ClinGen Gene Curation Standardized Evidence Summary Text

***Instructions**: The following are suggestions for how to word the elements important for inclusion in an evidence summary. Choose the text that is relevant for the gene curation at hand (not all text will be relevant for every curation). Bold text and/or text in capital letters acts as a placeholder and should be replaced with specific information; please feel free to edit the suggested text for flow, clarity, etc. as needed.*

[GENE ID] was FIRST reported in relation to **[mode of inheritance] [disease]** in **[year] (Author et al., PMID)**. At least # UNIQUE VARIANTS variants (e.g. missense, in-frame indel, nonsense, frameshift, large deletion, complex rearrangement, etc) have been reported in humans. Evidence **supporting/refuting [edit as appropriate]** this gene-disease relationship includes **DATA-TYPE (e.g. case-level data, case-control data, segregation data, experimental data [choose all that apply])**.

AND

Summary of Case Level Data: **X POINTS**
Variants in this gene have been reported in at least **# or %** probands in **#** publications (**PMIDs**).
Variants in this gene segregated with disease in **#** additional family members.

AND/OR

Summary of Case-Control Data: **X POINTS**
This gene-disease relationship has been studied in at least **XXX** case-control studies (**PMIDs**) at the single variant level and **XXX** case-control studies at the aggregate variant level.
Comment on overall statistics or OR.

For STRONG/DEFINITIVE genes (if applicable):
More evidence is available in the literature, but the maximum score for genetic evidence and/or experimental evidence (12 pts.) has been reached.

The mechanism for disease is **(unknown)** OR **(homozygous loss of function/ haploinsufficiency/ heterozygous gain of function, etc.) (Reference, if available)**

Of note, this gene has also been implicated in **(other disease associations)**. These **will be/have been** assessed separately.

EXPERIMENTAL EVIDENCE
This gene-disease association is supported by **(relevant experimental evidence used e.g. animal models, expression studies, *in vitro* functional assays, etc.)**

Summary Statement
Directions: Choose the one summary statement that corresponds to the appropriate clinical validity.

LIMITED
In summary, there is limited evidence to support this gene-disease relationship. Although more evidence is needed to support a causal role, no convincing evidence has emerged that contradicts the gene-disease relationship. ***(Add relationship-specific comments here)***

MODERATE
In summary, there is moderate evidence to support this gene-disease relationship. While more evidence is needed to establish this relationship definitively, no convincing contradictory evidence has emerged.

STRONG
In summary, there is strong evidence to support the relationship between **GENE** and **DISEASE (mode of inheritance)**. **(Additional reports in humans are needed)/(Three years must elapse from the first proposal of the association)** to reach a definitive classification.

DEFINITIVE
In summary, **GENE** is definitively associated with **INHERITANCE & DISEASE**.
Optional: This has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time.

REFUTED
In summary, there is convincing evidence refuting the relationship between **GENE** and **INHERITANCE & DISEASE**, which significantly outweighs the evidence supporting the relationship.

DISPUTED
In summary, there is convincing evidence disputing the relationship between **GENE** and **INHERITANCE & DISEASE**. More evidence is needed to either support or refute the role **GENE** plays in this disease.

NO REPORTED EVIDENCE
No convincing evidence for a causal role for **GENE** in **INHERITANCE AND DISEASE** has been reported. Although this gene-disease association is supported by (relevant experimental evidence e.g. animal models, expression studies, *in vitro* functional assays, etc.), no reports have directly implicated the gene in humans.

AND

ADD to ALL CURATIONS

This classification was approved by the ClinGen **(Clinical Domain)** Working Group on **DATE** (SOP Version XXX).

Please add any other important notes of interest

If applicable, add text regarding LUMPING AND SPLITTING CONSIDERATIONS:

List OMIM and Orphanet disease entity names.

LUMPING:
Per criteria outlined by the ClinGen Lumping and Splitting Working Group, we found no difference in molecular mechanism(s) AND/OR inheritance pattern AND/OR phenotypic variability.

ADD GENE-SPECIFIC CURATION RATIONALE.

Therefore, all of the disease entities have been lumped into one disease entity, **DISEASE ENTITY NAME**

SPLITTING:
Per criteria outlined by the ClinGen Lumping and Splitting Working Group, we found differences in molecular mechanism(s) AND/OR inheritance pattern AND/OR phenotypic variability.

ADD GENE-SPECIFIC CURATION RATIONALE.

