# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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**SUPPLEMENTARY APPENDIX**

Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation

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## SECTION S1

### R code used in statistical analysis of multiple molecular diagnoses

```
error.bar <- function(x, y, upper, lower=upper, length=0.1,...){
if(length(x) != length(y) | length(y) !=length(lower) | length(lower) != length(upper))
stop("vectors must be same length")
arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}

### Poisson model.  Rate parameter estimator is the Sample Mean of the observations
lam<-(1975*1+92*2+3*3+1*4)/7374

### variance of the rate parameter estimate is the variance of the sample mean:
V(xbar)=V(X)/N = lambda/N.  #SE(hat(lambda))= sqrt(lambda/N)
#sde<-sqrt(lam/7374)


### the estimator of theta = rate of singleton diagnoses.
theta<-1975/7374
### Constructing a probability mass distribution on 0,1,2,3,4 for the independence model
 idist<-c(theta,theta^2,theta^3,theta^4)
idists<-c(1-sum(idist),idist)

### the random variable for count_singletons is a binomial variate with variance = p*(1-p) where
p=idists[1]


#### Using Taylor Approximations to obtain variance estimate for the frequency of multiple diagnoses
### using the parameter estimates from each model
v.pmodel<-function(k,theta=0.2959045,N=7374){
N*theta*1/(prod(k)^2)*(-exp(-theta)*theta^k + k*theta^(k-1)*exp(-theta))^2
}

v.imodel<-function(k,theta=idists[2],N=7374){
k^2*N*theta^(2*k-1)*(1-theta)
}


e.idist<-7374*idists[-1]
e.pdist<-7374*dpois(1:4,lam)

intervals.poisson<-sapply(seq(1:4),function(k){c(e.pdist[k]-1.96*sqrt(v.pmodel(k)),
e.pdist[k]+1.96*sqrt(v.pmodel(k)))})
intervals.independence<-sapply(seq(1:4),function(k){c(e.idist[k]-1.96*sqrt(v.imodel(k)),
e.idist[k]+1.96*sqrt(v.imodel(k)))})
```

```
ip<-apply(intervals.poisson,2,function(x){paste('(',round(x[1],2),',',round(x[2],2),')',sep='')})
ii<-apply(intervals.independence,2,function(x){paste('(',round(x[1],2),',',round(x[2],2),')',sep='')})

forTable<-cbind(c(1,2,3,4),round(e.pdist,2),round(e.idist,2),ip,ii)
colnames(forTable)<-
c('Diagnoses','Poisson.Expected','Alternative.Expected','CI.95_Poisson','CI.95.Alternative')
write.table(forTable,file='vals_withCI.txt',sep='\t',col.names=T,row.names=F,quote=F)

png('SupplementalFigure_barplot.png')
b<-
barplot(rbind(c(1975,97,3,1),e.pdist,e.idist),beside=T,col=c('black','darkgrey','lightgrey'),ylim=c(0,2500))
legend(10,2000,legend=c("Observed",'Poisson','Independent'),fill=c('black','darkgrey','lightgrey'))
 error.bar(b[2,1],e.pdist[1],upper=1.96*sqrt(v.pmodel(1)),lower=0)
error.bar(b[2,2],e.pdist[2],upper=1.96*sqrt(v.pmodel(2)),lower=0)
error.bar(b[2,3],e.pdist[3],upper=1.96*sqrt(v.pmodel(3)),lower=0)
error.bar(b[2,4],e.pdist[4],upper=1.96*sqrt(v.pmodel(4)),lower=0)

error.bar(b[3,1],(idists[2])*7374,upper=1.96*sqrt(v.imodel(1)),lower=0)
error.bar(b[3,2],idists[3]*7374,upper=1.96*sqrt(v.imodel(2)),lower=0)
error.bar(b[3,3],idists[4]*7374,upper=1.96*sqrt(v.imodel(3)),lower=0)
error.bar(b[3,4],idists[5]*7374,upper=1.96*sqrt(v.imodel(4)),lower=0)
dev.off()
```

**SECTION S2**

## METHODS

### Variant Analysis

The false positive error rate for variants identified by WES is 2.68% for single nucleotide variants (SNV) and 14.84% for indels. Sanger sequencing was used as an orthogonal approach to eliminate next generation sequencing (NGS)-based false positive calls and segregate candidate diagnostic SNVs and short indels in the proband and available family members. The false positive error rate resulting from classification of variants identified by WES and confirmed by Sanger sequencing represents an additional source of error, and a recent study concordance in variant classification across nine diagnostic laboratories demonstrates only 34% concordance across laboratories.[1]

American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines for variant interpretation in Mendelian disorders (2015)[2,3] recommend a five-tier system (pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, benign) for variant classification, but acknowledge that additional tiers for sub-classification of VUS may be added.[2,3] The Baylor Genetics (BG) diagnostic laboratory uses a modified set of criteria that was developed before professional society guidelines were established and extends beyond the current ACMG guidelines (**File S3)**.[4,5] All variants reported here were re-analyzed by these optimized criteria. Pathogenic, likely pathogenic, or VUS favoring likely pathogenic status in Mendelian disease genes consistent with the expected inheritance and observed phenotype were required for a case to be considered to have a molecular diagnosis (**Table S5**).[4,5] Phenotypic information was provided upon referral to WES, and in many cases, physicians were re-contacted following issue of the initial WES report to investigate whether the clinical impression supported pathogenicity for the reported variants.

Reported deletions were confirmed by PCR following detection through WES read depth analysis, or known prior to WES analysis (5 cases, **Table S3**). A single reported copy number gain was identified by concurrent WES and cSNP array analysis.

**Phenotype Analyses**

To determine phenotypic similarity between expected clinical findings associated with each of the dual molecular diagnoses, we used the set of OMIM diseases that have been mapped to the Human Phenotype Ontology (HPO) database,[6,7] http://human-phenotype-ontology.github.io/downloads.html. Copy number variation (CNV) encompassing multiple genes and for which a single candidate gene has not been identified as being responsible for the phenotype were excluded. For the 80 dual molecular diagnoses for which OMIM diseases have been mapped to the HPO database,[7,8] HPO terms were analyzed to compute phenotype similarity scores between disease pairs using a symmetrized Resnik method (**File S4**).[8,9] Similarity scores were re-scaled to a value between 0 and 1. GeneMANIA was used to visualize physical interactions between protein pairs.[10]

**Statistical modeling of multiple diagnoses**

No established formalism exists for a statistical model of multiple diagnoses for exome-wide genetic diagnostics in a referral laboratory testing population. There are many attributes to consider in developing such a model. First, the genotypic substrate of multiple diagnoses is the exome-wide exposure of each individual to mutation and transmission of genetic variation that cause disease. This genetic variation must be ascertainable in the exome without causing

embryonic lethality. Many combinations of genetic disease causing variations may exclude viability. The determination of viable and non-viable combinations of variation cannot be ethically determined in humans by experimental means. Second, the referral population comprises the set of individuals and families who have phenotypes consistent with suspected genetic disease and yet who also have socio-economic, geographic or other means to access expensive exome-wide diagnostics. Moreover, the set of diagnoses we are considering are constrained to the reporting criteria as set forth by the ACMG. Recent papers have shown the application of these standards does not produce equivalent results across independent laboratories. Given the challenges set forth above, it is necessary to proceed empirically to establish a statistical model.

Poisson Model

The Poisson model presumes each proband's genome is exposed to a large number of independent potential genetic diagnoses (variations) and that each of these arises rarely in each case, so that the product of the number of potential diagnostic variations and the chance of these occurring converges to a modest non-zero value, which is the Poisson rate parameter. Under a set of well-established probabilistic axioms, such processes yield counting variables with the Poisson distribution, which gives probability values to any non-negative integer number of diagnoses in an individual case. The limitations of this model are that it does not consider exclusionary properties between pairs of diagnostic variations; the model also does not treat the inhomogeneous nature of mutation across the exome, nor does it consider the population load of recessive alleles and the complexity of dominant, recessive or sex-linked modes of inheritance. Still the model forms a strong heuristic to initiate the inquiry. The estimation of the rate parameter of the Poisson is the sample mean number of diagnoses per individual observed across

the exome cohort. The model does not constrain the maximum number of diagnoses. For a distribution with rate parameter $\lambda$, the notation below gives the probability for the chance of $k$ diagnoses per individual:

$$P(X = k) = \frac{e^{-\lambda}\lambda^{k}}{k!}$$

Independence Model

To provide an alternative to the Poisson model, we considered a more direct and constrained independence model for the occurrence of multiple diagnoses. Under this model we considered that singleton diagnoses occur at a rate $\theta$. We then model the chance of doubleton diagnoses as $\theta^2$, triples as $\theta^3$ and quads as $\theta^4$. We limit the maximum number of diagnoses at four and considered the chance of no diagnosis as $1 - \theta - \theta^2 - \theta^3 - \theta^4$. The resulting probability distribution places positive mass on the five states S={0,1,2,3,4}. We infer $\theta$ using the number of singleton diagnoses observed in our cohort, which in the case of this model is a binomial random variable with rate $\theta$. We then use this estimate of $\theta$ to determine the expected chance of 2, 3, and 4 diagnoses by squaring, cubing, and raising to the fourth power, respectively.

Confidence intervals:

To determine confidence intervals for the number of multiple diagnoses estimated under the Poisson and alternative statistical models we use the statistical error of the parameter estimate for each model together with established methods for approximating the variance of functions of random variables – in this case these are functions that determine the expected amounts of multiple diagnoses under each model. We then employ the normal approximation to determine interval boundaries.

The first step is to determine the variance of the parameter estimates. For the Poisson model, we have the variance of the sample mean: $V(\hat{\lambda}) = \frac{\lambda}{N}$ . For the independence model, we have

$(\hat{\theta}) = \frac{\theta(1-\theta)}{N}$, because our estimate is derived from N observations of a binomial variable with rate $\theta$. We can use the plugin values of the parameter estimates themselves to determine these quantities.

Next we use Taylor approximation to the variance of a function of a random variable, which in this case will be our parameter estimates. Considering $\hat{\lambda}$ as a generic estimator, we have:

$$V\left(f(\hat{\lambda})\right) = V(\hat{\lambda})[f'(\hat{\lambda})]^2$$

In the case of each model the probability mass function of the distribution determines *f*, and we can use differentiation and evaluation at the parameter estimate to obtain the variance values. Then, using a normal approximation we obtain the factor of 1.96 to determine a 95% confidence interval around the expected number of diagnoses for each model.

