on segregation evidence).

B. 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.

C. Points should be adjusted depending on statistical expectation of *de novo* variation in the gene in question for variants.

D. As described in the 2015 ACMG/AMP sequence variant interpretation guidelines[5], 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 – genes associated with severe, pediatric-onset disorders are more likely to show constraint than adult-onset conditions where overall fitness is not impacted.

E. For variants NOT considered to be "null" (typically missense), at least some impact to gene function must be demonstrated for the case to count. Impact based on predictions only would score less than the default 0.5 points and impact based on functional validation can score 0.5 or above (up to 1.5/case) depending on the validation quality and biological representativeness of the functional assay.

F. 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. Below are guidelines for calculating estimated LOD scores in the appropriate scenarios are included in the standard operating procedure available online.

G. Case-control studies should be independently assessed to evaluate the quality of the study design preferably in concert with an expert.

H. Case-control studies are classified based on how variation in cases and controls is evaluated: single variant analysis or aggregate variant analysis. Studies presenting both types of analyses may be counted in either category at the discretion of the curator/expert, but the same variants should not be counted in both categories.

   a. *Single variant analysis* studies 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 should be independently assessed with appropriate statistical correction for multiple testing.

   b. *Aggregate variant analysis* studies are those in which the statistical enrichment of two or more variants as an aggregate is assessed in cases compared to controls. This comparison could be accomplished by genotyping specific variants or by sequencing the entire gene.

I. 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. The following are some factors that should be considered:

      i. Are there systematic differences between individuals selected for study and individuals not selected for study?

      ii. Are the cases and controls matched by demographic information (e.g., age, ethnicity, location of recruitment, etc.)?

      iii. Are the cases and controls matched for genetic ancestry, if not did investigators account for genetic ancestry in the analysis?

      iv. Have the cases and controls been equivalently evaluated for presence or absence of a phenotype, and/or family history of disease?

   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

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	**0.5**	**3**	**3.5**	**NO**
CALCULATED CLASSIFICATION		**LIMITED**	**1-6**	
		MODERATE	**7-11**	
		STRONG	**12-18**	
		DEFINITIVE	**12-18 AND replication over time**	
Valid contradictory evidence (Y/N)*	YES	Piton A et al. 2013 Aug 8 (23871722)[6]; (Piton et al. refutes the original gene-disease assertion and ExAC data demonstrates that almost all of the variants identified are common in the general population.)		
CALCULATED CLASSIFICATION (DATE)		**LIMITED**		
MODIFY CALCULATED CLASSIFICATION		**YES**		
CURATOR CLASSIFICATION (DATE)		**DISPUTED 10/10/2016**		
EXPERT CURATION (DATE)		**DISPUTED 11/16/16 "Disputed" based on Pitton et al. 2013[6] and ExAC[7] data.**		

Figure S3: Summary matrix and classification for *AGTR2* and X-linked intellectual disability.

AKAP9 and autosomal dominant long QT syndrome

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	0.5	0.5	Chen L et al. 2007 Dec 26 (18093912)[8]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								0.5	

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	0.5	0.5	Marx SO et al. 2002 Jan 18 (11799244)[9]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	1.0	1	Chen L et al. 2007 Dec 26 (18093912)[8]
		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 Experimental Evidence Points (Maximum 6)						1.5	

Figure S4: Summary of evidence supporting a relationship between *AKAP9* and autosomal dominant long QT syndrome.

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	0.5	1.5	2	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 04/05/16		
	EXPERT CURATION (DATE)	LIMITED 12/15/16		

Figure S5: Summary matrix and classification for *AKAP9* and autosomal dominant long QT syndrome.

ARSD and chondrodysplasia punctata

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	0.0		
				Proband with predicted or proven null variant		1.5	0-2	10	0.0		
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	0.0	0	
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12		0	
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5		0.0		
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7	0.0		
					2	4					
					1.5	3					
					1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Guidelines: Points/Study		Guidelines: Max	Scores: Points	Scores: Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12	0.0		
		Aggregate Variant Analysis				0-6		12	0.0		
		Total Genetic Evidence Points (Maximum 12)								0	No reports of variants in this gene associated with this condition.

	Evidence Category	Evidence Type	Guidelines: Default	Guidelines: Range	Guidelines: Max	Scores: Points	Scores: Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2			
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	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 Experimental Evidence Points (Maximum 6)						0	

Figure S6: Summary of evidence supporting a relationship between *ARSD* and chondrodysplasia punctata.

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		0	0	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		NO REPORTED EVIDENCE 07/14/2016		
EXPERT CURATION (DATE)		NO REPORTED EVIDENCE 11/15/16		

Figure S7: Summary matrix and classification for *ARSD* and chondrodysplasia punctata.

ATF6 and autosomal recessive achromatopsia

		Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12	9.0	12	Ansar M et al. 2015 Sep (26063662); Kohl S et al. 2015 Jul (26029869)[10; 11]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5		3.5		
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	7.0	7	Ansar M et al. 2015 Sep (26063662); Kohl S et al. 2015 Jul (26029869)[10; 11]
						2	4					
						1.5	3					
						1	1.5					

		Case-Control Study Type	Case-Control Quality Criteria	Guidelines Points/Study	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Genetic Evidence	Case-Control Data	Single Variant Analysis	1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6	12			
		Aggregate Variant Analysis		0-6	12			
		Total Genetic Evidence Points (Maximum 12)					**14**	

	Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	0.5	0.5	Ansar M et al. 2015 Sep (26063662)[11]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	0.5	0.5	Ansar M et al. 2015 Sep (26063662)[11]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	1.0	1	Kohl S et al. 2015 Jul (26029869)[10]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						**2**	

Figure S8: Summary of evidence supporting a relationship between *ATF6* and autosomal recessive achromatopsia.

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	12	2	14	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	STRONG 06/01/2016		
	EXPERT CURATION (DATE)	STRONG 11/16/2016		

Figure S9: Summary matrix and classification for *ATF6* and autosomal recessive achromatopsia.

