Published in final edited form as:
Neuron. 2011 June 9; 70(5): 863–885. doi:10.1016/j.neuron.2011.05.002.

Multiple recurrent *de novo* copy number variations (CNVs), including duplications of the 7q11.23 Williams-Beuren syndrome region, are strongly associated with autism

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Summary

Given prior evidence for the contribution of rare copy number variations (CNVs) to autism spectrum disorders (ASD), we studied these events in 4,457 individuals from 1,174 simplex families, composed of parents, a proband and, in most kindreds, an unaffected sibling. We find significant association of ASD with *de novo* duplications of 7q11.23, where the reciprocal deletion causes Williams-Beuren syndrome, featuring a highly social personality. We identify rare recurrent *de novo* CNVs at five additional regions including two novel ASD loci, 16p13.2 (including the genes *USP7* and *C16orf72*) and *Cadherin13*, and implement a rigorous new approach to evaluating the statistical significance of these observations. Overall, we find large *de novo* CNVs carry substantial risk (OR=3.55; CI =2.16-7.46, $p=6.9 \times 10^{-6}$); estimate the presence of 130-234 distinct ASD-related CNV intervals across the genome; and, based on data from multiple studies, present compelling evidence for the association of rare *de novo* events at 7q11.23, 15q11.2-13.1, 16p11.2, and *Neurexin1*.

Introduction

Autism spectrum disorders (ASD) are defined by impairments in reciprocal social interaction, communication, and the presence of stereotyped repetitive behaviors and/or highly restricted interests. A genetic contribution is well established from twin studies (Bailey et al., 1995; Lichtenstein et al., 2010) in which the very large difference between the monozygotic and dizygotic concordance rates is consistent with the contribution of *de novo* mutation and/or complex inheritance. In addition, the over-representation of ASD in monogenic developmental disorders (Klauck et al., 1997; Smalley et al., 1992), gene discovery in families with Mendelian forms of the syndrome (Morrow et al., 2008; Strauss et al., 2006), and long-standing evidence for an increased burden of gross chromosomal abnormalities (Bugge et al., 2000; Veenstra-Vanderweele et al., 2004; Vorstman et al., 2006; Wassink et al., 2001) all point to the importance of genetic risks.

Over the last several years, dramatic advances have emerged from the study of copy number variants (CNVs) such as submicroscopic chromosomal deletions and duplications (Iafrate et al., 2004; Sebat et al., 2004). Sebat et al. (2007) first noted that “large” (mean size of 2.3Mb), rare (<1% frequency in the general population), *de novo* events were more frequent in ASD probands from families with only a single affected child (i.e. simplex families) than

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in controls, as well as in comparison to probands from families with more than one affected individual (i.e. multiplex families).

The over-representation of large *de novo* CNVs in ASD has been replicated in studies ranging from 60 to 393 simplex trios (Itsara et al., 2010; Marshall et al., 2008; Pinto et al., 2010), and two of the three studies (Marshall et al., 2008; Pinto et al., 2010) have confirmed a greater abundance in simplex versus multiplex ASD families. The burden of rare *de novo* CNVs in simplex probands (i.e. the percentage of individuals carrying ≥1 rare events) has ranged from 5.0-11% (Table S1). Rare structural variants, both transmitted and *de novo*, also show varying degrees of evidence for association with ASD. These include deletions and/or duplications at specific loci, including: 1q21.1, 15q11.2-13.1, 15q13.2-13.3, 16p11.2, 17q12, and 22q11.2 as well as recurrent structural variations involving one or a small number of genes, including: *Neurexin 1* (*NRXN1*), *Contactin 4* (*CNTN4*), *Neuroligin 1* (*NLGN1*), *Astrotactin 2* (*ASTN2*), and the contiguous genes *Patched Domain Containing 1* (*PTCHD1*) and *DEAD box Protein 53* (*DDX53*) (Bucan et al., 2009; Glessner et al., 2009; Kumar et al., 2008; Marshall et al., 2008; Moreno-De-Luca et al., 2010; Noor et al., 2010; Pinto et al., 2010; Weiss et al., 2008).

To date, the number of definitive replicated findings from these studies has remained small and all evidence has pointed to a highly heterogeneous allelic architecture as no risk variant is present in more than ~1% of affected individuals. In addition, examples of incomplete penetrance (not all mutation carriers have disease), and affected siblings not sharing the same risk variant, have been the rule rather than the exception. Moreover, remarkably diverse outcomes have been identified for apparently identical CNVs. For example, chromosome 16p11.2 deletions and duplications have been found in individuals with ASD and intellectual disability (ID) (Weiss et al., 2008), seizure disorder (Mefford et al., 2009), obesity (Bochukova et al., 2010), macrocephaly, and schizophrenia (McCarthy et al., 2009). These complexities suggest that the use of association strategies to demonstrate an excess of specific *de novo* CNVs will play an important role in definitively implicating loci in ASD.