**SECTION S3**

**Baylor Genetics**

**Procedure Manual**

| | | |
|---|---|---|
| Document Type: | SOP | Effective date: Oct. 2015 |
| Title: | Criteria for Variant Classification-Modified ACMG Criteria | |
| Applies to: | BG Molecular Tests | |
| Author | Yaping Yang, Jing Wang, Eric Schmitt, Fan Xia, Ping Fang, Magdalena Walkiewicz, Jinglan Zhang, Jennifer Scull, Liu Liu, Fangyuan Li and other BG molecular directors | |

## PURPOSE

Provide criteria for variant classification based on the 2015 ACMG/AMP guidelines (Richards S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine (2015) 17, 405-423. PMID: 25741868).

**BG approved criteria are labeled with "[BG]"**

## 1. Criteria for Classifying Pathogenic Variants

**This section is based on "Table 3. Criteria for Classifying Pathogenic Variants" of the 2015 ACMG guidelines**

Notes [**BG**]

- Avoid applying similar evidence twice: e.g. PS2 and PM6 (*de novo* criteria) cannot be both invoked for a variant in a patient; if all evidences from the literature were considered and counted and related criteria are invoked, then PP5 or BP6 cannot be invoked again as an additional line of evidence.
- Criterial can be "upgraded" or "downgraded" based on the strength of the evidence using lab director's professional judgements. Suggested classification in BG: "FINAL CLASSIFICATION_ORIGINAL CRITERION", e.g. PM_PS3 (meaning an evidence considered using PS3 is downgraded to PM as the final classification).

**Very strong evidence of pathogenicity**

PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3' end of a gene.
- [**BG]**: Changes in the first and last exon will be auto-alerted in the analysis pipeline and considered on a case by case basis.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts
- [**BG]**: For splice sites, the change should affect the invariant GT/AG (+/-1 or 2 position) sequences for U2 introns
- [**BG]**: A start codon may not be qualified as a *bona fide* pathogenic variant if there is another Methionine downstream and close by. Classification should be on a case-by-case basis

**Strong evidence of pathogenicity**

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val->Leu caused by either G>C or G>T in the same codon
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, *etc.* can contribute to nonmaternity

Note [**BG]**: use this rule for AD, X-linked disorders or mitochondrial disorders. For AR disorder, PM3 should also be considered.

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product *

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established

* [**BG**]: Enzyme studies on the patient samples do not necessarily connect a particular change with biochemical results. Mutagenesis studies may be needed. For carrier, the report should only consider well established clinical tests that have confirmative value as valid functional study.

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.

Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

PS5 [**BG**]: Mosaicism in a parent for AD or X-linked disorders

PS6 [**BG**]: ≥2 independent occurrences of PM6 (assumed *de novo*, but without confirmation of paternity and maternity), from internal and/or published data

PS7 [**BG**]: ≥2 independent occurrences of PM7 (PVS1 variant in a gene in which a truncating variant or large deletion was previously reported in a patient) from internal and/or published data

PS8 [**BG**]: in-frame single exon skipping, or gross deletion results in in-frame single exon deletion in a domain of known function

**Moderate evidence of pathogenicity**

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation

PM2 Absent from controls (or at extremely low frequency if recessive) (see Table 6) in Exome Sequencing Project, 1000 Genomes or ExAC
Caveat: Population data for indels may be poorly called by next generation sequencing

Note [**BG**]: Population specific data can be used

PM3 For recessive disorders, detected in *trans* with a pathogenic variant

Note: This requires testing of parents (or offspring) to determine phase

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

PM5 Novel missense change at an amino acid residue where a different

missense change determined to be pathogenic has been seen before

Example: Arg156His is pathogenic; now you observe Arg156Cys
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PM6 Assumed *de novo*, but without confirmation of paternity and maternity

PM7 [**BG**]: PVS1 variant in a gene in which a truncating variant or large deletion was previously reported in a patient

PM8 [**BG**]: Small in-frame changes that are located in a region where similar in-frame change(s) were previously reported in patient(s)

**Supporting evidence of pathogenicity**

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

Note: May be used as stronger evidence with increasing segregation data

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Note [**BG]**: Current consensus among CSER sites is “all or none”: apply this rule if all prediction programs used in a lab support the variant to be “deleterious” or “benign”. If there are conflicting predictions, then don’t invoke this criterion.

PP4 Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation

Note [**BG]**: only invoke when there is new evidence from the source that was not considered for other criteria during the variant evaluation.

## 2. Criteria for Classifying Benign Variants

**This section is based on "Table 4. Criteria for Classifying Benign Variants" of the 2015 ACMG guidelines**

**Stand-Alone evidence of benign impact**

BA1 Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC

Note [**BG**]: Population specific data can be used

**Strong evidence of benign impact**

BS1 Allele frequency is greater than expected for disorder (see table 6)

Note [**BG**]: Population specific data can be used

BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age

Note [**BG]**: per CSER, it is preferred not to apply both BS1 and BS2 based on the same set of data. e.g. if BS1 is applied based on ExAC data, then other data (such as parental data) is preferred to use if BS2 is to be applied too.

BS3 Well-established *in vitro* or *in vivo* functional studies shows no damaging effect on protein function or splicing

BS4 Lack of segregation in affected members of a family

Caveat: The presence of phenocopies for common phenotypes (*i.e.* cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

**Supporting evidence of benign impact**

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease

BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern

BP3 In-frame deletions/insertions in a repetitive region without a known

function

BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Note [**BG]**: Current consensus among CSER sites is “all or none”: apply this rule if all prediction programs used in a lab support the variant to be “deleterious” or “benign”. If there are conflicting predictions, then don’t invoke this criterion.

BP5 Variant found in a case with an alternate molecular basis for disease

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation

Note [**BG]**: only invoke when there is new evidence from the source that was not considered for other criteria during the variant evaluation

BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

## 3. Rules for Combining Criteria

**This section is based on "Table 5. Rules for Combining Criteria" of the 2015 ACMG guidelines**

**Pathogenic**

1) 1 Very Strong (PVS1) *AND*
    a) ≥1 Strong (PS1-PS4) *OR*
    b) ≥2 Moderate (PM1-PM6) *OR*
    c) 1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) *OR*
    d) ≥2 Supporting (PP1-PP5)

2) ≥2 Strong (PS1-PS4) *OR*

3) 1 Strong (PS1-PS4) *AND*
    a) ≥3 Moderate (PM1-PM6) *OR*
    b) 2 Moderate (PM1-PM6) *AND* ≥2 Supporting (PP1-PP5) *OR*
    c) 1 Moderate (PM1-PM6) *AND* ≥4 Supporting (PP1-PP5)

**Likely Pathogenic**

1) 1 Very Strong (PVS1) *AND* 1 Moderate (PM1-PM6) *OR*
2) 1 Strong (PS1-PS4) *AND* 1-2 Moderate (PM1-PM6) *OR*
3) 1 Strong (PS1-PS4) *AND* ≥2 Supporting (PP1-PP5) *OR*
4) ≥3 Moderate (PM1-PM6) *OR*
5) 2 Moderate (PM1-PM6) *AND* ≥2 Supporting (PP1-PP5) *OR*
6) 1 Moderate (PM1-PM6) *AND* ≥4 Supporting (PP1-PP5)

**Benign**

1) 1 Stand-Alone (BA1) *OR*
2) ≥2 Strong (BS1-BS4)

**Likely Benign**

1) 1 Strong (BS1-BS4) and 1 Supporting (BP1-BP7) *OR*
2) ≥2 Supporting (BP1–BP7)

***Variants should be classified as Uncertain Significance if other criteria are unmet or the criteria for benign and pathogenic are contradictory.**

**SECTION S4**

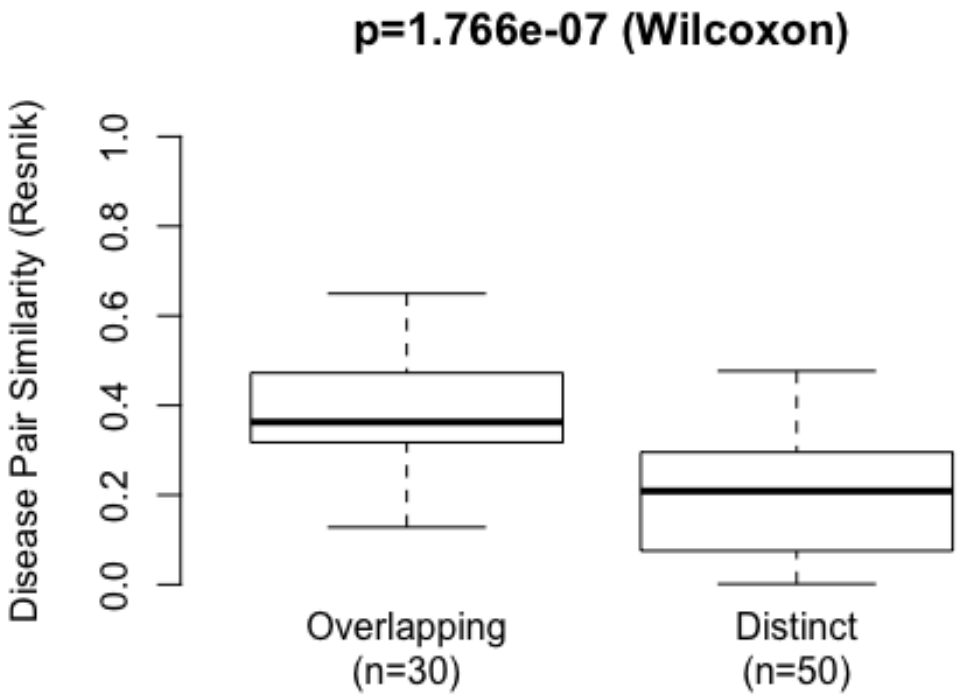
## R code used in analysis of phenotypic similarity

