Parental Co-Segregation Analysis Significantly Reduces but Does not Eliminate VUS Rate in Multi-Gene Panel Testing

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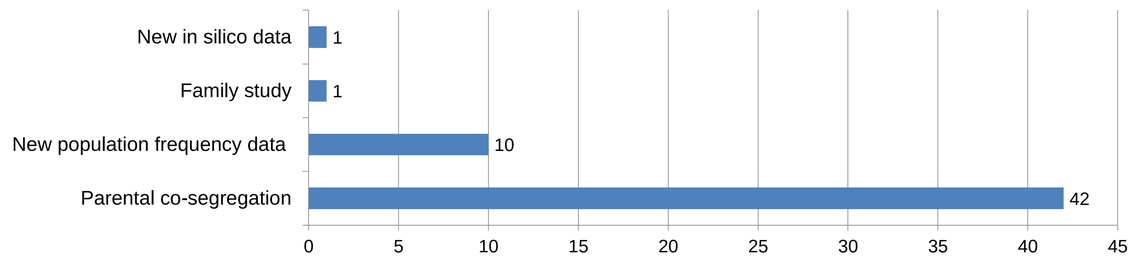
BACKGROUND

- Parental co-segregation analysis is a powerful tool for classification of variants of unknown significance (VUS).
- VUS found to be *de novo* in a proband can usually be upgraded to likely pathogenic.
- VUS inherited from an asymptomatic parent can often, but not always, be downgraded to likely benign.
- We explored the factors that contribute to the utility of parental co-segregation results in reclassifying variants of unknown significance in patients with neurodevelopmental disorders.

Fig 1. Evidence resulting in VUS downgrade to likely benign (N=54)



METHODS

- Retrospective analysis of 79 cases with neurodevelopment multigene panel testing with parental. variant analysis completed July 2016-March 2017.
- VUS in autosomal dominant (AD) and X-linked (XL). genes were eligible for parental testing.

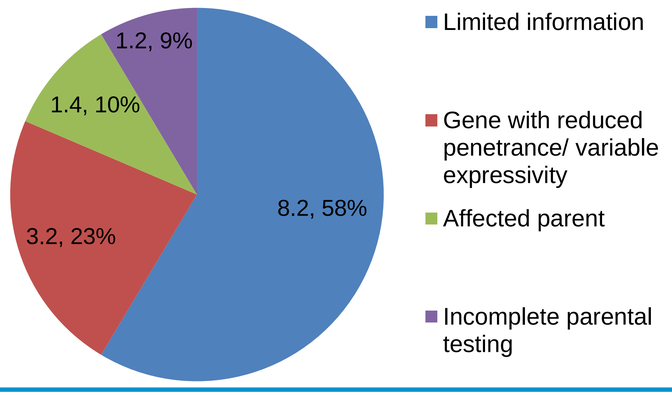
RESULTS

- 154 variants were detected.
- 6/154 (4%) upgraded to likely pathogenic (Table 1).
- 54/154 VUS (35%) downgraded to likely benign (Fig 1).
- 94/154 (61%) remained VUS.

Table 1. Summary of Variants Reclassified to Likely Pathogenic (all *de novo* in proband)

Gene	Alteration	Molecular diagnosis	Proband clinical summary (abbreviated)
ATP1A2	p.R937H (c.2810G>A)	*ATP1A2*-related neurological disorder	Teenage female: global DD, ID, autism, epilepsy syndrome (NOS), macrocephaly, dysmorphic features, melanoma, precocious puberty
CREBBP	p.R1498Q (c.4493G>A)	Rubinstein-Taybi syndrome	School-age female: autism, speech/language deficit, short stature, dysmorphic facial features and flat facial appearance, abnormalities of fingers and toes
KCNQ2	p.Y280C (c.839A>G)	*KCNQ2*-related neonatal epilepsy	Toddler female: Infantile/epileptic spasms @ 12 hours of life, hypsarrhythmia variant on EEG
KCNQ2	p.R332G (c.994A>G)	*KCNQ2*-related neonatal epilepsy	School-age female: generalized idiopathic epilepsy @ 3 days of life, global DD/ID, cortical visual impairment, static encephalopathy, dysphagia, hypothyroidism, GERD, allergies
KCNQ2	p.V543M (c.1627G>A)	*KCNQ2*-related neonatal epilepsy	Infant male: myoclonic seizures @ 4 months, DD, hypotonia, lachrimal problems, short stature, external hydrocephaly on head CT
PCDH19	p.R198P (c.593G>C)	*PCDH19*-related epilepsy	Toddler female: developmental and speech delay, dysmorphic facial features, focal seizures, asymmetric myelination on brain MRI, family history of seizures (maternal, paternal)

Figure 2: Barriers to VUS Downgrade



Variants Remaining VUS

- At least 2 pieces of supportive evidence are required before a variant can be downgraded to likely benign[1] (chart below).
- Presence of a variant in an unaffected parent is only one piece of evidence in support of benign classification of the variant.
- The majority of variants that were not downgraded (58%) were missing a second piece of supportive evidence (Figure 2).

E 2 needed (supportive)	(i) Cooccurrences with mutations in the same gene (phase unknown) (ii) Cooccurrences with mutations in other high penetrant genes that clearly explain a proband's phenotype (iii) Subpopulation frequency in support of benign classification (iv) Intact protein function observed in appropriate functional assay(s) (v) *In silico* models in agreement (benign) (vi) Not segregating with disease in family study (genes with incomplete penetrance) (vii) No disease association in small case-control study (viii) Other data supporting benign classification

TAKE-HOME POINTS

- Parental co-segregation analysis was informative in 39% of VUS, including 4/6 VUS upgraded to likely pathogenic in genes with therapeutic associations (*KCNQ2*[2] and *PCDH19*[3]).
- Independent of parental results, lack of evidence on the variant and/or gene-specific characteristics limited the ability to reclassify.
- Further delineation of these scenarios may result in more efficient application of parental co-segregation analysis and greater yield of informative results.

REFERENCES

1. Pesaran T et al. *Int J Breast Cancer*. 2016;2016:2469523. Epub 2016 Oct 16.
2. Pisano T et al. *Epilepsia*. 2015 May;56(5):685-91
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