

The evaluation of the molecular basis of disease (EMBoDy) in clinical reporting: What genetic counselors need to know for counseling and return of novel genetic etiology results identified via diagnostic exome sequencing

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BACKGROUND

- Exome sequencing serves a dual role as a diagnostic and a discovery tool,¹⁻³ and case reports of patients in whom a novel genetic etiology is identified on a clinical basis are becoming abundant.^{2,4-7}
- A novel genetic etiology is a newly described gene-disease relationship.
- In our laboratory, a novel genetic etiology is reported in roughly 1/10 patients.
- The analysis and assessment criteria used to evaluate novel genetic etiologies for clinical reporting are markedly different than those used for a clinically characterized gene.
- Given the implications for genetic counseling, it is important for genetic counselors to be aware of these differences.

TERMINOLOGY

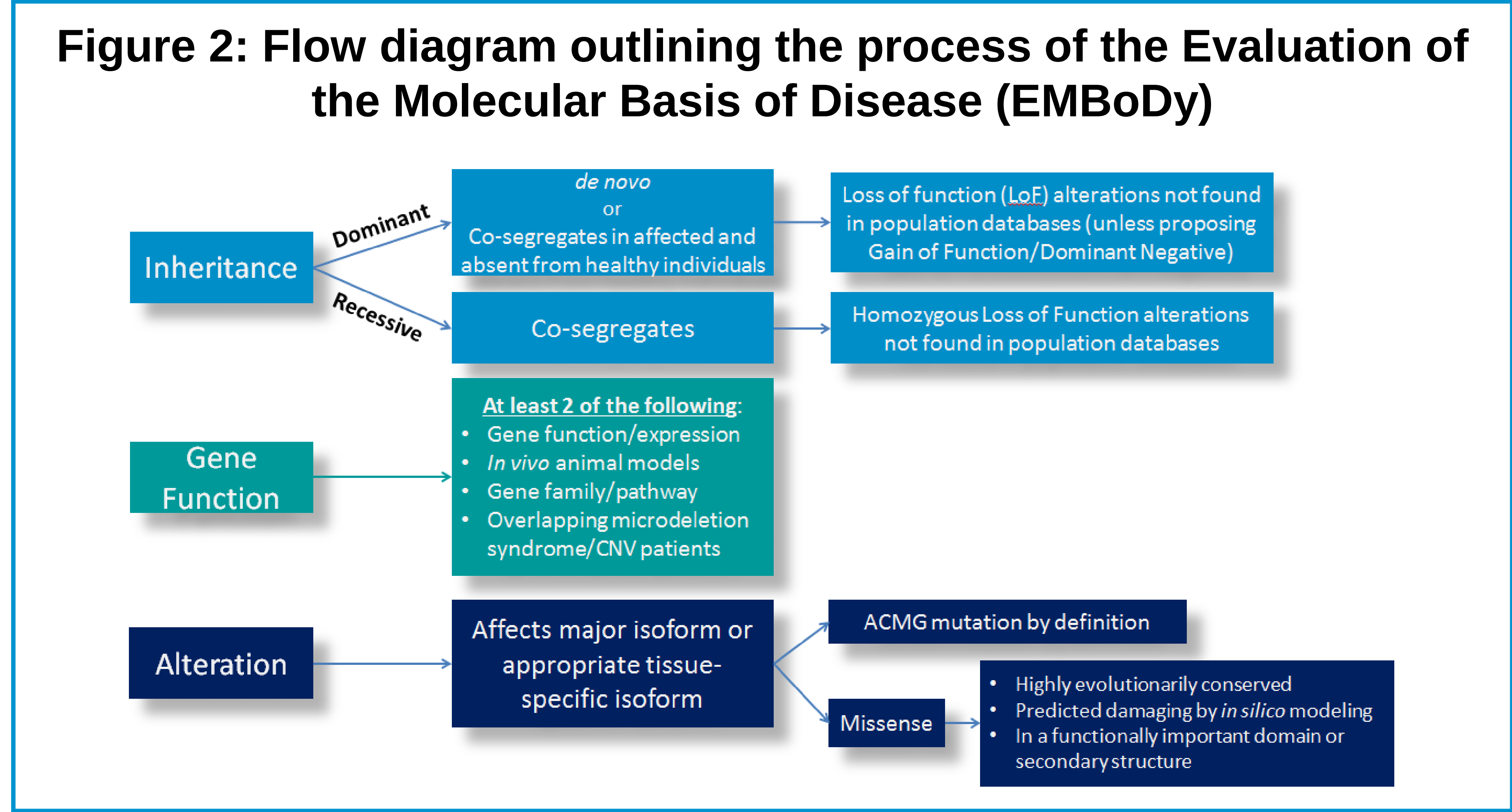
- Characterized Mendelian disease gene:** A gene known to underlie at least one Mendelian genetic condition.
- Novel Mendelian disease gene:** A gene that is not currently known to underlie a Mendelian genetic condition.
- Evaluation of the Molecular Basis of Disease (EMBoDy):** The analysis of new gene-disease relationships.
- Clinical Validity:** The determination that a particular disease is truly caused by pathogenic variants in a particular gene.
- Novel genetic etiology:** A gene-disease relationship and/or mechanism not previously proposed or with limited evidence based on ClinGen clinical validity assessment criterion (<http://www.clinicalgenome.org/knowledge-curation/clinical-validity-classifications/>)
- Characterized Disease:** A disease or phenotype whose underlying molecular etiology is established with at least moderate level of evidence based on ClinGen clinical validity assessment criterion (<http://www.clinicalgenome.org/knowledge-curation/clinical-validity-classifications/>)
- Idiopathic Disease:** A disease or phenotype whose underlying molecular etiology or etiologies have not been established. For heterogeneous conditions, there may be multiple etiologies.