BAG3 and autosomal dominant myofibrillar myopathy

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	7.0	7	Selcen D et al. 2009 Jan (19085932); Odgerel Z et al. 2010 Jul (20605452); Semmler AL et al. 2014 Aug 1 (25208129); Konersman CG et al. 2015 May (25728519); Kostera-Pruszczyk A et al. 2015 Dec (26545904); D et al. 2016 Jun (27443559); Jaffer F et al. 2012 Jun (22734908)[12-18]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									7	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.0	1	Homma S et al. 2006 Sep (16936253); Selcen D et al. 2009 Jan (19085932)[12; 19]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	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	6.0	4	Homma S et al. 2006 Sep (16936253); Hishiya A et al. 2010 Nov 12 (20884878)[19; 20]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									5	

Figure S10: Summary of evidence supporting a relationship between *BAG3* and autosomal dominant myofibrillar myopathy.

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	7	5	12	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE		
	EXPERT CURATION (DATE)	DEFINITIVE 12/18/2016		

Figure S11: Summary matrix and classification for *BAG3* and autosomal dominant myofibrillar myopathy.

C1QB and autosomal recessive immunodeficiency due to an early component of complement deficiency

					Guidelines			Scores		
		Evidence Type		Case Information Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo	2	0-3	12			
				Proband with predicted or proven null variant	1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact	0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant	2	0-3	12	8.0	10	Petry F et al. 1997 Dec (9476130); Marquart HV et al. 2007 Jul (17513176); McAdam RA et al. 1988 (2894352); Troedson C et al. 2013 May (23651859); Higuchi Y et al. 2013 Oct 28 (24160257); van Schaarenburg RA et al. 2015 Mar (25454803)[21-26]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans	1	0-1.5		2.0		
		Segregation Evidence		Evidence of segregation in one or more families — LOD Score Examples: 3	5	0-7	7	3.5	3.5	Marquart HV et al. 2007 Jul (17513176); Higuchi Y et al. 2013 Oct 28 (24160257)[23; 25]
				2	4					
				1.5	3					
				1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria	Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6		12			
		Aggregate Variant Analysis			0-6		12			
		Total Genetic Evidence Points (Maximum 12)							12	

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	1.0	1	van Schaarenburg RA et al. 2015 Mar (25454803); Higuchi Y et al. 2013 Oct 28 (24160257); McAdam RA et al. 1988 (2894352); Petry F et al. 1997 Dec (9476130); Marquart HV et al. 2007 Jul (17513176); van Schaarenburg RA et al. 2015 Mar (25454803))[21-24; 26]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	2.0	2	McAdam RA et al. 1988 (2894352)[21]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	2.0	2	Miura-Shimura Y et al. 2002 Aug 1 (12133956)[27]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						5	

Figure S12: Summary of evidence supporting a relationship between *C1QB* and autosomal recessive immunodeficiency due to an early component of complement deficiency.

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	12	5	17	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 06/13/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 01/09/2017		

Figure S13: Summary matrix and classification for *C1QB* and autosomal recessive immunodeficiency due to an early component of complement deficiency.

CD3E and autosomal recessive severe combined immunodeficiency

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12	6.0	6	Soudais C et al. 1993 Jan (8490660); de Saint Basile G et al. 2004 Nov (15546002); Fuehrer M et al. 2014 May (24515816)[28-30]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	0.0	0	
						2	4					
						1.5	3					
						1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									6	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.5	1.5	Manolios N et al. 1991 Jul (1828760); Thoenes G et al. 1992 Jan 5 (1370449); Fuehrer M et al. 2014 May (24515816)[30-32]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	0 - 2				
		Functional Alteration		Cells from affected individual			1	0 - 2	2	2.0	2	de Saint Basile G et al. 2004 Nov (15546002)[29]
				Engineered cells			0.5	0 - 1				
		Models & Rescue		Animal model			2	0 - 4	4	2.0	2	Wang B et al. 1994 Sep 27 (7937778)[33]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									5.5	

Figure S14: Summary of evidence supporting a relationship between *CD3E* and autosomal recessive severe combined immunodeficiency

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	6	5.5	11.5	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		DEFINITIVE 05/26/2016		
EXPERT CURATION (DATE)		**DEFINITIVE** 01/09/2017 Expert agrees with decision to round up to "Definitive," and is aware of additional unpublished genetic evidence to corroborate this claim.		

Figure S15: Summary matrix and classification for *CD3E* and autosomal recessive severe combined immunodeficiency.

CHD1L and autosomal dominant renal or urinary tract malformation (CAKUT)

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	2.5	2.5	Hwang DY et al. 2014 Jun (24429398)[34]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									3.5	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.0	1	Brockschmidt A et al. 2012 Jun (22146311)[35]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	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 Experimental Evidence Points (Maximum 6)									1	

Figure S16: Summary of evidence supporting a relationship between *CHD1L* and autosomal dominant renal or urinary tract malformation (CAKUT).

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	2.5	1	3.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 05/25/2016		
	EXPERT CURATION (DATE)	LIMITED 11/18/2016		

Figure S17: Summary matrix and classification for *CHD1L* and autosomal dominant renal or urinary tract malformation (CAKUT).

COL2A1 and autosomal dominant Spondyloepiphyseal dysplasia (Stanescu type)

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12	2.0	2	Jurgens J et al. 2015 Oct (26183434)[36]
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	1.0	1	Jurgens J et al. 2015 Oct (26183434); Hammarsjö A et al. 2016 Jan (26420734)[36; 37]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									9	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2			
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	0 - 2				
		Functional Alteration		Cells from affected individual			1	0 - 2	2	2.0	2	Chan D et al. 1993 Jul 15 (8325895); Vandenberg P et al. 1991 Sep 1 (1881905); Garofalo S et al. 1991 Nov 1 (1946380)[38-40]
				Engineered cells			0.5	0 - 1				
		Models & Rescue		Animal model			2	0 - 4	4	4.0	4	Vandenberg P et al. 1991 Sep 1 (1881905); Garofalo S et al. 1991 Nov 1 (1946380)[39-40]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									6	

Figure S18: Summary of evidence supporting a relationship between *COL2A1* and autosomal dominant Spondyloepiphyseal dysplasia (Stanescu type).

