

APPENDIX B: EXPERIMENTAL EVIDENCE EXAMPLES

FUNCTION

Biochemical function:

- **Example: *MYH7* and hypertrophic cardiomyopathy (HCM)**
Variants in *MYH7* have been identified in patients with HCM. *MYH7* encodes the beta-myosin heavy chain, the major protein comprising the thick filament of the cardiac sarcomere. Genes encoding other thick filament cardiac sarcomeric proteins, including *MYBPC3*, *MYL2*, *MYL3*, have been definitively associated with HCM. Therefore, the function of *MYH7* is shared with other known genes in the disease of interest. (Default: 0.5 points)
- **Example: *Biallelic mutations in DRAM2* cause retinal dystrophy.**
El-Asrag et al, 2016 [1] report variants in *DRAM2* in patients with retinal dystrophy. The authors review previous experimental evidence suggesting that *DRAM2* is involved in autophagy, and discuss the importance of autophagy in normal photoreceptor function. Localization of *DRAM2* in the inner segment of the photoreceptor layer and the apical surface of the retinal pigment epithelium is consistent with a role in photoreceptor autophagy. Therefore, the predicted function of *DRAM2* is consistent with the disease process. (Default: 0.5 points)
- **Example: *GAA* and Pompe disease**
Pompe disease (glycogen storage disease type II) is characterized by accumulation of glycogen in lysosomes. *GAA* encodes acid alpha-glucosidase, a lysosomal enzyme which breaks down glycogen. The function of acid alpha-glucosidase is therefore consistent with the disease process. (Default: 0.5 points)

Protein interaction:

- **Example: *KCNJ8* and Cantu syndrome**
The products of the *KCNJ8* and *ABCC9* genes interact to form ATP-sensitive potassium channels. Gain of function variants in *ABCC9* were reported in about 30 individuals with Cantu syndrome. Subsequently, gain of function variants in *KCNJ8* were also reported in individuals with Cantu syndrome (Brownstein et al, 2013 [2]; Cooper et al, 2014, [3]). Protein interaction points can be awarded to *KCNJ8* due to interaction of the gene product with a protein implicated in the disease (encoded by *ABCC9*). (Default: 0.5 points)

Expression:

- **Example: *TMEM132E* and autosomal recessive sensorineural hearing loss**
Li et al, 2015 [4] used qPCR to demonstrate that *TMEM132E* is highly expressed in the cochlea and the brain, two tissues that can be affected by hearing loss. Western blotting confirmed that the protein is expressed in these tissues. (Default: 0.5 points)
- **Example: *PDE10A* and childhood onset chorea with bilateral striatal lesions**
Mencacci et al, 2016 [5] reported variants in *PDE10A* in individuals with childhood onset chorea. They report that microarray data from post-mortem brain tissue showed

exceptionally high expression in the putamen, consistent with data in the Allen Mouse Brain Atlas and previous publications showing high and selective PDE10A expression in human striatum at both the RNA and protein levels [6, 7]. While PDE10A is transcribed in many tissues, the highest expression is in brain

(<https://gtexportal.org/home/gene/PDE10A>). Points can be awarded because PDE10A expression is relevant to the disease of interest. (Default: 0.5 points)

- **Example: Leptin and Severe early-onset obesity**

Leptin is a hormone secreted by adipose tissue that signals satiety. Montague et al 1997 [8], examined two severely obese children from a consanguineous Pakistani family. Circulating leptin levels were measured by ELISA and were found to be very low compared with controls and unaffected family members. (Default: 0.5 points)

FUNCTIONAL ALTERATION

- **Example: Functional alteration, patient cells**
FBN1 variants in Marfan Syndrome

Granata et al, 2016 [9] studied smooth muscle cells derived from isolated pluripotent stem cells from patients with Marfan syndrome and variants in *FBN1* (p.Cys1242Tyr and p.Gly880Ser). *FBN1* deposition into the extracellular matrix (ECM) and contractility of the differentiated smooth muscle cells in response to carbachol stimulation were measured. Results indicated that the ECM is destabilized for cells with the variant. Destabilization of the ECM in muscle cells is a hallmark of aortic aneurysm. Because aortic aneurysm is a phenotypic feature of Marfan syndrome, changes to ECM organization support the disease mechanism and this evidence can be counted as functional alteration. (Default: 1 point)