Figure 1: Results categories for characterized versus novel genetic etiology results

Alteration Pathogenicity + Gene Overlap = Overall Results Category

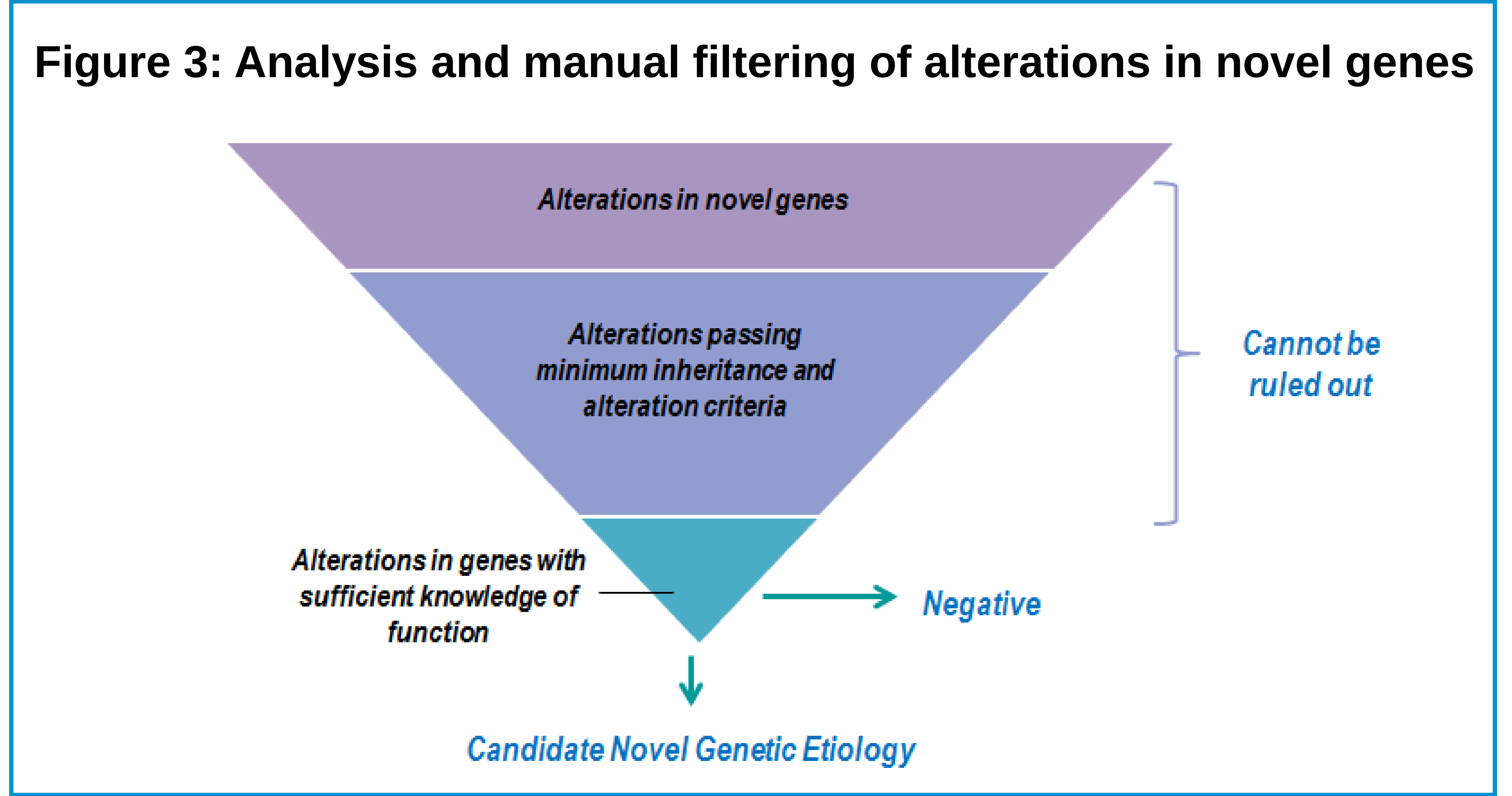
CHARACTERIZED GENES	NOVEL GENETIC ETIOLOGIES
Positive: relevant alteration(s) detected	Likely Positive: relevant alteration(s) detected
Likely Positive: relevant alteration(s) detected	Possibly Positive: alteration(s) of uncertain clinical significance detected
Uncertain: alteration(s) of uncertain clinical significance detected	Negative: No relevant alterations detected
Negative: No relevant alterations detected among characterized genes	