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	3	6	9	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	MODERATE 05/25/2016		
	EXPERT CURATION (DATE)	MODERATE 12/01/2016		

Figure S19: Summary matrix and classification for *COL2A1* and autosomal dominant Spondyloepiphyseal dysplasia (Stanescu type).

DICER1 and autosomal dominant pleuropulmonary blastoma

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	12.0	12	Hill DA et al. 2009 Aug 21 (19556464); Doros L et al. 2012 Sep (22180160); Stewart DR et al. 2014 Nov (25118636)[41-43]
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7		
						2	4				
						1.5	3				
						1	1.5				
						Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)		12
						Guidelines			Scores		
Experimental Evidence		Evidence Category		Evidence Type		Default	Range	Max	Points	Tally	PMIDs/Notes
		Function		Biochemical Function		0.5	0 - 2	2	2.0	2	Hill DA et al. 2009 Aug 21 (19556464); Harris KS et al. 2006 Feb 14 (16452165)[41; 44]
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	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	2.0	2	Harris KS et al. 2006 Feb 14 (16452165)[44]
				Cell culture model system		1	0 - 2				
				Rescue in animal model		2	0 - 4				
				Rescue in engineered equivalent		1	0 - 2				
									Total Experimental Evidence Points (Maximum 6)		4

Figure S20: Summary of evidence supporting a relationship between *DICER1* and autosomal dominant pleuropulmonary blastoma.

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	12	4	16	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 05/06/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 01/08/2017		

Figure S21: Summary matrix and classification for *DICER1* and autosomal dominant pleuropulmonary blastoma.

FGFR3 and autosomal dominant achondroplasia

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	10.0	10	Rousseau F et al. 1994 Sep 15 (8078586); Shiang R et al. 1994 Jul 29 (7913883)[45; 46] (Additional cases are available beyond those in these references.)
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	1.0	1	Rousseau F et al. 1994 Sep 15 (8078586)[45] (Additional cases are available beyond those in this reference.)
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	7	2.0	2	Rousseau F et al. 1994 Sep 15 (8078586)[45]
						2	4				
						1.5	3				
						1	1.5				
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Guidelines			Scores		PMIDs/Notes
						Points/Study		Max	Points	Tally	
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								12	Additional genetic evidence is available beyond this maximum score.
Experimental Evidence		Evidence Category		Evidence Type		Guidelines			Scores		PMIDs/Notes
						Default	Range	Max	Points	Tally	
		Function		Biochemical Function		0.5	0 - 2	2			
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	0 - 2				
		Functional Alteration		Cells from affected individual		1	0 - 2	2	1.0	1	Naski MC et al. 1998 Dec (9811582); Cho JY et al. 2004 Jan 13 (14699054)[47; 48]
				Engineered cells		0.5	0 - 1				
		Models & Rescue		Animal model		2	0 - 4	4	2.0	2	Wang Y et al. 1999 Apr 13 (10200283)[49]
				Cell culture model system		1	0 - 2				
				Rescue in animal model		2	0 - 4				
				Rescue in engineered equivalent		1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)								3	Additional experimental data may be available.

Figure S22: Summary of evidence supporting a relationship between *FGFR3* and autosomal dominant achondroplasia.

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	12	3	15	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 04/05/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 12/01/2016		

Figure S23: Summary matrix and classification for *FGFR3* and autosomal dominant achondroplasia.

HNRNPK and autosomal dominant Au-Kline syndrome

					Guidelines			Scores		
		Evidence Type		Case Information Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo	2	0-3	12	6.0	6	Au PY et al. 2015 Oct (26173930); Lange L et al. 2016 Sep (26954065)[50; 51]
				Proband with predicted or proven null variant	1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact	0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant	2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans	1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families — LOD Score Examples: 3	5	0-7	7			
				2	4					
				1.5	3					
				1	1.5					
					Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria	Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6		12			
		Aggregate Variant Analysis			0-6		12			
								Total Genetic Evidence Points (Maximum 12)		6.5
					Guidelines			Scores		
Experimental Evidence		Evidence Category		Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
		Function		Biochemical Function	0.5	0 - 2	2	0.5	0.5	Fan X et al. 2015 Dec 7 (26638989)[52]
				Protein Interaction	0.5	0 - 2				
				Expression	0.5	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 Experimental Evidence Points (Maximum 6)		0.5

Figure S24: Summary of evidence supporting a relationship between *HNRNPK* and autosomal dominant Au-Kline syndrome.

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	6	0.5	6.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		MODERATE		
EXPERT CURATION (DATE)		MODERATE 11/15/2016		

Figure S25: Summary matrix and classification for *HNRNPK* and autosomal dominant Au-Kline syndrome. Evidence is rapidly emerging supporting the association between *HNRNPK* and Au-Kline syndrome. Gallardo, et al. published a paper in 2015[53] describing an Hnrnpk +/- haploinsufficient mouse, which they developed to study its role in tumorigenesis. Personal communication with the senior author of that paper, Sean Post, in August 2016, revealed that the haploinsufficient mice appeared to have "significant reduction in overall size and had numerous structural/bone abnormalities," remniscient of the human phenotype, though he clarified that his group is not able to formally assess them for these types of phenotypes. Additionally, we are aware of at least one additional unpublished case - this evidence is not being formally considered, as it is not part of the public domain.