Therefore, we have split curations for the disease entities, DISEASE ENTITY NAMES

Example 1: *TMC1* and autosomal recessive sensorineural hearing loss
Variants in the *TMC1* gene has been reported in more than 20 probands with autosomal recessive (AR) sensorineural hearing loss since 2002 (Kurima *et al.*). More than 20 unique variants predicted to cause a loss of function of the protein have been reported, suggesting homozygous loss of function is the mechanism of hearing loss for this gene. This gene-disease relationship is supported by animal models, rescue experiments, and expression studies. In summary, *TMC1* is **definitively** associated with AR sensorineural hearing loss. This has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time. This classification was approved by the ClinGen Hearing Loss Working Group on 2/15/2017.

Example 2: *DMXL2* and autosomal dominant sensorineural hearing loss
A variant in the *DMXL2* gene has been reported in 1 proband with autosomal dominant sensorineural hearing loss in a single publication (Chen *et al.,* 2016). The variant in this gene

was identified by linkage analysis followed by whole-exome sequencing and Sanger confirmation, and segregated with disease in 16 additional family members. This gene-disease relationship is further supported by a zebrafish model and expression studies. In summary, there is **moderate** evidence to support this gene-disease association. While more evidence is needed to establish this relationship definitively, no convincing contradictory evidence has emerged. This classification was approved by the ClinGen Hearing Loss Working Group on 2/6/2017.

Example 3: *CEP78* and Cone-rod dystrophy with hearing loss

The *CEP78* gene has been reported in 9 probands with autosomal recessive cone-rod dystrophy in 3 different publications. Seven unique variants predicted to cause a loss of function of the protein, and variants segregated with disease in 3 additional family members. Variants in *CEP78* were first reported in individuals with this disease in 2016 (Nikopoulos et al, Namburi et al). This gene-disease relationship is supported by evidence of expression in the inner ear and retina and interaction with a known retinitis pigmentosa gene, *FAM161A*. In summary, there is **strong** evidence to support the association between CEP78 and AR cone-rod dystrophy with hearing loss. Three years must elapse from the first proposal of the association to reach a definitive classification.

Example 4: *PLN*-Intrinsic Cardiomyopathy

OMIM: Cardiomyopathy, dilated, 1P (MIM: 609909)
Cardiomyopathy, hypertrophic, 18 (MIM:613874)

Orphanet: Familial isolated dilated cardiomyopathy (ORPHA:154)

Variants in the *PLN* gene have been observed in individuals with hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy (van der Zwaag, 2012 PMID: 22820313) and heart failure of unknown etiology. The typical inheritance for *PLN*-related cardiomyopathy is autosomal dominant. Autosomal recessive inheritance has been noted, however, and appears to follow a dosage effect or semidominance, as loss of both alleles results in an earlier and more severe phenotypic presentation (Haghighi, 2003 PMID: 12639993). *PLN* is encoded by one exon, and missense, nonsense, and frameshift variants in the coding exon and the promoter region have been reported. Given that *PLN* only has one exon, many of the variants (even LOF) result in a protein product. *PLN* encodes the 52 amino acid protein, phospholamban, that functions to regulate *SERCA2* function in the sarcoplasmic reticulum. Phospholamban exists in both monomeric and homopentameric forms. The monomeric form is thought to inhibit *SERCA2* activity. *PLN*-mediated *SERCA2* inhibition is released upon phosphorylation of monomeric PLN by either *PKA* or *CAMKII*, resulting in the stabilization of the pentameric form (reviewed in Haghighi, 2014 PMID: 25451386, Young, 2015 PMID: 25563649). While the distinct genetic mechanism of *PLN*-mediated cardiomyopathy is unclear, the overall disease mechanism for *PLN* associated cardiomyopathy is dysregulation of *SERCA2* function, Ca^{2+} handling, and disrupted relaxation and contraction of the heart. Multiple cases of *PLN*-mediated cardiomyopathy are reported in the literature, extending beyond the

maximum score for genetic evidence (12 pts). This gene-disease association is supported by the function of the gene product, alteration of normal function in non-patient cells expressing patient-derived mutant *PLN*, and animal models. In summary, *PLN* is definitively associated with intrinsic cardiomyopathy. This association has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time. **This classification was approved by the Hypertrophic Cardiomyopathy Gene Curation committee on September 19, 2017.**