### R Markdown

```
#### DEFINE GOAL. ####

  #' Compare distinct and overlapping dual-diagnosis cases by:
  #' 1. Loading precomputed pairwise disease scores.
  #' 2. Normalizing the pairwise disease score for each case pair.
  #' 3. Comparatively plotting the two disease classes.

#### Load necessary libraries. ####
  library("SemanticSimilarity")

## Loading required package: parallel

## Loading required package: tools

## Loading required package: RMySQL

## Loading required package: DBI

## Loading required package: RJSONIO

## Loading required package: RCurl

## Loading required package: bitops

#### Load necessary objects. ####
  # Cohort summary table.
    load("allSolvedCases_MasterSummaryTable.RData")
  # Precomputed semantic similarity scores between all pairs of HPO-annotated
OMIM diseases.
    load("allVsAllDiseaseSimilaritiesResnik.RData")

#### Create necessary functions. ####
  computeNormalizedResnikSimilarityScoreBetweenDiseasePair <- function(diseas
eMIMid1,diseaseMIMid2)
  {
    # Convert MIM IDs into disease names.
      diseaseName1 <- diseasesInBothHPOandOMIMwithIdentityProperty[diseaseMIM
id1,2]
      diseaseName2 <- diseasesInBothHPOandOMIMwithIdentityProperty[diseaseMIM
id2,2]
    # Extract relativized scores: The pair score divided by the max score pos
sible between
```

```
    #   them (min of max for each)
      resnikSimilarityRelativized <- allVsAllDiseaseSimilaritiesResnik[
        diseaseName1,diseaseName2] /
        min(
          max(allVsAllDiseaseSimilaritiesResnik[diseaseName1,]),
          max(allVsAllDiseaseSimilaritiesResnik[diseaseName2,])
        )
    # Return the relativized score.
      return(resnikSimilarityRelativized)
  }

#### Compute the normalized Resnik similarity score between each disease pair
per diagnosis class. ####
  # Display the names of the summary table columns.
    colnames(allSolvedCases_MasterSummaryTable)

## [1] "Dual Diagnosis?"      "Dual Diagnosis Class" "Qty. Diagnoses"
## [4] "Gene"                 "OMIM Disease ID"      "OMIM Disease Name"
## [7] "HPO Phenotype Name"   "HPO Phenotype ID"

  # Identify all dual-diagnosis cases with both diseases mapped to the HPO.
    dualDiagnosisCasesWithHPOterms <- names(which(
      allSolvedCases_MasterSummaryTable[,"Qty. Diagnoses"]==2 &
        unlist(lapply(allSolvedCases_MasterSummaryTable[,"OMIM Disease ID"],l
ength))==2
    ))
  # Identify the case IDs in each disease diagnosis class (overlapping vs. di
stinct).
    casesPerClass <- split(
      x=dualDiagnosisCasesWithHPOterms,
      f=unlist(
        allSolvedCases_MasterSummaryTable[
          dualDiagnosisCasesWithHPOterms,"Dual Diagnosis Class"
        ]
      )
    )
  # Compute the normalized Resnik similarity per disease pair for each diagno
sis class.
    normalizedDiseasePairSimilaritiesPerClass <- rev(lapply(
      casesPerClass,
      function(currClass)
      {
        unlist(lapply(
          currClass,
          function(currCaseID)
          {
            currCaseDiagnosisMIMids <- allSolvedCases_MasterSummaryTable[[
              currCaseID,"OMIM Disease ID"
            ]]
            #print(currCaseDiagnosisMIMids)
```

```
            computeNormalizedResnikSimilarityScoreBetweenDiseasePair(
              diseaseMIMid1=currCaseDiagnosisMIMids[1],
              diseaseMIMid2=currCaseDiagnosisMIMids[2]
            )
          }
        ))
      }
    ))
    save(
      normalizedDiseasePairSimilaritiesPerClass,
      file="normalizedDiseasePairSimilaritiesPerClass.RData"
    )

#### Plot the normalized scores. ####
  # Similarity scores.
    #pdf(file="plots/disease_pair_similarity_per_diagnosis_class_updated.pdf"
,width=7,height=5)
    #par(mfrow=c(1,2),mar=c(3,5,3,3))
    groupNames <- c(
      paste("Overlapping\n(n=",length(casesPerClass$Overlapping),")",sep=""),
      paste("Distinct\n(n=",length(casesPerClass$Distinct),")",sep="")
    )
    yAxisLimits <- c(0,1)
    # Resnik (relativized)
      scoreName <- "Resnik Similarity (relativized)"
      pValueResnikRelativized <- paste(
        "p=",
        round(
          wilcox.test(
            normalizedDiseasePairSimilaritiesPerClass$Overlapping,
            normalizedDiseasePairSimilaritiesPerClass$Distinct
          )$p.value,
          digits=10
        ),
        " (Wilcoxon)",
        sep=""
      )
      boxplot(
        normalizedDiseasePairSimilaritiesPerClass,
        ylab="Disease Pair Similarity (Resnik)", main=pValueResnikRelativized
,
        ylim=yAxisLimits, outline=FALSE, axes=FALSE
      )
      axis(side=2)
      axis(side=1,labels=groupNames,at=1:2,lwd=0)
```


p=1.766e-07 (Wilcoxon)
Disease Pair Similarity (Resnik)
0.0
0.2
0.4
0.6
0.8
1.0
Overlapping
(n=30)
Distinct
(n=50)

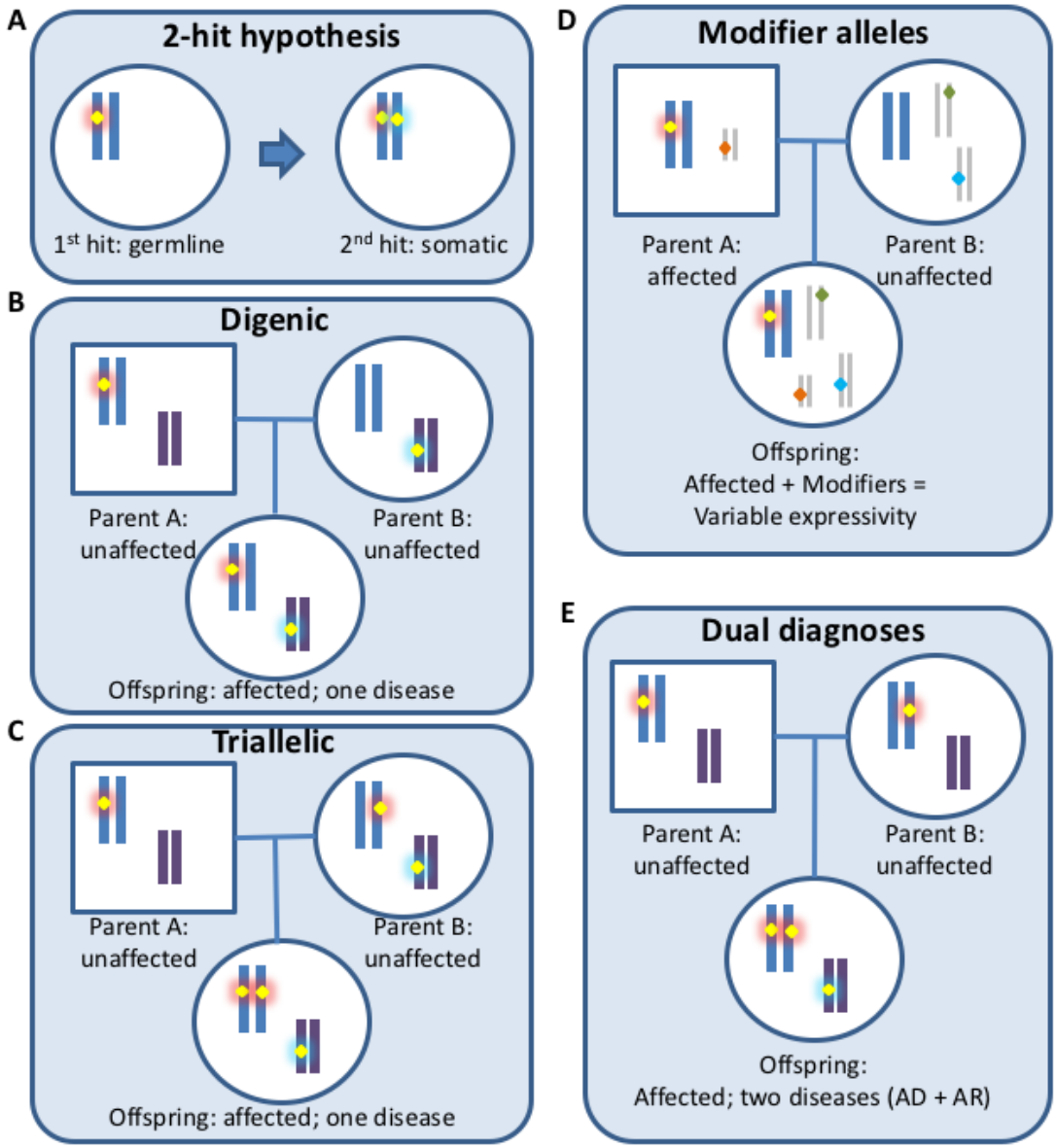
```
    #dev.off()
```

# R code used in statistical analysis of multiple molecular diagnoses

```
error.bar <- function(x, y, upper, lower=upper, length=0.1,...){
if(length(x) != length(y) | length(y) !=length(lower) | length(lower) != length(upper))
stop("vectors must be same length")
arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}

### Poisson model.  Rate parameter estimator is the Sample Mean of the observations
lam<-(1975*1+92*2+3*3+1*4)/7374

### variance of the rate parameter estimate is the variance of the sample mean:
V(xbar)=V(X)/N = lambda/N.  #SE(hat(lambda))= sqrt(lambda/N)
#sde<-sqrt(lam/7374)


### the estimator of theta = rate of singleton diagnoses.
theta<-1975/7374
### Constructing a probability mass distribution on 0,1,2,3,4 for the independence model
 idist<-c(theta,theta^2,theta^3,theta^4)
idists<-c(1-sum(idist),idist)

### the random variable for count_singletons is a binomial variate with variance = p*(1-p) where
p=idists[1]


#### Using Taylor Approximations to obtain variance estimate for the frequency of multiple diagnoses
### using the parameter estimates from each model
v.pmodel<-function(k,theta=0.2959045,N=7374){
N*theta*1/(prod(k)^2)*(-exp(-theta)*theta^k + k*theta^(k-1)*exp(-theta))^2
}

v.imodel<-function(k,theta=idists[2],N=7374){
k^2*N*theta^(2*k-1)*(1-theta)
}


e.idist<-7374*idists[-1]
e.pdist<-7374*dpois(1:4,lam)

intervals.poisson<-sapply(seq(1:4),function(k){c(e.pdist[k]-1.96*sqrt(v.pmodel(k)),
e.pdist[k]+1.96*sqrt(v.pmodel(k)))})
intervals.independence<-sapply(seq(1:4),function(k){c(e.idist[k]-1.96*sqrt(v.imodel(k)),
e.idist[k]+1.96*sqrt(v.imodel(k)))})

ip<-apply(intervals.poisson,2,function(x){paste('(',round(x[1],2),',',round(x[2],2),')',sep='')})
ii<-apply(intervals.independence,2,function(x){paste('(',round(x[1],2),',',round(x[2],2),')',sep='')})
```

```
forTable<-cbind(c(1,2,3,4),round(e.pdist,2),round(e.idist,2),ip,ii)
colnames(forTable)<-
c(‘Diagnoses','Poisson.Expected','Alternative.Expected','CI.95_Poisson','CI.95.Alternative')
write.table(forTable,file='vals_withCI.txt',sep='\t',col.names=T,row.names=F,quote=F)

png('SupplementalFigure_barplot.png')
b<-
barplot(rbind(c(1975,97,3,1),e.pdist,e.idist),beside=T,col=c('black','darkgrey','lightgrey'),ylim=c(0,2500))
legend(10,2000,legend=c("Observed",'Poisson','Independent'),fill=c('black','darkgrey','lightgrey'))
 error.bar(b[2,1],e.pdist[1],upper=1.96*sqrt(v.pmodel(1)),lower=0)
error.bar(b[2,2],e.pdist[2],upper=1.96*sqrt(v.pmodel(2)),lower=0)
error.bar(b[2,3],e.pdist[3],upper=1.96*sqrt(v.pmodel(3)),lower=0)
error.bar(b[2,4],e.pdist[4],upper=1.96*sqrt(v.pmodel(4)),lower=0)

error.bar(b[3,1],(idists[2])*7374,upper=1.96*sqrt(v.imodel(1)),lower=0)
error.bar(b[3,2],idists[3]*7374,upper=1.96*sqrt(v.imodel(2)),lower=0)
error.bar(b[3,3],idists[4]*7374,upper=1.96*sqrt(v.imodel(3)),lower=0)
error.bar(b[3,4],idists[5]*7374,upper=1.96*sqrt(v.imodel(4)),lower=0)
dev.off()
```