LAMB1 and autosomal recessive lissencephaly 5

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type		Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12	4.0	4	Radmanesh F et al. 2013 Mar 7 (23472759)[54]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples: 3	5	0-7	7	4.0	4	Radmanesh F et al. 2013 Mar 7 (23472759)[54]
					2	4					
					1.5	3					
					1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								8	
Experimental Evidence		Evidence Category		Evidence Type		Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function		0.5	0 - 2	2			
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	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	1.0	1	Lee J et al. 2007 Jun (17525174)[55]
				Cell culture model system		1	0 - 2				
				Rescue in animal model		2	0 - 4				
				Rescue in engineered equivalent		1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)								1	

Figure S26: Summary of evidence supporting a relationship between *LAMB1* and autosomal recessive lissencephaly 5.

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	8	1	9	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	MODERATE 11/03/2016		
	EXPERT CURATION (DATE)	MODERATE 11/15/2016		

Figure S27: Summary matrix and classification for *LAMB1* and autosomal recessive lissencephaly 5.

LBR and autosomal recessive anadysplasia-like, spontaneously remitting spondylometaphyseal dysplasia

		Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12	4.0	4	Sobreira N et al. 2015 Jan (25348816); Borovik L et al. 2013 Aug (23824842)[56; 57]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									4	

	Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	1.0	1	Olins AL et al. 2010 Jan-Feb (21327105)[58]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	0.5	0.5	Zwerger M et al. 2010 Jan 15 (19940018)[59]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	1.0	1	Shultz LD et al. 2003 Jan 1 (12490533)[60]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						2.5	

Figure S28: Summary of evidence supporting a relationship between *LBR* and autosomal recessive anadysplasia-like, spontaneously remitting spondylometaphyseal dysplasia.

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	4	2.5	6.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		MODERATE		
EXPERT CURATION (DATE)		MODERATE 12/01/2016		

Figure S27: Summary matrix and classification for *LBR* and autosomal recessive anadysplasia-like, spontaneously remitting spondylometaphyseal dysplasia.

MYO9A and autosomal recessive arthrogryposis

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type		Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12	2.0	2	Bayram Y et al. 2016 Feb (26752647)[61]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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		Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								2	
Experimental Evidence		Evidence Category		Evidence Type		Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function		0.5	0 - 2	2	1.0	1	Chieregatti E et al. 1998 Dec 18 (9819351); Gorman SW et al. 1999 Jul 15 (10409426)[62; 63]
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	0 - 2				
		Functional Alteration		Cells from affected individual		1	0 - 2	2	0.5	0.5	Omelchenko T et al. 2012 Feb 21 (22305756)[64]
				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 Experimental Evidence Points (Maximum 6)								1.5	

Figure S30: Summary of evidence supporting a relationship between *MYO9A* and autosomal recessive arthrogryposis.

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	2	1.5	3.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 09/08/2016		
	EXPERT CURATION (DATE)	LIMITED 11/24/2016 The expert scored this at 3 points, which corresponded to a solid Limited classification.		

Figure S31: Summary matrix and classification for *MYO9A* and autosomal recessive arthrogryposis.

NGLY1 and autosomal recessive congenital disorder of deglycosylation

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12	10.0	10	Need AC et al. 2012 Jun (22581936); Caglayan AO et al. 2015 Jan (25220016); Enns GM et al. 2014 Oct (24651605); Bosch DG et al. 2016 May (26350515); Heeley J et al. 2015 Apr (25707956)[65-69]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples: 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)		2

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	1.0	1	Need AC et al. 2012 Jun (22581936); He P et al. 2015 Aug (25900930)[65; 70]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	2.0	2	Need AC et al. 2012 Jun (22581936); Enns GM et al. 2014 Oct (24651605); Heeley J et al. 2015 Apr (25707956)[65; 67; 69]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	4.0	4	Huang C et al. 2015 Feb 3 (25605922)[71]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
						Total Experimental Evidence Points (Maximum 6)		6

Figure S32: Summary of evidence supporting a relationship between *NGLY1* and autosomal recessive congenital disorder of deglycosylation.

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	10	6	16	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 06/02/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 12/01/2016		

Figure S33: Summary matrix and classification for *NGLY1* and autosomal recessive congenital disorder of deglycosylation.

NHP2 and autosomal recessive dyskeratosis congenital

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12	1.0	2	Vulliamy T et al. 2008 Jun 10 (18523010)[72] (Variant points were downgraded because later papers suggest that the null variant may still result in functional protein product.)
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5		1.0		
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									2	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.5	1.5	Trahan C et al. 2010 Mar 1 (20008900); Freund A et al. 2014 Dec 4 (25467444)[73; 74]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	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	2.5	2.5	Dez C et al. 2001 Feb 1 (11160879); Vulliamy T et al. 2008 Jun 10 (18523010); Vulliamy T et al. 2008 Jun 10 (18523010)[72; 75]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									4	

Figure S34: Summary of evidence supporting a relationship between *NHP2* and autosomal recessive dyskeratosis congenital.

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	2	4	6	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 08/04/2016		
	EXPERT CURATION (DATE)	LIMITED 01/25/2017 During expert review, the expert added more experimental evidence; however, the clinical validity classification remained limited.		

Figure S35: Summary matrix and classification for *NHP2* and autosomal recessive dyskeratosis congenital.

PALB2 and autosomal dominant hereditary breast cancer

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10	7.5	7.4	Erkko H et al. 2007 Mar 15 (17287723); Heikkinen T et al. 2009 May 1 (19383810); Casadei S et al. 2011 Mar 15 (21285249); Hartley T et al. 2014 (25225577); Janatova M et al. 2013 Dec (24136930) [76-80]
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	0.0	0	
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	3.0	3	Hartley T et al. 2014 (25225577); Janatova M et al. 2013 Dec (24136930) [79; 80]
						2	4					
						1.5	3					
						1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12	5.0	5	Erkko H et al. 2007 Mar 15 (17287723); Heikkinen T et al. 2009 May 1 (19383810) [76; 77]
		Aggregate Variant Analysis					0-6		12	4.0	4	Cybulski C et al. 2015 Jun (25959805) [81]
		Total Genetic Evidence Points (Maximum 12)									17	

Experimental Evidence	Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
	Function	Biochemical Function	0.5	0 - 2	2	1.0	1	Xia B et al. 2006 Jun 23 (16793542) [82]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	2.0	2	Erkko H et al. 2007 Mar 15 (17287723) [76]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	2.0	2	Bowman-Colin C et al. 2013 May 21 (23657012) [83]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						5	

Figure S36: Summary of evidence supporting a relationship between *PALB2* and autosomal dominant hereditary breast cancer.