Lumping and Splitting: Per criteria outlined by the ClinGen Lumping and Splitting Working Group, we found no difference in molecular mechanism(s) underlying the disease entities: (1) Cardiomyopathy, dilated, 1P (MIM: 609909) and (2) Cardiomyopathy, hypertrophic, 18 (MIM:613874). Furthermore, a progressive cardiomyopathy beginning with hypertrophic and leading to dilated has been observed in a proband (Haghighi, 2003 PMID: 12639993). Both interfamilial and intrafamilial variability were observed between *PLN* variants (Haghighi, 2003 PMID: 12639993; van der Zwaag, 2012 PMID: 22820313). For clinical management, no striking differences should occur, as all of the phenotypes and conditions associated with *PLN* are of cardiovascular nature, and individuals should be monitored appropriately. Therefore, all of the disease entities have been lumped into one disease entity, ***Intrinsic cardiomyopathy.***

Data on the occurrence of cardiomyopathy in individuals with *PLN* associated intrinsic cardiomyopathy was collected by the Hypertrophic Cardiomyopathy Gene Curation Committee (subgroup of the Cardiovascular Working Group). Mutation of *PLN* results in the development of an intrinsic cardiomyopathy that can present with left ventricular hypertrophy.

Example 5: ***TTR*-Hereditary ATTR (Transthyretin Amyloidosis) Amyloidosis**

OMIM:	Amyloidosis, hereditary, transthyretin-related (MIM:105210)
	Carpal tunnel syndrome, familial (MIM:115430)
	Dystransthyretinemic hyperthyroxinemia (MIM:145680)
Orphanet:	ATTRV122I amyloidosis (ORPHA:85451)
	ATTRV30M amyloidosis (ORPHA:85447)
	Hereditary ATTR amyloidosis (ORPHA:271861)

Variants in the *TTR* gene have been observed in individuals with amyloidosis, an autosomal dominant syndromic disorder characterized by deposition of extracellular transthyretin amyloid deposits throughout the body. Of note, autosomal recessive inheritance has been reported (reviewed in Jacobson et al., 2016, PMID:27652282) . Individuals with *TTR*- related amyloidosis can present with multi-systemic phenotypes including, polyneuropathy, carpal tunnel syndrome, cardiomyopathy, gastrointestinal features, autonomic insufficiency, and renal insufficiency. Familial amyloidosis was first described in 1952 (Andrade et al.; PMID 12978172), but it was not until 1984 (Saraiva et al.; PMID 6736244) that *TTR* was put forth as the causative gene for this initial report of familial amyloidosis. There are >100 known variants in *TTR* reported in humans world-wide as documented in Mutations in Hereditary Amyloidosis Registry (http://www.amyloidosismutations.com) and the Indiana University- Purdue University

Indianapolis (IUPUI), USA Amyloidosis Research group site (http://www.iupui.edu/~amyloid/amy_info.html). All reported variants are missense (Rowczenio et al., 2014). Multiple cases are reported in the literature but the maximum score for genetic evidence (12 pts) has been reached. The mechanism for disease is gain of function; disease is caused by an accelerated increase in the ability of ATTR to accumulate as amyloid fibrils in tissue (Sekijima, 2015; PMID 25604431). Note, wild-type ATTR (wtATTR), the protein product of the *TTR* gene, can cause amyloidosis (senile cardiac amyloidosis) on its own, but this form is age-dependent, and often occurs at a much greater age of onset than individuals who harbor a pathogenic variant in *TTR*. This gene-disease relationship is supported by the function of the gene product, decreased myocyte functional activity in non-patient cells treated with fibril forming *TTR*, animal models, rescue experiments, and studies of the clinical impact of anti-sense-oligonucleotides (ASOs) for the treatment of amyloidosis in humans (http://ttrstudy.com). In summary, *TTR* is definitively associated with Amyloidosis. This association has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time. This classification was approved by Jonathan Berg, M.D., Ph.D. on August 30, 2017.

Lumping and Splitting: Per criteria outlined by the ClinGen Lumping and Splitting Working Group, we found that no difference in molecular mechanism underlying the two disease entities: (1) Amyloidosis, hereditary, transthyretin-related (MIM:105210), and (2) Carpal tunnel syndrome, familial (MIM:115430). Furthermore, carpal tunnel syndrome is often a presenting phenotype that occurs in hereditary amyloidosis (MIM:105210). Therefore, the two entities (MIM:105210 and MIM:115430) have been lumped into one disease entity, ***Hereditary ATTR amyloidosis***.