- **Example: Functional alteration, non-patient cells**
FHL1 and Emery-Dreifuss Muscular Dystrophy (EDMD)

Some patients with EDMD develop hypertrophic cardiomyopathy. Freidrich et al, 2012[10] transduced neonatal murine cardiomyocytes with AAV constructs with FHL1 p.Lys45Serfs and p.Cys276Ser variants. Variant FHL1 proteins were mislocalized and did not incorporate into the sarcomere. Localization and incorporation into the sarcomere for MYBPC3, a known causative gene for HCM, was also perturbed. Because MYBPC3 is known to be involved in HCM, and sarcomere disruption is a hallmark of HCM, the changes in its expression and localization of mutant FHL1 in cultured non-patient cells is experimental evidence to support the disease mechanism. (Default: 0.5 points)

MODELS AND RESCUE

- **Example: Animal model**

- TMEM132E and autosomal recessive sensorineural hearing loss***

Li et al, 2015 [4] knocked down *TMEM132E* in zebrafish using antisense morpholino oligos. The morpholino animals displayed delayed startle response and reduced extracellular microphonic potentials, suggesting hearing loss. (Default: 2 points)

- Example: Cell culture model**
FHL1 and Emery-Dreifuss Muscular Dystrophy (EDMD)
 Some patients with EDMD develop hypertrophic cardiomyopathy. Freidrich et al, 2012 [10] measured contraction in AAV transduced **rat engineered heart tissue** (rEHT) expressing FHL1 variants. rEHT tissue expressing the mutant FHL1 constructs had significantly altered contraction parameters. Hypercontractility and diastolic dysfunction are hallmarks of HCM, therefore changes to these parameters due to mutant FHL1 expression support the disease mechanism. (Default: 1 point)
- Example: Rescue in human**
Leptin and Severe early-onset obesity
 The LEP gene encodes leptin, a satiety hormone that is secreted by adipose tissue. Montague et al reported that two severely obese children from a consanguineous Pakistani family had frameshift variants in LEP [8]. When one of these children was treated with recombinant Leptin for 12 months (Farooqi et al 1999)[12], hyperphagia ceased and the amount of body fat lost was 15.6kg (accounting for 95% of the weight lost).(Default: 2 points)
- Example: Rescue in an animal model**
TMEM132E and autosomal recessive sensorineural hearing loss
 Li et al, 2015 [4] injected human TMEM132E mRNA into antisense oligo knockdown zebrafish. This partially rescued the hearing defects in those fish. (1 point was given instead of 2, default, because the mRNA only partially rescues the phenotype).
- Example: Rescue in patient cells**
COL3A1 and Ehlers-Danlos, vascular type
 EDS Type IV is caused by dominant-negative mutations in the procollagen type III gene, COL3A1. Müller et al, 2012[12] studied cultured fibroblasts from a patient with EDS type IV who was heterozygous for p.Gly252Val in COL3A1 and from a healthy control. The authors identified a single siRNA that was able to knockdown the mutant COL3A1 mRNA (>90%) in the patient-derived fibroblasts without affecting wild type COL3A1. Prior to treatment with the siRNA, the mutant cells showed disorganized bundles of collagen fibers. After treatment with siRNA, the morphology of the extracellular matrix more closely resembled healthy control fibroblasts. (Default: 1 point)
- Example: Rescue in humans**
 Pompe disease is caused by deficient activity of acid-alpha glucosidase (GAA). Patients with the infantile onset form typically die by one year of age if untreated. Kishnani et al, 2006 [13] report clinical improvements in 8 patients with infantile-onset Pompe disease who received a weekly intravenous infusion of recombinant GAA for 52 weeks. Clinical improvements included amelioration in cardiomyopathy, improved growth, and acquisition of new motor skills in 5 patients, including independent walking in three of them. Although four patients died after the initial study phase, the median age at death was significantly later than expected for patients who were not treated. Treatment was safe and well tolerated. (4 points)

References:

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