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	12	5	17	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 06/02/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 12/01/2016		

Figure S37: Summary matrix and classification for *PALB2* and autosomal dominant hereditary breast cancer.

PMS2 and pancreatic cancer

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	0.0	0	
				Proband with predicted or proven null variant		1.5	0-2	10	0.0	0	
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	0.0	0	
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12	0.0	0	
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5		0.0		
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7	0.0	0	
					2	4					
					1.5	3					
					1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Guidelines: Points/Study		Guidelines: Max	Scores: Points	Scores: Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12	0.0	0	
		Aggregate Variant Analysis				0-6		12	0.0	0	
		Total Genetic Evidence Points (Maximum 12)								0	No reports of variants in this gene associated with this condition.

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	0.0	0	
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	0.0		
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	0.0		
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						0	Experimental evidence not evaluated. Since no genetic evidence has been reported, the classification is automatically "No Evidence Reported."

Figure S38: Summary of evidence supporting a relationship between *PMS2* and pancreatic cancer.

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		0	0	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		NO REPORTED EVIDENCE 07/18/2016		
EXPERT CURATION (DATE)		NO REPORTED EVIDENCE 12/01/2016		

Figure S39: Summary matrix and classification for *PMS2* and pancreatic cancer.

PSD3 and autosomal dominant antecubital pterygium syndrome

Genetic Evidence

Case-Level Data

Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
		Proband with predicted or proven null variant			1.5	0-2	10			
		Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	0.5	0.5	Bayram Y et al. 2016 Feb (26752647)[61] (Only unrelated probands considered.)
	Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
		Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	4.0	4	Bayram Y et al. 2016 Feb (26752647)[61] (LOD score 1.8)
				2	4					
				1.5	3					
				1	1.5					

Case-Control Data

Case-Control Study Type	Case-Control Quality Criteria	Guidelines Points/Study	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Single Variant Analysis	1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6	12			
Aggregate Variant Analysis		0-6	12			

Total Genetic Evidence Points (Maximum 12): 4.5

Experimental Evidence

Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Function	Biochemical Function	0.5	0 - 2	2			
	Protein Interaction	0.5	0 - 2				
	Expression	0.5	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 Experimental Evidence Points (Maximum 6): 0

Figure S40: Summary of evidence supporting a relationship between *PSD3* and autosomal dominant antecubital pterygium syndrome.

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	4.5	0	4.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 06/03/2016		
	EXPERT CURATION (DATE)	LIMITED 11/24/2016		

Figure S41: Summary matrix and classification for *PSD3* and autosomal dominant antecubital pterygium syndrome.

RAD51C and autosomal recessive Fanconi anemia

		Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12		1	Vaz F et al. 2010 May (20400963)[84]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5		1.0		
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	1.0	1	Vaz F et al. 2010 May (20400963)[84]
						2	4					
						1.5	3					
						1	1.5					

		Case-Control Study Type	Case-Control Quality Criteria	Guidelines Points/Study	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Genetic Evidence	Case-Control Data	Single Variant Analysis	1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6	12			
		Aggregate Variant Analysis		0-6	12			
		Total Genetic Evidence Points (Maximum 12)					**2**	

	Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	0.5	0.5	Somyajit K et al. 2012 Jan 27 (22167183)[85]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	3.0	2	Vaz F et al. 2010 May (20400963); Somyajit K et al. 2012 Jan 27 (22167183) [84; 85]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	3.0	3	Vaz F et al. 2010 May (20400963)[84]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
	Total Experimental Evidence Points (Maximum 6)						**5.5**	

Figure S42: Summary of evidence supporting a relationship between *RAD51C* and autosomal recessive Fanconi anemia.

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	2	5.5	7.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	MODERATE 06/01/2016		
	EXPERT CURATION (DATE)	MODERATE 01/05/2017		

Figure S43: Summary matrix and classification for *RAD51C* and autosomal recessive Fanconi anemia.

RAD51D and autosomal dominant hereditary breast cancer

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
				Proband with predicted or proven null variant			1.5	0-2	10	1.0	1	Baker JL et al. 2015 Feb (25445424); Loveday C et al. 2011 Aug 7 (21822267); Pelttari LM et al. 2012 Jul (22652533); Osher DJ et al. 2012 Apr 10 (22415235)[86-89] (Due to the common nature of the disease, opting to give 0.1 points per case.)
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									4.5	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.5	1.5	Schild D et al. 2000 Jun 2 (10749867); Martin RW et al. 2007 Oct 15 (17942895)[90; 91]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	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	2.0	2	Smiraldo PG et al. 2005 Mar 15 (15781618)[92]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									3.5	

Figure S44: Summary of evidence for a relationship between *RAD51D* and autosomal dominant hereditary breast cancer.

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	1	3.5	4.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	YES	Dowty JG et al. 2008 Nov (18058226); Jara L et al. 2010 Aug (20054644); Wickramanayake A et al. 2012 Dec (22986143); Gutiérrez-Enríquez S et al. 2014 May 1 (24130102)[93-96] (These studies have reported OR/HR that indicate no association between RAD51D and breast cancer, or found any truncating variants in breast cancer-only cases/families.)		
	CALCULATED CLASSIFICATION (DATE)	CONFLICTING EVIDENCE REPORTED		
EXPERT CURATION (DATE)		LIMITED 12/01/2016		

Figure S45: Summary matrix and classification for *RAD51D* and autosomal dominant hereditary breast cancer. The discrepancy between the experts and original biocurators is due to interpretation of the case-control studies. According to the experts consulted, current studies are not large enough to address the question of whether or not variants in *RAD51D* are relevant to breast cancer. Experimental evidence shows a link between *RAD51D* and homologous recombination, a function of other genes, such as *BRCA1* and *BRCA2*, known to be involved in hereditary breast cancer.