Data on the occurrence of hypertrophic cardiomyopathy (HCM) in individuals with Hereditary ATTR amyloidosis was collected by the Hypertrophic Cardiomyopathy Gene Curation Committee (subgroup of the Cardiovascular Working Group). Some individuals with Hereditary ATTR amyloidosis present with features of hypertrophic cardiomyopathy (HCM) and/or restrictive cardiomyopathy (RCM). The prevalence of HCM and/or RCM in Hereditary ATTR amyloidosis is high but exact percentages have not been accurately defined due the high prevalence of HCM and RCM (~25%) development in senile cardiac amyloidosis, which occurs due to accumulation of wildtype TTR accumulation in the heart with age (Galant et al., 2017 PMID 28213611; Meier-Ewert et al., 2011 PMID 21989745; Perlini et al, 2016 PMID 27900617; Ruberg and Berk, 2012 PMID 22949539). Several variants including TTR-V30M, TTR-T60A, and TTR-V122L are the most commonly associated with cardiac amyloidosis (Siddiqi and Ruberg, 2017 PMID 28739313).

Example 6: *CRYAB*-Alpha-crystallinopthy

OMIM:	Cardiomyopathy, dilated, 1II (MIM: 615184)
	Cataract 16, multiple types (MIM: 613763)
	Myopathy, myofibrillar, 2 (MIM: 608810)

Myopathy, myofibrillar, fatal infantile hypertrophy, alpha-B crystallin-related (MIM: 613869)

Orphanet: **Alpha-crystallinopathy (ORPHA:98910**)

Alpha-B crystallin-related late-onset distal myopathy (ORPHA:399058)
Early-onset lamellar cataract (ORPHA:441452)
Early-onset nuclear cataract (ORPHA:98991)
Early-onset posterior polar cataract (ORPHA:98993)
Familial isolated dilated cardiomyopathy (ORPHA:154)
Fatal infantile hypertonic myofibrillar myopathy (ORPHA: 280553)

Variants in the *CRYAB* gene have been reported in individuals with syndromic myofibrillar myopathy, a condition with a variable clinical presentation. Alpha-B-crystallin (*CRYAB*)-related myofibrillar myopathy is typically characterized by muscle weakness, cardiomyopathy, cataracts, respiratory failure, dysphagia, and dysphonia. Typical inheritance for *CRYAB*-related myopathy is autosomal dominant; however, autosomal recessive inheritance has been noted, and appears to follow a dosage effect or semidominance, as loss of both alleles results in earlier and more severe phenotypic presentation (OMIM; MIM:613869). Three percent of reported cases of myofibrillar myopathies are attributed to *CRYAB* mutations (Selcen and Engel, 2005; GeneReviews PMID: 20301672). The majority of causative variants are missense, but nonsense and frameshift variants have also been reported. Given that *CRYAB* has only three exons, most variants (even LOF) still produce a protein product. *CRYAB* belongs to the small heat shock protein family, and functions to prevent protein aggregation in cells (Sanbe, 2004 PMID: 22040875). Multiple cases are reported in the literature but the maximum score for genetic evidence (12 pts) has been reached. The mechanism for disease is unclear and could involve both loss of function and gain of function, but abnormal aggregation of CRYAB proteins in cells is associated with disease (Sanbe, 2004 PMID: 22040875). This gene-disease relationship is supported by the function of the gene product, alteration in normal function in cells expressing patient-derived mutant CRYAB, and animal models. In summary, *CRYAB* is definitively associated with Alpha-crystallinopathy. This association has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time. This classification was approved by Jonathan Berg, M.D., Ph.D.on August 30, 2017.

Lumping and Splitting: Per criteria outlined by the ClinGen Lumping and Splitting Working Group, we found no difference in molecular mechanism underlying the disease entities: (1) Cardiomyopathy, dilated, 1II (MIM: 615184); (2) Cataract 16, multiple types (MIM: 613763); (3) Myopathy, myofibrillar, 2 (MIM: 608810), (4) Myopathy, myofibrillar, fatal infantile hypertrophy, alpha-B crystallin-related (MIM: 613869). Furthermore, the fatal infantile myofibrillar myopathy that is inherited in an autosomal recessive manner functions in a dose-dependent or semi-dominant manner. Interfamilial phenotypic variability is also observed between *CRYAB* variants. Therefore, all of the disease entities have been lumped into one disease entity, ***Alpha-crystallinopathy.***

Data on the occurrence of cardiomyopathy in individuals with Alpha-crystallinopathy was collected by the Hypertrophic Cardiomyopathy Gene Curation Committee (subgroup of the Cardiovascular Working Group). Protein-altering variation in *CRYAB* results in the development of cardiomyopathy (hypertrophic, restrictive, and dilated). The mechanism for *CRYAB*-induced cardiomyopathy is attributed to progressive mitochondrial dysfunction, as well as induction of apoptosis in cardiomyocytes, leading to cell death and reduced contractile function (Sanbe 2004, PMID: 15220483; Sanbe, 2004 PMID: 22040875).