The ASD phenotype of individuals with *CHD8*-regulated gene mutations: Utilizing family trait contribution to understand variability amongst genetic etiologies of ASD

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Background

Chromodomain helicase protein 8 (*CHD8*) is one of the most frequently identified *de novo* likely gene disrupting mutations (LGDM) occurring in simplex ASD (Iossifov et al, 2014; O'Roak et al, 2012). *CHD8* is a key regulator for a network of associated ASD risk genes involved in neurodevelopment (Cotney et al, 2014). Distinct phenotypic patterns have emerged for *CHD8*, yet considerable variability in ASD presentation remains (Bernier et al, 2014). Considering this variability in observed phenotype, the impact of *CHD8* and associated LGDMs may be better captured by the extent that observed performance deviates from expected outcomes. Given high familial heritability of phenotypic traits such as social behavior (Constantino, 2005), parental functioning serves as a measurement of expected outcome and informs penetrance of *de novo* gene variants (Moreno-de-Luca et al, 2015). This promising approach can help quantify genetic contribution to phenotypic variability for mutations to *CHD8* and *CHD8*-target genes.

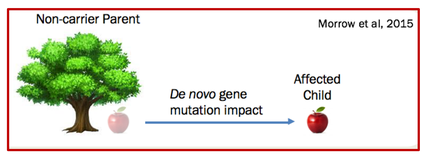
We aim to:

1) **Understand the impact of *CHD8* and *CHD8* target gene mutations on social behavior and atypical mannerisms, by quantifying observed-expected (e.g., proband-parent) discrepancies and comparing this profile to those of other ASD-associated gene-disrupting mutations and cases of idiopathic ASD (no know genetic event).**

2) **Conduct exploratory analyses of individual subscales of social behavior across groups to illuminate potential social behaviors that may be differentially impacted by *CHD8*-regulated LGD mutations.**

Methods

Figure 1. Model for determining genetic impact from unaffected parent phenotype



Participants

Simplex families of children who met strict criteria for ASD and either of the following gene statuses: (1) a *de novo* mutation of *CHD8* and *CHD8* target genes (as defined by Cotney et al, 2015), (2) other non-*CHD8*-related LGDMs, or (3) no known gene event (i.e. idiopathic).

Table 1. Sample Characteristics

	***CHD8* Target**		**Other LGDM**		**Idiopathic**	
	n	**%**	**n**	**%**	**n**	**%**
Male	**37**	**80.43**	**260**	**83.07**	**1732**	**87.43**
Female	**9**	**19.57**	**53**	**16.93**	**249**	**12.57**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (mos)	118.31	44.94	111.11	42.86	107.34	42.40

Group differences by gender, $\chi^2(2) = 6.07$, $p = .048$. Groups did not differ by age. Despite differing gender ratios between groups, gender not controlled for in analyses; females present with less impairment in ASD symptomology compared to males (Bolte, Duketis, Poustka, & Holtmann, 2011; Szatmari et al, 2012), which likely tempers effect sizes by group and provides a more conservative group comparison.

Measures

Social Responsiveness Scale (SRS), a 65-item questionnaire measuring social awareness, cognition, motivation, communication, and behavioral mannerisms (SRS; Constantino, 2005). Proband SRS completed by primary caregiver and parent SRS completed by a partner, close friend, or family member. Raw scores used to capture greater variability at the lower and higher ends of the scale. Biparental mean SRS scores used for families when data from both parents were available. When only one parent was available, that parent's data was used instead of biparental mean.

Results

We examined social responsiveness differences between the proband's observed and expected (i.e. parental scores) by looking at the effect size between probands and parents. One-way ANOVA compared group differences in observed-expected discrepancy between proband and parents for raw SRS scores.

Table 2. Proband and Parent SRS scores and corresponding effect sizes by group

	Proband		**Parents**		
	Mean	SD	Mean	SD	Effect Size
***CHD8* Target**	107.22	25.84	29.58	17.06	4.55
Other LGDM	99.10	26.19	29.74	18.10	3.83
Idiopathic	97.48	26.76	29.95	17.48	3.86

Observed-expected discrepancies between groups were significantly different, with the *CHD8* target group showing the greatest discrepancy, $F(2, 2325) = 2.92$, $p = .050$.