RPS10 and autosomal dominant Diamond-Blackfan anemia

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12	4.0	4	Doherty L et al. 2010 Feb 12 (20116044); Smetanina NS et al. 2015 Sep (25946618)[97; 98]
				Proband with predicted or proven null variant			1.5	0-2	10	10.0	10	Doherty L et al. 2010 Feb 12 (20116044); Smetanina NS et al. 2015 Sep (25946618); Yazaki M et al. 2012 May (22510774)[97-99]
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	0.0	0	
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									12	
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.5	1.5	Havugimana PC et al. 2012 Aug 31 (22939629); Kristensen AR et al. 2012 Sep (22863883); Doherty L et al. 2010 Feb 12 (20116044)[97; 100; 101]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	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 Experimental Evidence Points (Maximum 6)									1.5	

Figure S46: Summary of evidence supporting a relationship between *RPS10* and autosomal dominant Diamond-Blackfan anemia.

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	12	1.5	13.5	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 07/04/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 01/19/2017		

Figure S47: Summary matrix and classification for *RPS10* and autosomal dominant Diamond-Blackfan anemia.

RPS24 and autosomal dominant Diamond-Blackfan anemia

						Guidelines			Scores		PMIDs/Notes
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	6.0	6	Quarello P et al. 2010 Feb (19773262); Landowski M et al. 2013 Nov (23812780); Smetanina NS et al. 2015 Sep (25946618)[98; 102; 103]
				Proband with predicted or proven null variant		1.5	0-2	10	4.5	4.5	Gazda HT et al. 2006 Dec (17186470)[104]
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		PMIDs/Notes
		Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	
	Case-Control Data	Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)	10.5	
		Evidence Category		Evidence Type		Guidelines			Scores		PMIDs/Notes
						Default	Range	Max	Points	Tally	
Experimental Evidence		Function		Biochemical Function		0.5	0 - 2	2	1.5	1.5	Choesmel V et al. 2008 May 1 (18230666); Havugimana PC et al. 2012 Aug 31 (22939629); Gazda HT et al. 2006 Dec (17186470)[100; 104; 105]
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	0 - 2				
		Functional Alteration		Cells from affected individual		1	0 - 2	2	2.0	2	Choesmel V et al. 2008 May 1 (18230666)[105]
				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 Experimental Evidence Points (Maximum 6)	3.5	

Figure S48: Summary of evidence supporting a relationship between *RPS24* and autosomal dominant Diamond-Blackfan anemia.

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	10.5	3.5	14	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 07/04/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 01/17/2017		

Figure S49: Summary matrix and classification for *RPS24* and autosomal dominant Diamond-Blackfan anemia.

SCN4B and autosomal dominant Long QT Syndrome

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	1.0	1	Medeiros-Domingo A et al. 2007 Jul 10 (17592081)[106] (Only unrelated probands considered.)
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7	1.0	1	Medeiros-Domingo A et al. 2007 Jul 10 (17592081)[106]
					2	4					
					1.5	3					
					1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Guidelines			Scores		PMIDs/Notes
						Points/Study		Max	Points	Tally	
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								3	
Experimental Evidence		Evidence Category		Evidence Type		Guidelines			Scores		PMIDs/Notes
						Default	Range	Max	Points	Tally	
		Function		Biochemical Function		0.5	0 - 2	2	1.0	1	Medeiros-Domingo A et al. 2007 Jul 10 (17592081)[106] (8x increase in late sodium current by mutant form)
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	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 Experimental Evidence Points (Maximum 6)								1	

Figure S50: Summary of evidence supporting a relationship between *SCN4B* and autosomal dominant Long QT Syndrome.

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	2	1	3	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		LIMITED 06/14/2016		
EXPERT CURATION (DATE)		LIMITED 12/15/16		

Figure S51: Summary matrix and classification for *SCN4B* and autosomal dominant Long QT Syndrome.

SKI and autosomal dominant Shprintzen-Goldberg syndrome

Genetic Evidence	Case-Level Data	Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12	12.0	12	Carmignac V et al. 2012 Nov 2 (23103230); Doyle AJ et al. 2012 Nov (23023332)[107; 108]
				Proband with predicted or proven null variant			1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	5.0	5	Doyle AJ et al. 2012 Nov (23023332); Carmignac V et al. 2012 Nov 2 (23103230)[107; 108]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	2.0	2	Carmignac V et al. 2012 Nov 2 (23103230)[107]
						2	4					
						1.5	3					
						1	1.5					
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria			Guidelines Points/Study		Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance			0-6		12			
		Aggregate Variant Analysis					0-6		12			
		Total Genetic Evidence Points (Maximum 12)									16.5	Additional genetic evidence available beyond 12 point maximum score.
Experimental Evidence		Evidence Category		Evidence Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
		Function		Biochemical Function			0.5	0 - 2	2	1.5	1.5	Doyle AJ et al. 2012 Nov (23023332)[108]
				Protein Interaction			0.5	0 - 2				
				Expression			0.5	0 - 2				
		Functional Alteration		Cells from affected individual			1	0 - 2	2	1.0	1	Doyle AJ et al. 2012 Nov (23023332)[108]
				Engineered cells			0.5	0 - 1				
		Models & Rescue		Animal model			2	0 - 4	4	2.0	2	Doyle AJ et al. 2012 Nov (23023332)[108]
				Cell culture model system			1	0 - 2				
				Rescue in animal model			2	0 - 4				
				Rescue in engineered equivalent			1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)									4.5	Additional experimental data may be available.