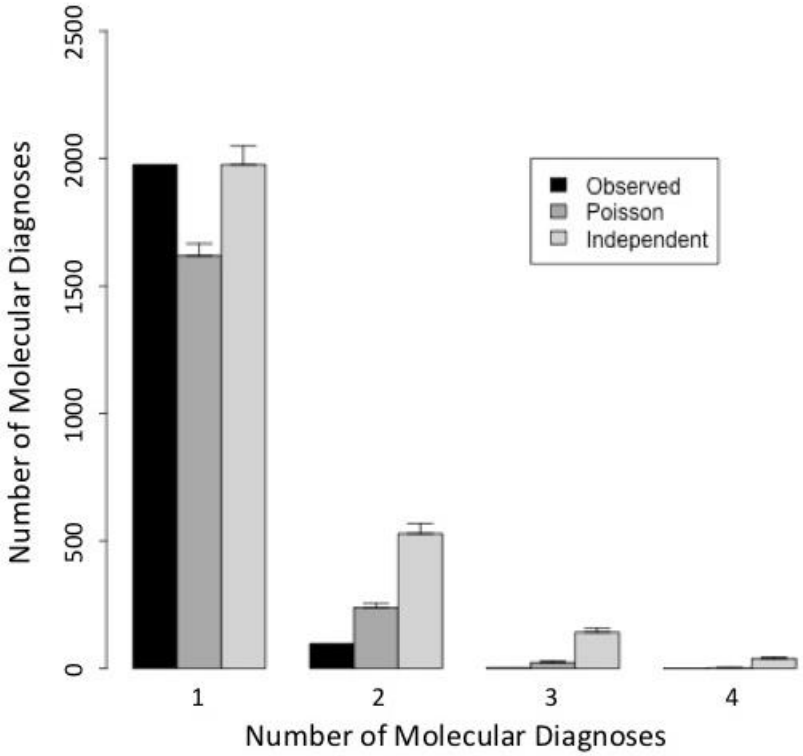
**FIGURE S1**




**SUPPLEMENTAL FIGURE 1**. (A-E) Models of non-Mendelian inheritance. (**A**) Two-hit hypothesis describing inheritance of one pathogenic allele, followed by a somatic event in the second allele. (**B**) Digenic inheritance of pathogenic alleles at different loci from each parent results in an affected offspring. (**C**) Triallelic inheritance of pathogenic alleles at two loci resulting in an affected offspring. (**D**) Genomic mutational burden leading to a modification of a parentally inherited phenotype. (**E**) Dual molecular diagnoses, in this case one recessive

diagnosis inherited from carrier parents and one *de novo* event resulting in a second, independent AD diagnosis.

**FIGURE S2**

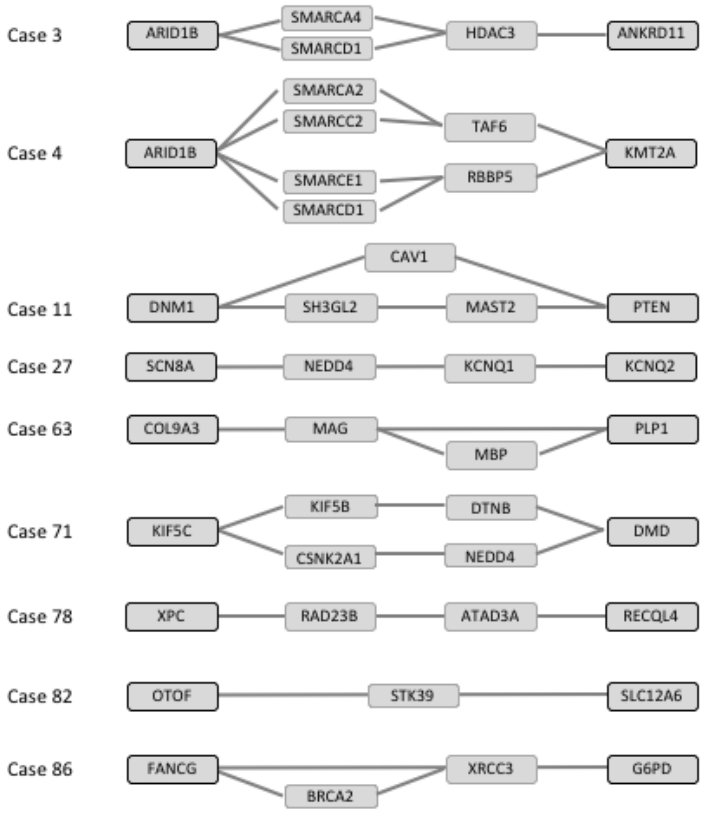



| | | | | |
|---|---|---|---|---|
| **Observed** | 1975 | 97 | 3 | 1 |
| **Poisson** | 1617.85<br>1569.6-1665.8 | 238.27<br>221.1-255.44 | 23.39<br>18.02-28.77 | 1.72<br>0.09-3.36 |
| **Independent** | 1975<br>1900.47-2049.53 | 528.97<br>489.05-568.89 | 141.68<br>125.64-157.72 | 37.95<br>32.22-43.67 |

**SUPPLEMENTAL FIGURE 2**. Observed and expected rates of 1, 2, 3, and 4 molecular diagnoses per case in a cohort of 7374 individuals with 2182 independently occurring molecular diagnoses. Expected proportions of molecular diagnoses were determined by estimation of a Poisson model and an alternative model considering independent occurrence of multiple diagnoses: Poisson model, 14.0%, [238.27 + 23.39 + 1.72]/ [1617.85 + 238.27 + 23.39 + 1.72]; independent model, 26.4%, [528.97 + 141.68 + 37.95]/[1975.0 + 528.97 + 141.68 + 37.95]; P<0.0001 by onesided

binomial test for both models. 95% confidence intervals are listed below each value for the two models.

**FIGURE S3**




**SUPPLEMENTAL FIGURE 3**. Simplified diagrams of the analysis of physical interactions performed using the GeneMANIA prediction server. Third-degree interactions are observed when the protein products of nine molecular diagnosis pairs are input: ARID1B and ANKRD11 (case 3), ARID1B and KMT2A (case 4), DMN1 and PTEN (case 11), SCN8A AND KCNQ2 (case 27), and COL9A3 and PLP1 (case 63), KIF5C and DMD (case 71), RECQL4 and XPC (case 78), OTOF and SLC12A6 (case 82), and FANCG and G6PD (case 86).

**TABLE S1. The rate of multiple molecular diagnoses reported in prior patient cohort studies.**[4,5,11-13]

| Study type | % Dx'd Cases with ≥2 Mol Dx | # Cases with ≥2 Mol Dx | # Cases with Mol Dx | Total # cases | Reference |
|---|---|---|---|---|---|
| Pilot, all ages, consecutive cases | **6.5%** | 4 | 62 | 250 | 4 |
| All ages, consecutive cases | **4.6%** | 23 | 504 | 2000 | 5 |
| Age ≥ 18y, consecutive cases | **7.1%** | 6 | 85 | 486 | 11 |
| All ages, proband- and trio-WES, consecutive cases | **7.2%** | 11 | 152 | 500 | 12 |
| All ages, proband- and trio-WES, consecutive cases | **~3.2%** | 28 | ~875 | 3040 | 13 |

**TABLE S2**

Specialty of referring physician for 101 cases with more than one molecular diagnosis.

| Specialty | Number of Cases | Percent of cases |
|---|---|---|
| Medical Genetics | 82 | 81.2% |
| Neurology | 13 | 12.9% |
| Neurogenetics | 2 | 2.0% |
| Allergy/Immunology | 1 | 1.0% |
| Unknown | 3 | 3.0% |

**TABLE S3**

Cases for which prior knowledge of a molecular diagnosis, or strong suspicion of a specific genetic diagnosis, was provided at the time of sample submission. The evidence for a clinically suspected diagnosis, when provided, has been included in the table.

| **Molecular diagnosis** | **Clinically suspected diagnosis** |
|---|---|
| 16p11.2 deletion (array CGH) | Alexander disease |
| 16p13.11 deletion (CMA) | Beta-thalassemia minor |
| 20p11.21 deletion of unclear clinical significance (CMA) | Ethylmalonic aciduria |
| Homozygous p.F508del *CFTR* variant | Factor VII deficiency |
| Xp22.31 deletion encompassing *STS* (CMA) | G6PD deficiency (2 cases) |
| Xp22.31 deletion encompassing *STS* (CMA) | Lysosomal storage disease (absent beta-hexaminidase A activity) |
| | Medium chain acyl-CoA dehydrogenase deficiency |
| | Ohtahara syndrome |
| | Pseudoxanthoma elasticum |
| | Tyrosinase-negative oculocutaneous albinism |

CGH – comparative genomic hybridization; CMA – chromosomal microarray

**TABLE S4**

Table demonstrating the number of diagnoses for predicted modes of inheritance (AD, AR, XL). Numbers of diagnoses reported as *de novo*, inherited, or for which parental testing was unavailable (n/a) are indicated.

| Inheritance | | Total Dx | Inheritance status | | | Dx with inheritance ascertained | % *de novo* variants |
|---|---|---|---|---|---|---|---|
| | | | *De novo* | N/A | Inh | | |
| Monoallelic | **AD** | 112 | 61 | 22 | 29 | 90 | **67.8%** |
| | **XL** | 34 | 15 | 5 | 14 | 29 | **51.7%** |
| | **TOTAL AD + XL** | 146 | 76 | 27 | 43 | 119 | **63.9%** |
| Other | **AR** | 61 | 0 | 8 | 53 | 53 | |