We have conducted a genome-wide analysis focusing primarily on the study of rare *de novo* CNVs in 4,457 individuals comprising 1,174 simplex ASD families from the Simons Simplex Collection (Fischbach and Lord, 2010), nearly three-fold larger than previously reported simplex cohorts. Each family is extensively phenotyped, with a single affected offspring, unaffected parents, and, in the majority of cases, at least one unaffected sibling. This ascertainment strategy is designed to enrich for rare *de novo* risk variants, the family-based case-control comparisons mitigate a wide range of technical and methodological confounders that have plagued association study designs (Altshuler et al., 2008), and we have developed a new rigorous approach to evaluating the genome-wide significance of recurrent rare *de novo* events. Consequently, the scale and design of this study provides an extraordinary opportunity to investigate the relative contributions of rare *de novo* and rare transmitted variants in simplex families, to identify novel ASD risk loci, to evaluate the relationship between rare structural variation and social and intellectual disability (ID), and to place these findings in the context of previous ASD data, particularly with regards rare *de novo* CNVs.

Results

Simons Simplex Collection summary characteristics

A total of 4,457 individuals from 1,174 families were included in the study. Data from 1,124 families passed all quality control; 872 families were quartets that included two unaffected parents, a proband, and one unaffected sibling; 252 families were trios that included two unaffected parents and a proband (Figure 1).

The male to female ratio for probands was 6.2:1. All had confirmed ASD diagnoses based on well-accepted research criteria (Risi et al., 2006), including autism: 1,006 (89.5%), Pervasive Developmental Disorder-Not Otherwise Specified: 96 (8.5%), and Asperger Syndrome: 22 (2%). The mean age at inclusion was 9.1 years for probands (4-18 years) and 10.0 years (3.5-26 years) for siblings. The mean (±95% CI) full-scale IQ in probands was 85.1±1.5, however the range was considerable (<20-167, Figure 3); the mean verbal IQ was 81.9±1.7 and the mean non-verbal IQ was 88.4±1.4. Self-reported ancestry was as follows: White, non-hispanic: 74.5%; Mixed: 9.3%; Asian: 4.3%; White, Hispanic: 4.0%; African American: 3.8%; Other: 4.2%. Additional phenotypic data may be found in recent publications (Fischbach and Lord, 2010) and at www.sfari.org/simons-simplex-collection.

Illumina 1M arrays accurately detect both rare de novo *and transmitted CNVs*

DNA samples derived from whole blood (N=4,381), cell lines (N=68), or saliva (N=8) were genotyped on the Illumina IMv1 (334 families) or Illumina IMv3 Duo Bead-arrays (840 families), which share 1,040,853 probes in common. CNV prediction was performed by PennCNV (PN) (Wang et al., 2007), QuantiSNP (QT) (Colella et al., 2007), and GNOSIS (GN), (www.CNVision.org) (Figure 1). 115 predicted rare (≤50% of the span of the event found at >1% in the database of genomic variation (DGV)) CNVs were evaluated by quantitative polymerase chain reaction (qPCR). A higher positive predictive value was observed for CNVs predicted by PN and QT, with or without GN (PPV=97% with GN, PPV=83% without) than for other combinations of algorithms, irrespective of the number of probes mapping within the structural variation (Table S2, Figure S1); these “high-confidence” criteria were subsequently used to identify all rare transmitted CNVs.

However, given the likely importance of *de novo* variation, and the relative challenge of accurately detecting these CNVs (Lupski, 2007), we focused on this small subset of predictions and further optimized our detection strategy using the first 585 quartets with complete genotyping data (Figure 1). We predicted putative *de novo* events from the group of rare high-confidence CNVs based on the combination of within-family intensity and genotypic data and used a blinded qPCR confirmation process (Figure S1). 53% of *de novo* predictions based on ≥20 probes (N=94) were confirmed compared with 2.6% with <20 probes (N=430). 82% of failures were false-positive predictions in offspring, 18% were false-negatives in parents. The data from this experiment were used to refine *de novo* prediction thresholds (supplementary materials). In addition, given the large number of predictions of small CNVs, and the low yield of true positives in the pilot data set (Figure S1), we elected to restrict all further statistical analysis to rare *de novo* events encompassing ≥20 probes that were also confirmed by qPCR in whole-blood DNA (Figure S1).