Figure 2: Flow diagram outlining the process of the Evaluation of the Molecular Basis of Disease (EMBoDy)



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graph LR
    Inheritance -- Dominant --> C1[de novo or Co-segregates in affected and absent from healthy individuals]
    Inheritance -- Recessive --> C2[Co-segregates]
    C1 --> L1[Loss of function (LoF) alterations not found in population databases (unless proposing Gain of Function/Dominant Negative)]
    C2 --> L2[Homozygous Loss of Function alterations not found in population databases]
    GeneFunction[Gene Function] --> C3[At least 2 of the following: Gene function/expression, In vivo animal models, Gene family/pathway, Overlapping microdeletion syndrome/CNV patients]
    C3 --> A1[Affects major isoform or appropriate tissue-specific isoform]
    A1 --> A2[ACMG mutation by definition]
    A1 --> A3[Missense]
    A3 --> A4[Highly evolutionarily conserved, Predicted damaging by in silico modeling, In a functionally important domain or secondary structure]
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Figure 3: Analysis and manual filtering of alterations in novel genes



The funnel diagram shows the process of filtering alterations in novel genes. The top section is 'Alterations in novel genes'. The middle section is 'Alterations passing minimum inheritance and alteration criteria'. The bottom section is 'Alterations in genes with sufficient knowledge of function'. A bracket on the right side of the top two sections indicates they 'Cannot be ruled out'. An arrow points from the bottom section to 'Candidate Novel Genetic Etiology', and another arrow points from the bottom section to 'Negative'.

RESULTS

- For alterations in characterized genes in which the patient’s phenotype is consistent with the known clinical and molecular spectrum, the reportable findings are categorized as positive, likely positive, or uncertain (**Figure 1**).
- Because no previous patients have been reported for novel genetic etiologies, a unique set of scientific criteria are used to make the case for the gene’s potential implication in the patient’s phenotype (**Figure 2**).
- Evaluated evidence includes relevant human microdeletion syndromes, gene function and expression profiles, co-localization/interaction with gene products known to cause similar presentations, animal models, gene family/pathway information, and possible mutational mechanism inferred from distribution of variants in control populations (**Figure 2**).
- The analysis of alterations among novel genes involves first filtering out genes with insufficient knowledge of gene function (**Figure 3**).
- These gene findings with insufficient available evidence are provided in a supplemental list in the patient’s report. *These are not negative findings, and the possibility exists for sufficient scientific evidence to emerge in the future.* (**Figure 4**).

Figure 4: Comparison of analysis of characterized vs novel genes

Analysis of Novel Genetic Etiologies
- Rule out alterations likely benign - Genes with insufficient knowledge of function cannot be ruled out – <i>Provided in supplemental section of report</i> - Rule out alterations in genes unlikely to explain the patient’s phenotype from among genes with sufficient knowledge of function
Analysis of Characterized Genes
- Rule out alterations likely benign - Rule out alterations in genes in which the clinical association is inconsistent with the current patient

TAKE-HOME POINTS

- Negative characterized gene findings are fundamentally different than negative novel gene findings in that most alterations in novel genes cannot be ruled out.
- As new information becomes available about the function of genes, it is possible for a once novel gene to become relevant for a patient.
- Genetic counselors should remain in contact with the laboratory to gain new information, to perhaps find additional patients with alterations in the same gene, and obtain re-analysis.

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