**TABLE S5**

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Autosomal Dominant + Autosomal Dominant** | | | | | | | | | | | | | | |
| 1 | 2.8 | M | AD | *ADAR* | 615010 | Het | D | SNV | Missense | p.G1007R | Yes | PS1, PS2 | P | |
| 1 | 2.8 | M | AD | *APOB* | 615558 | Het | P | InDel | Frameshift | p.G997fs | Yes | PVS1, PS7, PM2, PP1 | P | |
| 2* | 20 | F | AD | *ANKRD11* | 148050 | Het | D | InDel | Frameshift | p.E800fs | Yes | PVS1, PS2 | P | |
| 2* | 20 | F | AD | *FLG* | 146700 | Het | P | InDel | Frameshift | p.S761fs | No | PVS1, PS1 | P | |
| 3* | 6.8 | F | AD | *ANKRD11* | 148050 | Het | D | InDel | Frameshift | p.K635fs | Yes | PVS1, PS2 | P | |
| 3* | 6.8 | F | AD | *ARID1B* | 135900 | Het | D | InDel | Frameshift | p.L2234fs | Yes | PVS1, PS2 | P | |
| 4 | 10.2 | M | AD | *ARID1B* | 135900 | Het | D | SNV | Missense | p.G6D | Yes | PS2, PM2 | LP | |
| 4 | 10.2 | M | AD | *KMT2A* | 605130 | Het | D | InDel | Frameshift | p.S774fs | Yes | PVS1, PS2 | P | |
| 5* | 2.2 | F | AD | *ASXL3* | 615485 | Het | M | SNV | Nonsense | p.R1117* | Yes | PVS1, PS7, PM2 | P | |
| 5* | 2.2 | F | AD | *ENG* | 187300 | Het | Unk | SNV | Splice site | c.1273-2A>G | No | PVS1, PS1, PM2 | P | |
| 6 | 4.9 | F | AD | *CACNA1A* | 108500 | Het | D | SNV | Missense | p.V1393M | Yes | PS2, PM2 | LP | |

* Previously reported case.