Figure S52: Summary of evidence supporting a relationship between *SKI* and autosomal dominant Shprintzen-Goldberg syndrome.

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	12	4.5	16.5	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 06/02/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 12/01/2016		

Figure S53: Summary matrix and classification for *SKI* and autosomal dominant Shprintzen-Goldberg syndrome.

SMAD3 and autosomal dominant aneurysm-osteoarthritis syndrome

Genetic Evidence

Case-Level Data

Evidence Type		Case Information Type			Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo			2	0-3	12			
		Proband with predicted or proven null variant			1.5	0-2	10	5.0	5	van de Laar IM et al. 2011 Feb (21217753); Regalado ES et al. 2011 Sep 2 (21778426); van de Laar IM et al. 2012 Jan (22167769)[109-111]
		Proband with other variant type with some evidence of gene impact			0.5	0-1.5	7	4.0	4	van de Laar IM et al. 2011 Feb (21217753); Regalado ES et al. 2011 Sep 2 (21778426); van de Laar IM et al. 2012 Jan (22167769)[109-111]
	Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant			2	0-3	12			
		Two variants (not predicted/proven null) with some evidence of gene impact in trans			1	0-1.5				
Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples	3	5	0-7	7	7.0	7	van de Laar IM et al. 2011 Feb (21217753); Regalado ES et al. 2011 Sep 2 (21778426)[109; 110]
				2	4					
				1.5	3					
				1	1.5					

Case-Control Data

Case-Control Study Type	Case-Control Quality Criteria	Guidelines Points/Study	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Single Variant Analysis	1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance	0-6	12			
Aggregate Variant Analysis		0-6	12			

Total Genetic Evidence Points (Maximum 12)	17	Additional genetic evidence is available, but not curated due to achievement of maximum genetic evidence score.

Experimental Evidence

Evidence Category	Evidence Type	Guidelines Default	Guidelines Range	Guidelines Max	Scores Points	Scores Tally	PMIDs/Notes
Function	Biochemical Function	0.5	0 - 2	2	1.0	1	Verrecchia F et al. 2007 Jun 14 (17589920); van de Laar IM et al. 2011 Feb (21217753)[109; 112]
	Protein Interaction	0.5	0 - 2				
	Expression	0.5	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	4.0	4	Yang X et al. 2001 Apr 2 (11285272); Tan CK et al. 2013 Jun 19 (23782924)[113; 114]
	Cell culture model system	1	0 - 2				
	Rescue in animal model	2	0 - 4				
	Rescue in engineered equivalent	1	0 - 2				

Total Experimental Evidence Points (Maximum 6)	5	

Figure S54: Summary of evidence supporting a relationship between *SMAD3* and autosomal dominant aneurysm-osteoarthritis syndrome.

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	12	5	17	YES
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	DEFINITIVE 03/30/2016		
	EXPERT CURATION (DATE)	DEFINITIVE 12/01/2016		

Figure S55: Summary matrix and classification for *SMAD3* and autosomal dominant aneurysm-osteoarthritis syndrome.

SMARCA1 and autosomal dominant syndromic intellectual disability with Coffin-Syris-like features

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10	1.5	1.5	Karaca E et al. 2015 Nov 4 (26539891)[115]
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	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		Guidelines			Scores		PMIDs/Notes
						Points/Study		Max	Points	Tally	
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								1.5	
Experimental Evidence		Evidence Category		Evidence Type		Guidelines			Scores		PMIDs/Notes
						Default	Range	Max	Points	Tally	
		Function		Biochemical Function		0.5	0 - 2	2	1.0	1	Lopes F et al. 2016 Mar (26740508)[116]
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	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	2.0	2	Lopes F et al. 2016 Mar (26740508)[116]
				Cell culture model system		1	0 - 2				
				Rescue in animal model		2	0 - 4				
				Rescue in engineered equivalent		1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)								3	

Figure S56: Summary of evidence supporting a relationship between *SMARCA1* and autosomal dominant syndromic intellectual disability with Coffin-Syris-like features.

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	1.5	3	4.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	LIMITED 06/14/2016		
	EXPERT CURATION (DATE)	MODERATE 11/15/2016		

Figure S57: Summary matrix and classification for *SMARCA1* and autosomal dominant syndromic intellectual disability with Coffin-Syris-like features.

SOS2 and autosomal dominant Noonan syndrome

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	4.0	4	Yamamoto GL et al. 2015 Jun (25795793); Cordeddu V et al. 2015 Nov (26173643)[117; 118]
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7	3.0	3	Yamamoto GL et al. 2015 Jun (25795793); Cordeddu V et al. 2015 Nov (26173643)[117; 118]
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)		7

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	0.5	0.5	Cordeddu V et al. 2015 Nov (26173643)[118]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	0.5	0.5	Cordeddu V et al. 2015 Nov (26173643)[118]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	0.0	0	Esteban LM et al. 2000 Sep (10938118)[119] (No points are given. A knock-out mouse described here and this disease mechanism is gain of function.)
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
						Total Experimental Evidence Points (Maximum 6)		1

Figure S58: Summary of evidence supporting a relationship between *SOS2* and autosomal dominant Noonan syndrome.

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	7	1	8	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
	CALCULATED CLASSIFICATION (DATE)	MODERATE 05/26/2016		
	EXPERT CURATION (DATE)	MODERATE 12/05/16		

Figure S59: Summary matrix and classification for *SOS2* and autosomal dominant Noonan syndrome.

TMPO and autosomal dominant dilated cardiomyopathy

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12	0.0	0	Taylor MR et al. 2005 Dec (16247757)[120] (c.2068C>T is classified as Benign/Likely Benign by ClinVar submitters.)
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12			
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5				
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		
		Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
	Case-Control Data	Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)		0
						Guidelines			Scores		
		Evidence Category		Evidence Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence		Function		Biochemical Function		0.5	0 - 2	2	0.5	0.5	Taylor MR et al. 2005 Dec (16247757) [120] (Interaction of mutated protein product with A-type Lamins)
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	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 Experimental Evidence Points (Maximum 6)		0.5

Figure S60: Summary of evidence for a relationship between *TMPO* and autosomal dominant dilated cardiomyopathy.