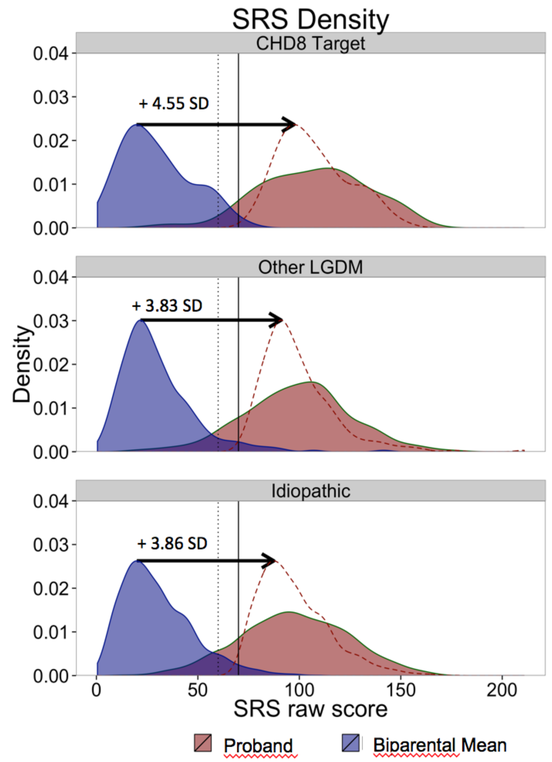


Figure 2. Density Distributions of ASD phenotype as measured by SRS for Probands and their Unaffected Parents. Dashed red line indicates the distribution predicted for probands when considering expected values (i.e. parental scores).

While differences emerge for discrepancy values between probands and parents, between group comparisons of proband SRS mean scores did not yield significant differences, $p > 0.05$.

Results Continued

Exploratory analyses were then conducted on individual subscales of the SRS, in an effort to better understand the elements of ASD phenotype that are driving differences between groups. *CHD8* Target group showed elevated mannerisms compared to other groups, trending toward significance after accounting for multiple comparisons, $F(2, 2293) = 3.420$, $p = 0.033$.

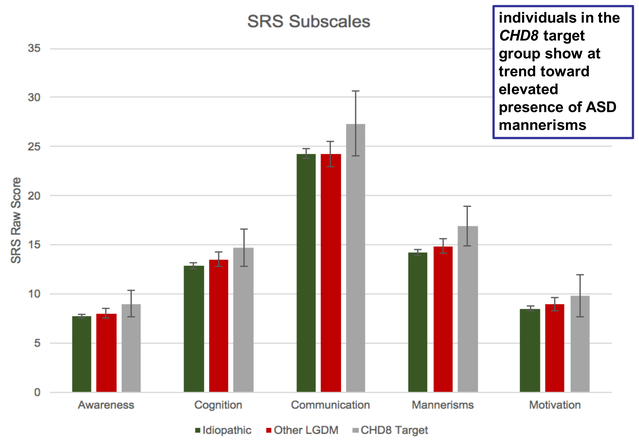


Figure 3. Proband-parent discrepancy scores for ASD phenotypic traits as measured by SRS subscales. Error bars represent 95% confidence intervals.

Discussion

When differentially expressed, *CHD8* and *CHD8*-regulated gene mutations appear to be more significantly impairing to the ASD phenotype compared to other identified *de novo* LGD risk genes or cases with no known genetic event. These differences are made apparent by comparing discrepancies between observed proband social behavior to "expected" parent social behavior, but not when looking only at proband social behavior scores alone. This suggests that quantifying *de novo* genetic impact through proband's phenotypic discrepancy from familial background provides unique insight into subtle differences in ASD symptomology. Importantly, these group differences support evidence for the study of ASD symptomology as a continuum, rather than a dichotomous classification of autism or no autism (Morrow, 2015).

Exploratory analyses of behavioral subdomains, as measured by SRS, suggest that the presence of repetitive and restricted mannerisms in individuals with *CHD8* and *CHD8* target mutations may be a key driver in the observed deficits in their behavior as reported by parent report. Further inquiry is needed to understand this relationship and the factors potentially contributing to elevated mannerisms in the *CHD8* target group (e.g. cognitive ability).

This present study provides evidence for the utility of proband-parent comparisons as a quantifying measure for *de novo* genetic impact. A larger sample of individuals with *CHD8* and *CHD8* target gene mutations is needed to increase power. Given currently small samples of individuals with a single shared gene mutation, further work is also needed to solidify appropriate groupings of individuals with ASD risk genes (e.g. mutation type, gene function, genetic load) that enhance understanding of genetic impact.

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