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 4.9 | F | AD | *SLC26A1* | n/a | Het | D | SNV | Missense | p.L496R | Yes | PS2 | VUS-LP | |
| 7* | 8.4 | F | AD | *CHD2* | 615369 | Het | D | SNV | Missense | p.G646E | Yes | PS2, PM2 | LP | |
| 7* | 8.4 | F | AD | *PRRT2* | 605751 | Het | P | InDel | Frameshift | p.T108fs | Yes | PVS1, PS7, PM2 | P | |
| 8 | 5 | F | AD | *CHD8* | 615032 | Het | Unk | SNV | Nonsense | p.S2173* | Yes | PVS1, PS7 | P | |
| 8 | 5 | F | AD | *COL5A1* | 130000 | Het | Unk | InDel | Frameshift | p.G1255fs | Yes | PVS1, PS7 | P | |
| 9 | 5.1 | F | AD | *COL4A1* | 607595 | Het | D | SNV | Missense | p.K1185K | Yes | PS2, PM2 | LP | |
| 9 | 5.1 | F | AD | *CRYGD* | 115700 | Het | M | InDel | Nonsense | p.Y56* | No | PVS1, PS1 | P | |
| 10* | 20.8 | F | AD | *CREBBP* | 180849 | Het | D | SNV | Missense | p.M1872V | Yes | PS2, PM2 | LP | |
| 10* | 20.8 | F | AD | *PRICKLE 2* | 613832 | Het | D | InDel | Frameshift | p.G127fs | Yes | PVS1, PS2 | P | |
| 11 | 2.4 | F | AD | *DNM1* | 616346 | Het | D | InDel | Nonframeshift | p.Q155_I156insM | Yes | PS2, PM2 | LP | |
| 11 | 2.4 | F | AD | *PTEN* | 153480 | Het | D | SNV | Nonsense | p.R335* | No | PVS1, PS1 | P | |
| 12 | 5.6 | F | AD | *FBN1* | 154700 | Het | M | SNV | Missense | p.R1170H | No | PS1, PP1 (moderate), PP3 | LP | |
| 12 | 5.6 | F | AD | *MYO1F* | n/a | Het | D | SNV | Missense | p.R594Q | Yes | PS2, PM2 | VUS-LP | |
| 13* | 18.1 | F | AD | *FLG* | 146700 | Het | Unk | InDel | Frameshift | p.S761fs | No | PVS1, PS1 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13* | 18.1 | F | AD | *MEF2C* | 613443 | Het | D | SNV | Missense | p.K23R | Yes | PS2, PM2 | LP | |
| 14 | 1.5 | M | AD | *FLG* | 146700 | Het | P | InDel | Frameshift | p.S761fs | No | PVS1, PS1 | P | |
| 14 | 1.5 | M | AD | *PACS1* | 615009 | Het | D | SNV | Missense | p.R203W | No | PS1, PS2 | P | |
| 15 | 4.1 | M | AD | *GDF6* | 118100 | Het | P | SNV | Missense | p.K424R | No | PS1, PS3, PM1, PP3 | P | |
| 15 | 4.1 | M | AD | *SOX10* | 611584 | Het | D | SNV | Missense | p.R106G | Yes | PS2, PM2 | LP | |
| 16* | 10.4 | M | AD | *GLI2* | 610829 | Het | Unk | InDel | Frameshift | p.S579fs | Yes | PVS1, PM2, PM7 | P | |
| 16* | 10.4 | M | AD | *IRF6* | 608864 | Het | Unk | SNV | Nonsense | p.R412* | No | PVS1, PS1 | P | |
| 17 | 8.2 | M | AD | *GLI2* | 610829 | Het | D | SNV | Missense | p.T1552S | Yes | PS2, PM2 | LP | |
| 17 | 8.2 | M | AD | *SCN2A* | 613721 | Het | D | InDel | Frameshift | p.K743fs | Yes | PVS1, PS2 | P | |
| 18 | 7.6 | M | AD | *HBB* | 613985 | Het | Unk | SNV | Nonsense | p.Q40* | No | PVS1, PS1 | P | |
| 18 | 7.6 | M | AD | *KANSL1* | 610443 | Het | Unk | InDel | Frameshift | p.L329fs | Yes | PVS1, PM2 | LP | |
| 19 | 2.4 | M | AD | *PTPN11* | 163950 | Het | D | SNV | Missense | p.G60V | Yes | PS2, PM5 (strong) | LP | |
| 19 | 2.4 | M | AD | *SHH* | 142945 | Het | D | InDel | Frameshift | p.T429fs | Yes | PS2, PS7, PM2 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20* | 10 | F | AD | *SCN1A* | 607208 | Het | D | SNV | Missense | p.P1171L | Yes | PS2, PM2 | LP | |
| 20* | 10 | F | AD | *SMARCA 2* | 601358 | Het | D | SNV | Missense | p.T756I | Yes | PS2, PM2 | LP | |
| 21 | 0.2 | M | AD | 16p11.2 | n/a | Het | Unk | Deletion | n/a | | No | | | 0.53 Mb deletion |
| 21 | 0.2 | M | AD | *KAT6A* | 616268 | Het | P | SNV | Nonsense | p.R1169* | Yes | PVS1, PM2 | LP | |
| 22 | 5.1 | M | AD | NOTCH1 | 109730 | Het | P | SNV | Nonsense | p.Y2216* | Yes | PVS1, PS7 | P | |
| 22 | 5.1 | M | AD | TTN | 604145 | Het | M | SNV | Nonsense | p.R4186* | Yes | PVS1, PS7 | P | |
| 23 | 1.5 | M | AD | *KCNQ2* | 613720 | Het | D | InDel | Frameshift | p.E778fs | Yes | PVS1, PS2 | P | |
| 23 | 1.5 | M | AD | *PRRT2* | 128200 | Het | P | InDel | Frameshift | p.R217fs | No | PVS1, PS1 | P | |
| 24 | 6.1 | F | AD | *ANKRD11* | 148050 | Het | P | SNV | Nonsense | p.S1041* | Yes | PVS1, PS7 | P | |
| 24 | 6.1 | F | AD | *SLC6A1* | 616421 | Het | M | SNV | Missense | p.R44Q | No | PS1 (moderate), PM2, PP1, PP2, PP3 | LP | |
| 25 | 1.8 | F | AD | *PTCH1* | 109400 | Het | D | InDel | Frameshift | p.L39fs | Yes | PVS1, PS2 | P | |
| 25 | 1.8 | F | AD | *TCF12* | 615314 | Het | P | SNV | Nonsense | p.R626* | Yes | PVS1, PS7 | P | |
| 26 | 7.6 | F | AD | *COL11A1* | 604841 | Het | Unk | SNV | Splice site | c.2754+5 G>A | No | PS3, PM2 | LP | |
| 26 | 7.6 | F | AD | *KRIT1* | 116860 | Het | M | InDel | Frameshift | p.R49fs | Yes | PVS1, PS7 | LP | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 5.7 | M | AD | *KCNQ2* | 613720 | Het | D | InDel | Frameshift | p.L766fs | Yes | PVS1, PS2 | P | |
| 27 | 5.7 | M | AD | *SCN8A* | 614558 | Het | D | SNV | Missense | p.N1468S | Yes | PS2, PM2 | LP | |
| 28 | 0.6 | F | AD | *NF1* | 162200 | Het | D | SNV | Missense | p.N1883S | Yes | PS2, PM2 | LP | |
| 28 | 0.6 | F | AD | *SOX9* | 114290 | Het | D | SNV | Missense | p.M476T | Yes | PS2, PM2 | LP | |
| 29 | 19.4 | F | AD | *MYH2* | 605637 | Het | D | SNV | Missense | p.A387V | Yes | PS2, PM2 | LP | |
| 29 | 19.4 | F | AD | *SMC1A* | 300590 | Het | D | SNV | Nonsense | p.S39* | Yes | PVS1, PS2 | P | |
| 30 | 0.1 | M | AD | *SCN1A* | 607208 | Het | D | SNV | Missense | p.V1784A | Yes | PS2, PM1, PM2 | LP | |
| 30 | 0.1 | M | AD | 16p13.11 | | Het | D | Deletion | n/a | | No | | | 1.166 Mb |
| 31 | 3.2 | M | AD | *CTNNB1* | 615075 | Het | D | SNV | Nonsense | p.R95X | No | PVS1, PS2 | P | |
| 31 | 3.2 | M | AD | *1q21.1q21.2* | | Het | Unk | Deletion | n/a | | No | | | 0.86 Mb |
| 32 | 8.9 | M | AD | *SOX11* | 615866 | Het | Unk | SNV | Nonsense | p.W429X | Yes | PVS1, PM2, PM7 | P | |
| 32 | 8.9 | M | AD | *17q11.2* | | Het | Unk | Duplication | n/a | | No | | | 1.07 Mb |
| 33* | 2.8 | F | AD | *SETBP1* | 269150 | Het | D | SNV | Missense | p.D868N | No | PS1, PS2 | P | |
| 33* | 2.8 | F | AD/AR | *CLCN1* | 255700 | Het | M | SNV | Nonsense | p.R894* | No | PVS1, PS1 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33* | 2.8 | F | AD/ AR | *CLCN1* | 255700 | Het | P | SNV | Missense | p.A849T | Yes | PM3 | VUS-LP | |
| **Autosomal Dominant + Autosomal Recessive** | | | | | | | | | | | | | | |
| 34* | 14.3 | F | AD | *KCNT1* | 614959 | Het | D | SNV | Missense | p.A934T | No | PS1, PS2 | P | |
| 34* | 14.3 | F | AR | *TTN* | 608807 | Het | P | SNV | Nonsense | p.R19931* | Yes | PVS1, PS7 | P | |
| 34* | 14.3 | F | AR | *TTN* | 608807 | Het | M | SNV | Missense | p.K12032N | Yes | PM3 | VUS-LP | |
| 35* | 9.1 | M | AD | *ABCC9* | 608569 | Het | M | InDel | Frameshift | p.V1525fs | No | PVS1 (strong), PS1, PS3 | P | |
| 35* | 9.1 | M | AR | *RAPSN* | 608931 | Het | P | SNV | Missense | p.G291D | No | PS1 (moderate), PM2, PM3, PP3 | LP | |
| 35* | 9.1 | M | AR | *RAPSN* | 608931 | Het | M | SNV | Missense | p.A153T | No | PS1 (moderate) | VUS-LP | |
| 36 | 5.2 | M | AD | ***ACTG1*** | 614583 | Het | D | SNV | Missense | p.H40Y | Yes | PS2, PM2 | LP | |
| 36 | 5.2 | M | AR | *WFS1* | 222300 | Het | M | SNV | Missense | p.M731V | Yes | PM2 | VUS-LP | |
| 36 | 5.2 | M | AR | *WFS1* | 222300 | Het | P | SNV | Missense | p.C360Y | Yes | PM2 | VUS-LP | |
| 37* | 47.5 | M | AD | *DES* | 601419 | Het | Unk | SNV | Missense | p.S13F | No | PS1, PS7, PM2, PP3 | P | |
| 37* | 47.5 | M | AR | *CLCN1* | 255700 | Het | Unk | SNV | Missense | p.F167L | No | PS1, PS3 (moderate), PP3 | LP | |
| 37* | 47.5 | M | AR | *CLCN1* | 255700 | Het | Unk | InDel | Missense | p.G190S | Yes | PM3 | VUS-LP | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | 5.8 | M | AD | ***GATAD2B*** | 615074 | Het | D | SNV | Nonsense | p.Q232* | Yes | PVS1, PS2 | P | |
| 38 | 5.8 | M | AR | *WWOX* | 614322 | Het | M | InDel | Nonframeshift | p.S411del | Yes | PM2 | VUS-LP | |
| 38 | 5.8 | M | AR | *WWOX* | 614322 | Het | P | SNV | Missense | p.C183F | Yes | PM2 | VUS-LP | |
| 39 | 4.4 | F | AD | ***GNAO1*** | 615473 | Het | D | SNV | Missense | p.Y231C | Yes | PS2, PM2 | LP | |
| 39 | 4.4 | F | AR | *ACADM* | 201450 | Het | M | SNV | Splice site | c.287-2A>G | Yes | PVS1, PS7, PM2 | P | |
| 39 | 4.4 | F | AR | *ACADM* | 201450 | Het | P | SNV | Missense | p.K329E | No | PS1, PS3, PM2, PM3 | P | |
| 40 | 4.4 | F | AD | *HBB* | 613985 | Het | D | SNV | Splice site | c.92+6T>C | No | PS1, PS4 | P | cSNP 142 Mb AOH |
| 40 | 4.4 | F | AR | *TJP2* | 607748 | Hom | M+P | InDel | Frameshift | p.S415fs | Yes | PVS1, PM2, PM3 | P | cSNP 142 Mb AOH |
| 41* | 6.3 | M | AD | ***KIF5C*** | 615282 | Het | D | SNV | Missense | p.Y135C | Yes | PS2, PM2 | LP | cSNP 170 Mb AOH |
| 41* | 6.3 | M | AR | *NRXN1* | 614325 | Hom | M+P | SNV | Missense | p.D612G | Yes | PM2, PP3 | VUS-LP | cSNP 170 Mb AOH |
| 42 | 1.6 | M | AD | ***KMT2D*** | 147920 | Het | D | SNV | Missense | p.L797V | Yes | PS2, PM2 | LP | |
| 42 | 1.6 | M | AR | *HEXA* | 272800 | Het | M | InDel | Frameshift | p.Y427fs | No | PVS1, PS1, PM3 | P | |
| 42 | 1.6 | M | AR | *HEXA* | 272800 | Het | P | SNV | Splice site | c.1073+1G>A | No | PVS1, PS1, PM3 | P | |
| 43* | 1.6 | M | AD | ***NF1*** | 162200 | Het | D | InDel | Frameshift | p.R1970fs | Yes | PVS1, PS2 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43* | 1.6 | M | AR | *MEGF8* | 614976 | Het | M | SNV | Missense | p.A2037S | Yes | PM2 | VUS-LP | |
| 43* | 1.6 | M | AR | *MEGF8* | 614976 | Het | P | SNV | Missense | p.P1020L | Yes | PM2 | VUS-LP | |
| 44* | 16.9 | F | AD | ***NF1*** | 162200 | Het | D | SNV | Missense | p.G2093C | Yes | PS2, PM2 | LP | cSNP 271 Mb AOH |
| 44* | 16.9 | F | AR | *GALNT3* | 211900 | Hom | M+P | SNV | Nonsense | p.R169* | Yes | PVS1, PM2, PM3 | P | cSNP 271 Mb AOH |
| 45 | 10 | M | AD | ***PUF60*** | 615583 | Het | D | SNV | Splice site | c.1381-2A>G | No | PVS1, PS2 | P | |
| 45 | 10 | M | AR | *LOXHD1* | 613079 | Het | M | SNV | Missense | p.A1406V | Yes | PM3 | VUS-LP | |
| 45 | 10 | M | AR | *LOXHD1* | 613079 | Het | P | SNV | Nonsense | p.K148* | No | PVS1, PS7 | P | |
| 46 | 2.6 | F | AD | ***SCN8A*** | 614306 | Het | D | SNV | Missense | p.V216G | Yes | PS2, PM2 | LP | |
| 46 | 2.6 | F | AR | *MAN2B1* | 248500 | Het | M | SNV | Missense | p.G928R | Yes | PM3 | VUS-LP | |
| 46 | 2.6 | F | AR | *MAN2B1* | 248500 | Het | P | SNV | Nonsense | p.Y461* | No | PVS1, PS1 | P | |
| 47 | 3 | M | AD | *SLC2A9* | 612076 | Het | Unk | SNV | Missense | p.R380W | No | PS1, PS3, PP3 | P | cSNP 226 Mb AOH |
| 47 | 3 | M | AR | *ETHE1* | 602473 | Hom | Unk | Deletion | n/a | n/a | | PVS1, PS1 | P | cSNP 226 Mb AOH |
| 48 | 0.5 | F | AD | ***SPRED1*** | 611431 | Het | D | SNV | Nonsense | p.R325* | No | PVS1, PS1 | P | cSNP 98 Mb AOH |
| 48 | 0.5 | F | AR | *MEGF10* | 614399 | Hom | M+P | InDel | Frameshift | p.W520fs | Yes | PVS1, PS7 | P | cSNP 98 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 49* | 11.9 | F | AD | ***SYNGAP1*** | 612621 | Het | D | SNV | Nonsense | p.R544* | Yes | PVS1, PS2 | P | |
| 49* | 11.9 | F | AR | *MTFMT* | 614947 | Het | M | SNV | Missense | p.S125L | No | PS1, PM1, PM2, PM3 | P | |
| 49* | 11.9 | F | AR | *MTFMT* | 614947 | Het | P | SNV | Missense | p.S209L | No | PVS1, PS1, PM2, PM3 | P | |
| 50 | 9.8 | M | AD | ***TGFB2*** | 614816 | Het | D | SNV | Missense | p.R153H | Yes | PS2, PM2 | LP | |
| 50 | 9.8 | M | AR | *TYR* | 203100 | Het | M | SNV | Missense | p.A355V | No | PS1, PM2, PM3, PP3, PP4 | P | |
| 50 | 9.8 | M | AR | *TYR* | 203100 | Het | P | SNV | Missense | p.R77W | No | PS1, PM2, PM3, PP3, PP4 | P | |
| 51 | 10.7 | M | AD | *GFAP* | 203450 | Het | D | SNV | Missense | p.E72G | Yes | PS2, PM2 | LP | cSNP 244 Mb AOH |
| 51 | 10.7 | M | AR | *MPZ* | 605253 | Hom | Unk | Deletion | n/a | Deletion exons 4-6 | Yes | PVS1, PS7 | P | cSNP 244 Mb AOH |
| 52 | 0.6 | F | AD | *ARHGEF10* | 608236 | Het | P | SNV | Missense | p.S688N | Yes | | VUS-LP | cSNP 124.7 Mb AOH |
| 52 | 0.6 | F | AR | *ECEL1* | 615065 | Hom | M+P | SNV | Nonsense | p.W490* | Yes | PVS1, PS7 | P | cSNP 124.7 Mb AOH |
| 53 | 3 | F | AD | *FLG* | 146700 | Het | Unk | SNV | Missense | p.A1427T | Yes | PM2 | VUS-LP | cSNP 0 Mb AOH |
| 53 | 3 | F | AR | *TECPR2* | 615031 | Hom | M+P | InDel | Frameshift | p.L440fs | Yes | PVS1, PS7 | P | cSNP 0 Mb AOH |
| 54 | 0.8 | F | AD | *TUBB3* | 614039 | Het | D | SNV | Missense | p.E328K | Yes | PS2, PM2 | LP | cSNP 183 Mb AOH |
| 54 | 0.8 | F | AR | *ISCA2* | 616370 | Hom | M+P | SNV | Missense | p.G77S | No | PS1, PS4, PM2, PM3 | P | cSNP 183 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | 0.1 | F | AD | *NLRC4* | 616050 | Het | D | SNV | Missense | p.S171F | Yes | PS2, PM2 | LP | |
| 55 | 0.1 | F | AR | *SERPINA 1* | 613490 | Het | M | SNV | Missense | p.E288V | No | PS1, PS3, PM3 | P | |