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		**0.5**	**0.5**	**NO**
CALCULATED CLASSIFICATION		**LIMITED**	**1-6**	
		MODERATE	**7-11**	
		STRONG	**12-18**	
		DEFINITIVE	**12-18 AND replication over time**	
Valid contradictory evidence (Y/N)*	YES	Taylor MR et al. 2005 Dec (16247757) [120] (The only variant that has been reported in association with human disease has been found at high publication frequency in ExAC.[7])		
CALCULATED CLASSIFICATION (DATE)		**CONFLICTING EVIDENCE REPORTED 10/07/16**		
EXPERT CURATION (DATE)		**REFUTED 11/30/2016**		

Figure S61: Summary matrix and classification for *TMPO* and autosomal dominant dilated cardiomyopathy.

VPS8 and autosomal recessive arthrogryposis

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12	0.0	0.5	Bayram Y et al. 2016 Feb (26752647)[61]
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5		0.5		
		Segregation Evidence		Evidence of segregation in one or more families	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		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
		Total Genetic Evidence Points (Maximum 12)								0.5	
Experimental Evidence		Evidence Category		Evidence Type		Default	Range	Max	Points	Tally	PMIDs/Notes
		Function		Biochemical Function		0.5	0 - 2	2	1.5	1.5	Horazdovsky BF et al. 1996 Dec 27 (8969229); Epp N et al. 2013 (23840658)[121; 122]
				Protein Interaction		0.5	0 - 2				
				Expression		0.5	0 - 2				
		Functional Alteration		Cells from affected individual		1	0 - 2	2	0.0	0	
				Engineered cells		0.5	0 - 1				
		Models & Rescue		Animal model		2	0 - 4	4	0.0	0	
				Cell culture model system		1	0 - 2				
				Rescue in animal model		2	0 - 4				
				Rescue in engineered equivalent		1	0 - 2				
		Total Experimental Evidence Points (Maximum 6)								1.5	

Figure S62: Summary of evidence supporting a relationship between *VPS8* and autosomal recessive arthrogryposis.

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	0.5	1.5	2	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		LIMITED		
EXPERT CURATION (DATE)		LIMITED 11/24/2016		

Figure S63: Summary matrix and classification for *VPS8* and autosomal recessive arthrogryposis.

WRAP53 and autosomal recessive dyskeratosis congenital

						Guidelines			Scores		
		Evidence Type		Case Information Type		Default	Range	Max	Points	Tally	PMIDs/Notes
Genetic Evidence	Case-Level Data	Variant Evidence	Autosomal Dominant or X- linked Disorder	Variant is de novo		2	0-3	12			
				Proband with predicted or proven null variant		1.5	0-2	10			
				Proband with other variant type with some evidence of gene impact		0.5	0-1.5	7			
			Autosomal Recessive Disease	Two variants in trans and at least one de novo or a predicted/proven null variant		2	0-3	12		3.5	Zhong F et al. 2011 Jan 1 (21205863)[123] (The expert chose to upgrade the variant points because of the specificity of the phenotype.)
				Two variants (not predicted/proven null) with some evidence of gene impact in trans		1	0-1.5		3.5		
		Segregation Evidence		Evidence of segregation in one or more families	LOD Score Examples 3	5	0-7	7			
					2	4					
					1.5	3					
					1	1.5					
						Guidelines			Scores		
	Case-Control Data	Case-Control Study Type		Case-Control Quality Criteria		Points/Study		Max	Points	Tally	PMIDs/Notes
		Single Variant Analysis		1. Variant Detection Methodology 2. Power 3. Bias and confounding 4. Statistical Significance		0-6		12			
		Aggregate Variant Analysis				0-6		12			
									Total Genetic Evidence Points (Maximum 12)		3.5

			Guidelines			Scores		
	Evidence Category	Evidence Type	Default	Range	Max	Points	Tally	PMIDs/Notes
Experimental Evidence	Function	Biochemical Function	0.5	0 - 2	2	2.5	2	Freund A et al. 2014 Dec 4 (25467444); Zhong F et al. 2011 Jan 1 (21205863); Mahmoudi S et al. 2010 Nov 2 (21072240)[74; 123; 124]
		Protein Interaction	0.5	0 - 2				
		Expression	0.5	0 - 2				
	Functional Alteration	Cells from affected individual	1	0 - 2	2	3.5	2	Zhong F et al. 2011 Jan 1 (21205863); Batista LF et al. 2011 May 22 (21602826)[123; 125]
		Engineered cells	0.5	0 - 1				
	Models & Rescue	Animal model	2	0 - 4	4	2.0	2	Zhong F et al. 2011 Jan 1 (21205863); Mahmoudi S et al. 2010 Nov 2 (21072240)[123; 125]
		Cell culture model system	1	0 - 2				
		Rescue in animal model	2	0 - 4				
		Rescue in engineered equivalent	1	0 - 2				
						Total Experimental Evidence Points (Maximum 6)		6

Figure S64: Summary of evidence supporting a relationship between *WRAP53* and autosomal recessive dyskeratosis congenital.

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	3.5	6	9.5	NO
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	
Valid contradictory evidence (Y/N)*	NO			
CALCULATED CLASSIFICATION (DATE)		MODERATE		
MODIFY CALCULATED CLASSIFICATION		YES		
CURATOR CLASSIFICATION (DATE)		LIMITED 08/04/2016		
EXPERT CURATION (DATE)		MODERATE 01/25/2017		

Figure S65: Summary matrix and classification for *WRAP53* and autosomal recessive dyskeratosis congenital. This gene/disease relationship was initially classified as limited by the curator. During expert review, the expert added more experimental evidence and it was increased to moderate.

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