| 55 | 0.1 | F | AR | *SERPINA 1* | 613490 | Het | P | SNV | Missense | p.E366K | No | PS1, PS3, PM3 | P | |
| 56 | 9.2 | F | AD | *CACNB4* | 607682 | Het | P | InDel | Frameshift | p.Q204fs | Yes | PVS1, PM2, PM7 | P | |
| 56 | 9.2 | F | AR | *TANGO2* | 616878 | Hom | M+P | Deletion | Deletion | Deletion exon 3-9 | Yes | PVS1, PS1 | P | |
| 57 | 63.7 | M | AD | *CPOX* | 121300 | Het | Unk | SNV | Missense | p.R447C | No | PS1, PS3 | P | cSNP 54 Mb AOH |
| 57 | 63.7 | M | AR | *ABCA1* | 205400 | Hom | Unk | SNV | Missense | p.N935D | Yes | PM1, PM2, PM5 | LP | cSNP 54 Mb AOH |
| 58 | 5.8 | M | AD | *GLI2* | 610829 | Het | D | InDel | Frameshift | p.T1088fs | Yes | PVS1, PS2, PS7 | P | |
| 58 | 5.8 | M | AR | *BLM* | 210900 | Het | M | SNV | Missense | p.S1380R | Yes | PM3 | VUS-LP | |
| 58 | 5.8 | M | AR | *BLM* | 210900 | Het | P | InDel | Frameshift | p.N515fs | Yes | PVS1, PS7 | P | |
| **Autosomal Dominant + X-linked** | | | | | | | | | | | | | | |
| 59 | 4.5 | F | AD | *CHD8* | 615032 | Het | D | SNV | Missense | p.R812W | Yes | PS2, PM2 | LP | |
| 59 | 4.5 | F | XL | *BRWD3* | 300659 | Het | D | SNV | Nonsense | p.R190* | Yes | PVS1, PS2 | P | |
| 60* | 10.4 | F | AD | ***ARID1B*** | 135900 | Het | D | InDel | Frameshift | p.D1630fs | Yes | PVS1, PS2 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 60* | 10.4 | F | XL | ***GRIA3*** | 300699 | Het | D | SNV | Missense | p.M706L | Yes | PS2, PM5 | LP | |
| 61 | 8.1 | F | AD | ***ARID1B*** | 135900 | Het | D | SNV | Splice site | c.3689+1 G>C | Yes | PVS1, PS2 | P | |
| 61 | 8.1 | F | XL | *G6PD* | 300908 | Het | M | SNV | Missense | p.S188F | No | PS1, PS3, PS4 | P | |
| 62 | 14.2 | M | AD | ***CHD7*** | 214800 | Het | D | SNV | Missense | p.I2688R | Yes | PS2, PM2 | LP | |
| 62 | 14.2 | M | XL | *DMD* | 310200 | Hem | M | InDel | Frameshift | p.R1884f s | Yes | PVS1, PS7 | P | |
| 63 | 23.6 | M | AD | *COL9A3* | 600969 | Het | M | SNV | Splice site | c.369+2T >C | Yes | PVS1, PS7 | P | |
| 63 | 23.6 | M | XL | *PLP1* | 312080 | Hem | M | SNV | Initiation codon | p.M1? | No | PVS1, PS1 | P | |
| 64 | 0 | F | AD | ***DNM1L*** | 614388 | Het | D | SNV | Missense | p.E379K | Yes | PS2, PM2 | LP | |
| 64 | 0 | F | XL | ***PDHA1*** | 312170 | Het | D | SNV | Missense | p.G150R | Yes | PS2 | VUS-LP | |
| 65* | 15.2 | F | AD | *EFHC1* | 254770 | Het | P | SNV | Nonsense | p.R538* | Yes | PVS1(moderat e) | VUS-LP | |
| 65* | 15.2 | F | XL | ***SMC1A*** | 300590 | Het | D | SNV | Missense | p.G100R | Yes | PS2, PM2 | LP | |
| 66 | 4.3 | M | AD | *GLI2* | 610829 | Het | M | InDel | Frameshift | p.Q297fs | Yes | PVS1, PS7 | P | |
| 66 | 4.3 | M | XL | ***KDM5C*** | 300534 | Hem | D | SNV | Missense | p.P480L | No | PS2, PM2 | LP | |
| 67 | 4 | M | AD | *GLI3* | 174200 | Het | P | InDel | Frameshift | p.D751fs | Yes | PVS1, PS7 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 67 | 4 | M | XL | ***LAS1L*** | | Hem | D | SNV | Missense | p.R415W | No | PS2, PM2 | LP | |
| 68 | 16.4 | M | AD | ***GRIN2B*** | 613970 | Het | D | SNV | Missense | p.W607C | Yes | PS2, PM2 | LP | |
| 68 | 16.4 | M | XL | *SHOXY* | 300582 | Hem | P | SNV | Splice site | c.544+1 G>A | Yes | PVS1, PS7 | P | |
| 69 | 8.3 | M | AD | ***PAFAH1B 1*** | 607432 | Het | D | SNV | Missense | p.G162S | No | PS2, PM2 | LP | |
| 69 | 8.3 | M | XL | ***FGD1*** | 305400 | Hem | D | InDel | Frameshift | p.P176fs | Yes | PVS1, PS2 | P | |
| 70 | 3.2 | M | AD | *SCN1A* | 607208 | Het | P | SNV | Missense | p.S570N | Yes | PM2, PP1, PP3 | VUS-LP | |
| 70 | 3.2 | M | XL | *PDHA1* | 312170 | Hem | M | SNV | Splice site | c.292-2A>G | No | PVS1, PS1 | P | |
| 71 | 7.3 | M | AD | *KIF5C* | 615282 | Het | Unk | SNV | Missense | p.E237K | No | PS3, PS6 | P | |
| 71 | 7.3 | M | XL | *DMD* | 310200 | Hem | Unk | Deletion | n/a | Deletion exon 49 | No | PVS1, PS1 | P | |
| 72 | 5.9 | F | AD | *MED13L* | 608808 | Het | D | SNV | Splice site | c.4956-2A>C | Yes | PVS1, PS2 | P | |
| 72 | 5.9 | F | XL | *HUWE1* | 300706 | Het | D | SNV | Missense | p.R1080H | Yes | PS2, PM2 | LP | |
| 73 | 2.4 | M | AD | *COL7A1* | 131750 | Het | P | SNV | Splice site | c.6900+4 A>G | No | PS1, PS3, PM2, PP1 | P | |
| 73 | 2.4 | M | XL | *DDX3X* | 300958 | Hem | M | SNV | Missense | p.R351Q | No | PS1, PM1, PM2, PP2, PP3 | P | |
| 74 | 9.8 | M | AD | *COL9A1* | 614135 | Het | Unk | SNV | Splice site | c.876+2T >A | Yes | PVS1, PM2, PM7 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 74 | 9.8 | M | XL | *ATRX* | 301040 | Hem | Unk | SNV | Missense | p.P190L | No | PS1, PM2 | LP | |
| **Autosomal Recessive + Autosomal Recessive** | | | | | | | | | | | | | | |
| 75 | 2.8 | M | AR | *AGL* | 232400 | Hom | M+P | SNV | Missense | p.W1451C | Yes | PS3, PM2, PP2, PP3 | LP | cSNP 367.5 Mb AOH |
| 75 | 2.8 | M | AR | *PCCA* | 606054 | Hom | M+P | SNV | Missense | p.G142D | No | PS1, PM2, PP3, PP4 | LP | cSNP 367.5 Mb AOH |
| 76 | 0.5 | M | AR | *HEXB* | 268800 | Hom | M+P | SNV | Missense | p.C91S | Yes | PM2, PP3 | VUS-LP | cSNP 103.9 Mb AOH |
| 76 | 0.5 | M | AR | *MCCC2* | 210210 | Hom | M+P | SNV | Missense | p.V339M | No | PS1, PS3 | P | cSNP 103.9 Mb AOH |
| 77 | 10.1 | M | AR | *MTPAP* | 613672 | Hom | M+P | SNV | Missense | p.V490L | Yes | PP1 | VUS-LP | cSNP 268 Mb AOH |
| 77 | 10.1 | M | AR | *NPC1* | 257220 | Het | M | InDel | Frameshift | p.L280fs | Yes | PVS1, PS7 | P | cSNP 268 Mb AOH |
| 77 | 10.1 | M | AR | *NPC1* | 257220 | Het | Unk | SNV | Missense | p.N916S | Yes | PM2, PP3 | VUS-LP | cSNP 268 Mb AOH |
| 78* | 15.7 | F | AR | *RECQL4* | 268400 | Hom | M+P | SNV | Nonsense | p.W383* | No | PVS1, PS7 | P | cSNP 187 Mb AOH |
| 78* | 15.7 | F | AR | *XPC* | 278720 | Hom | M+P | SNV | Splice site | c.1872+1G>C | Yes | PVS1, PS7 | P | cSNP 187 Mb AOH |
| 79 | 14.7 | M | AR | *TPO* | 274500 | Hom | M+P | SNV | Missense | p.R665Q | No | PS1, PM2, PM3, PP2, PP4 | P | cSNP 175 Mb AOH |
| 79 | 14.7 | M | AR | *MMP2* | 259600 | Hom | M+P | SNV | Missense | p.D180V | Yes | PP3 | VUS-LP | cSNP 175 Mb AOH |
| 80 | 1.3 | F | AR | *AGL* | 232400 | Hom | M+P | SNV | Splice site | c.3836+1G>A | Yes | PVS1, PS7 | P | cSNP 182 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80 | 1.3 | F | AR | *LPAR6* | 278150 | Hom | M+P | SNV | Missense | p.G146R | No | PS1, PS3, PM2 | LP | cSNP 182 Mb AOH |
| 81* | 7.4 | M | AR | PAPSS2 | 612847 | Hom | Unk | SNV | Nonsense | p.R329* | No | PVS1, PS1 | P | cSNP 610 Mb AOH |
| 81* | 7.4 | M | AR | TRDN | 615441 | Hom | Unk | SNV | Splice site | c.1187-2A>G | Yes | PVS1, PS7 | P | cSNP 610 Mb AOH |
| 82 | 4.5 | F | AR | OTOF | 601071 | Hom | M+P | SNV | Missense | p.R1172Q | Yes | PM2 | VUS-LP | cSNP 132 Mb AOH |
| 82 | 4.5 | F | AR | SLC12A6 | 218000 | Hom | M+P | InDel | Frameshift | p.S25fs | Yes | PVS1, PS7 | P | cSNP 132 Mb AOH |
| 83 | 0.4 | M | AR | AGPAT2 | 608594 | Het | M | InDel | Splice site | c.492+4_492+7delAGTG | Yes | PM2, PM3 | VUS-LP | |
| 83 | 0.4 | M | AR | AGPAT2 | 608594 | Het | P | SNV | Nonsense | p.W168* | Yes | PVS1, PS7 | P | |
| 83 | 0.4 | M | AR | *OBSL1* | 612921 | Het | M | InDel | Frameshift | p.V825fs | Yes | PVS1, PS7 | P | |
| 83 | 0.4 | M | AR | *OBSL1* | 612921 | Het | P | SNV | Nonsense | p.Q1319* | Yes | PVS1, PS7 | P | |
| **Autosomal Recessive + X-linked** | | | | | | | | | | | | | | |
| 84* | 3 | F | AR | *BBS10* | 209900 | Het | M | InDel | Frameshift | p.V230fs | Yes | PVS1, PS7 | P | |
| 84* | 3 | F | AR | *BBS10* | 209900 | Het | P | SNV | Missense | p.A479E | Yes | PM3 | VUS-LP | |
| 84* | 3 | F | XL | ***PDHA1*** | 312170 | Het | D | InDel | Frameshift | p.R343fs | Yes | PVS1, PS2 | P | |
| 85 | 3.3 | F | AR | *F7* | 227500 | Hom | M+P | SNV | Missense | p.A429T | No | PS1 (moderate), PM2, PP3, PP4 | LP | cSNP 79 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 85 | 3.3 | F | XL | ***MECP2*** | 312750 | Het | D | SNV | Missense | p.R111K | Yes | PS2, PM2 | LP | cSNP 79 Mb AOH |
| 86 | 0.7 | F | AR | *FANCG* | 614082 | Hom | M+P | InDel | Frameshift | p.Y213fs | No | PVS1, PS1 | P | cSNP 287 Mb AOH |
| 86 | 0.7 | F | XL | *G6PD* | 300908 | Het | P | SNV | Missense | p.S188F | No | PS1, PS3, PS4 | P | cSNP 287 Mb AOH |
| 87 | 16.6 | M | AR | *TCF12* | 615314 | Hom | M+P | SNV | Missense | p.K574Q | Yes | PM2 | VUS-LP | cSNP 180 Mb AOH |
| 87 | 16.6 | M | XL | *SLC35A2* | 300896 | Hem | M | InDel | Frameshift | p.Q206fs | Yes | PVS1, PS7 | P | cSNP 180 Mb AOH |
| 88* | 3.7 | M | AR | *TREX1* | 225750 | Het | M | InDel | Nonframeshift | p.A123dup | No | PS1, PM1, PM2, PM3, PM8 | P | |
| 88* | 3.7 | M | AR | *TREX1* | 225750 | Het | P | SNV | Missense | p.R114H | No | PS1, PS3 | P | |
| 88* | 3.7 | M | XL | *PHEX* | 307800 | Hem | M | SNV | Splice site | c.733-1G>C | Yes | PVS1, PS7 | P | |
| 89 | 3.7 | M | AR | *PLA2G6* | 256600 | Hom | Unk | Deletion | n/a | Deletion exon 2 | Yes | PVS1, PM2, PM7 | P | cSNP 0 Mb AOH, 1/17 exons deleted |
| 89 | 3.7 | M | XL | *BCAP31* | 300475 | Hem | M | SNV | Nonsense | p.S36* | Yes | PVS1, PS7 | P | cSNP 0 Mb AOH |
| 90* | 2.6 | F | AR | *CFTR* | 219700 | Hom | M+P | InDel | Nonframeshift | p.F508del | No | PVS1, PS1 | P | cSNP 0 Mb AOH |
| 90* | 2.6 | F | XL | *SMC1A* | 300590 | Het | D | SNV | Missense | p.F47C | Yes | PS2, PM2 | LP | cSNP 0 Mb AOH |
| 91 | 0.8 | M | AR | *SLC45A2* | 606574 | Hom | M+P | InDel | Frameshift | p.Y55fs | Yes | PVS1, PS7 | P | cSNP 309 Mb AOH |
| 91 | 0.8 | M | XL | *AVPR2* | 304800 | Hem | M (mosaic) | SNV | Missense | p.C112F | Yes | PS5, PM2 | LP | cSNP 309 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 92 | 0.1 | F | AR | *ALG6* | 603147 | Het | M | SNV | Missense | p.V330F | Yes | PM2, PM3 | VUS-LP | |
| 92 | 0.1 | F | AR | *ALG6* | 603147 | Het | P | SNV | Splice site | c.257+5 G>A | No | PS1, PS3, PM3 | P | |
| 92 | 0.1 | F | XL | *SHOX* | 127300 | Het | D | SNV | Missense | p.R173C | No | PS1, PS3, PM5 | P | |
| 93 | 12.4 | F | AR | *IRX5* | 611174 | Het | M | InDel | Nonframeshift | p.S81del | Yes | PM2, PM3 | VUS-LP | |
| 93 | 12.4 | F | AR | *IRX5* | 611174 | Het | P | InDel | Frameshift | p.K455fs | Yes | PVS1, PM2 | P | |
| 93 | 12.4 | F | XL | *HDAC8* | 300882 | Het | D | SNV | Missense | p.D176G | Yes | PS2, PM2 | LP | |
| 94 | 13.2 | M | AR | *BCS1L* | 124000 | Het | M | SNV | Missense | p.R69C | Yes | PM2, PP3 | VUS-LP | |
| 94 | 13.2 | M | AR | *BCS1L* | 124000 | Het | P | SNV | Nonsense | p.R200* | Yes | PVS1, PS7 | P | |
| 94 | 13.2 | M | XL | *NLGN4X* | 300495 | Hem | M | SNV | Nonsense | p.R101* | Yes | PVS1, PS7 | P | |
| **X-linked + X-linked** | | | | | | | | | | | | | | |
| 95 | 5.7 | M | XL | *KIAA2022* | 300912 | Hem | M | InDel | Frameshift | p.G1417fs | Yes | PVS1, PS7 | P | |
| 95 | 5.7 | M | XL | *PHF8* | 300263 | Hem | M | InDel | Frameshift | p.L126fs | Yes | PVS1, PS7 | P | |
| 96 | 0.6 | M | XL | *SMC1A* | 300590 | Hem | D | SNV | Missense | p.R196C | Yes | PS2, PM2 | LP | |
| 96 | 0.6 | M | XL | *DMD* | 310200 | Hem | Unk | Deletion | n/a | Deletion exons 49-51 | No | PVS1, PS1 | P | |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 97 | 1 | M | XL | *KIAA2022* | 300912 | Hem | D | InDel | Nonsense | p.C459* | Yes | PVS1, PS2 | P | |
| 97 | 1 | M | XL | Xp22.31 | | Hem | Unk | Deletion | n/a | | | | | 1.41 Mb deletion, includes STS |
| **Autosomal Dominant + Autosomal Dominant + X-linked** | | | | | | | | | | | | | | |
| 98* | 17.1 | M | AD | KMT2A | 605130 | Het | Unk | InDel | Frameshift | p.P1247fs | Yes | PVS1, PS7 | P | |
| 98* | 17.1 | M | AD | MYF6 | 614408 | Het | Unk | InDel | Frameshift | p.L197fs | Yes | PVS1, PS7 | P | |
| 98* | 17.1 | M | XL | Xp22.31 deletion | | Het | Unk | Deletion | | | | | | |
| **Autosomal Recessive + Autosomal Recessive + Autosomal Recessive** | | | | | | | | | | | | | | |
| 99* | 39.7 | M | AR | ABCC6 | 264800 | Het | M | SNV | Missense | p.R265G | No | PS1, PM3 | LP | cSNP 0 Mb AOH |
| 99* | 39.7 | M | AR | ABCC6 | 264800 | Het | P | SNV | Nonsense | p.R518* | No | PVS1, PS1 | P | cSNP 0 Mb AOH |
| 99* | 39.7 | M | AR | MTRR | 236270 | Het | M | SNV | Missense | p.L401I | Yes | PM2 | VUS-LP | cSNP 0 Mb AOH |
| 99* | 39.7 | M | AR | MTRR | 236270 | Het | P | SNV | Missense | p.I290T | Yes | PM2 | VUS-LP | cSNP 0 Mb AOH |
| 99* | 39.7 | M | AR | YARS2 | 613561 | Het | M | InDel | Frameshift | p.D121fs | Yes | PVS1, PS7 | P | cSNP 0 Mb AOH |
| 99* | 39.7 | M | AR | YARS2 | 613561 | Het | P | SNV | Missense | p.I251V | Yes | PM3 | VUS-LP | cSNP 0 Mb AOH |
| 100 | 21.2 | F | AR | CA2 | 259730 | Hom | M + P | SNV | Splice site | c.232+1G>A | No | PVS1, PS1 | P | cSNP 359 Mb AOH |

| No | Age | Sex | Inh | Gene | Dse MIM # | Zyg | Prnt orig | NT | AA | Variant | Nvl | BG Criteria | Class | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 | 21.2 | F | AR | MCCC2 | 210210 | Hom | M + P | SNV | Missense | p.V339M | No | PS1, PS3 | P | cSNP 359 Mb AOH |
| 100 | 21.2 | F | AR | SPG11 | 604360 | Hom | M + P | SNV | Nonsense | p.R2034* | No | PVS1, PS1 | P | cSNP 359 Mb AOH |
| **Autosomal Dominant + Autosomal Dominant + Autosomal Dominant + Autosomal Recessive** | | | | | | | | | | | | | | |
| 101* | 5.8 | F | AD | COL4A3 | 104200 | Het | M | SNV | Missense | p.P1109S | Yes | PP4 | VUS-LP | cSNP 0 Mb AOH |
| 101* | 5.8 | F | AD | SCN1B | 615377 | Het | P | SNV | Missense | p. G257R | Yes | PP1 | VUS-LP | cSNP 0 Mb AOH |
| 101* | 5.8 | F | AD | THRA | 614450 | Het | P | SNV | Splice site | c.54-1G>A | Yes | PVS1, PS7 | P | cSNP 0 Mb AOH |
| 101* | 5.8 | F | AR | DGAT | 615863 | Hom | M+P | SNV | Splice site | c.751+2T>C | No | PVS1, PS1 (moderate), PS3 | P | cSNP 0 Mb AOH |

**TABLE S6**

Combinations of modes of inheritance in 97 cases with two molecular diagnoses.

| Mode of Inheritance | AD | AR | XL |
|---|---|---|---|
| AD | 33 | 25 | 16 |
| AR | | 9 | 11 |
| XL | | | 3 |

**TABLE S7**

Parental ages in years at time of birth for 101 cases with more than one molecular diagnosis.

The number of *de novo* (DN) variants for each case is indicated.

| Number of DN diagnoses | Average Maternal Age | Average Paternal Age |
|---|---|---|
| 0 | 28.5 | 32.8 |
| 1 | 30.2 | 32.7 |
| 2 | 31.3 | 35.5 |

**APPENDIX REFERENCES**

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