At the conclusion of our study, we were able to evaluate this threshold further via a comparison of confirmed *de novo* CNVs identified here with those reported in 1,340 overlapping offspring (probands or siblings) using the Nimblegen 2.1M array, as described by Levy and colleagues in this issue (Levy et al., 2011). At a threshold defined by the presence of ≥20 Illumina probes within a genomic interval, a combined total of 58 rare *de novo* CNVs were identified across both studies, with each array type identifying 95% (n=55) of the total events. This suggests that both arrays have high sensitivity for such events at or above this threshold, and that the combined results are very likely to represent the complete set of large *de novo* CNVs present in the SSC. This situation is reversed below 20 probes: a total of 31 small rare *de novo* CNVs were identified between the two groups with approximately twice as many found using the 2.1M Nimblegen array vs. the IM Illumina array (23 CNVs vs. 12 CNVs respectively) as would be expected for the increased probe resolution. Of these 31 events only 13% (n=4) were identified by both groups, suggesting

that the sensitivity for small *de novo* events was low for both arrays and that, as expected, there is a pool of small *de novo* structural events that were not considered in our analyses.

Analysis of rare *de novo* CNVs in the Simons Simplex Collection (SSC)

***Rare*, de novo, genic CNVs are over-represented in simplex probands—**In light of strong prior evidence for an increased burden of *de novo* CNVS in simplex autism (Itsara et al., 2010; Marshall et al., 2008; Pinto et al., 2010; Sebat et al., 2007), we investigated these events in probands versus their unaffected siblings in all 872 quartets in this study (Figure 1). A total of 28,610 rare, high-confidence CNVs were identified; 97 were classified as rare and likely *de novo*, and 83 events were confirmed to be rare *de novo* CNVs by qPCR in whole-blood DNA (Table S4).

Rare *de novo* CNVs were significantly more common among probands than siblings. Overall 5.8% of probands (N=51 out of 872) had at least one rare *de novo* CNV compared with 1.7% of their unaffected siblings (N=15 out of 872) yielding an odds ratio (OR) of 3.55 (CI =2.16-7.46, $p=6.9 \times 10^{-6}$, Fisher's exact test) (Table 1 and Figure 2). When we considered the proportion of individuals carrying at least one rare *de novo* CNV that also contains known genes (genic CNVs), the OR increased to 4.02 (50 in probands vs. 12 in siblings; CI =1.98-6.36, $p=2 \times 10^{-6}$). These results remained consistent regardless of whether we analyzed total numbers of CNVs as opposed to the proportion of individuals with at least one (Figure 2), or increased the stringency of the threshold for “rarity” (supplementary materials).

Given the strong male predominance and increased rates of ASD in monogenic X-linked intellectual disability syndromes, we paid particular attention to rare *de novo* CNVs on the X chromosome but found only 2 events: one genic deletion present in a male at the gene *DDX53* and a duplication involving 6 genes in a female sibling (Xq11.1). This small number precluded meaningful group comparisons. Importantly, neither these, nor any subsequent statistical results reported here were substantively altered by the exclusion of 15 confirmed rare *de novo* CNVs identified during our detection optimization experiments that did not then meet our minimum probe criteria (Table S4). Of note, however, one of these was an exonic deletion of *NLGN3* on chromosome X in a male proband. (Table S4)

This burden of rare *de novo* CNVs in simplex families is remarkably similar to previously published results (Table S1) despite varying CNV discovery approaches and array densities from 85,000 (Sebat et al., 2007) to 1 million probes (Pinto et al., 2010). We reasoned that this was likely due to the particular importance of large *de novo* events, as their detection would be least sensitive to differences in probe number and distribution. Indeed, we found that rare *de novo* CNVs in probands tended to be larger than in siblings (mean 1.6Mb vs. 0.7Mb) (Figure 2, Figure S2) and to include a greater number of genes (16-fold increase in probands, and a 29-fold increase considering only deletions).

In fact, we found that *de novo* CNVs in probands were both larger and contained a greater number of genes when these measures were considered independently. We fit a series of stepwise linear models that increased in complexity from individual predictors to an analysis of covariance model, with size and affected status as predictors, to a three-term model that included the interaction of size and affected status. We confirmed a significant difference between probands and siblings with regard to the number of genes within CNVs (estimated $\beta=11.1$ more genes in a proband's *de novo* CNV; $p=0.025$) even after accounting for the strong effect of the size of the event (estimated $\beta=6.8$ genes per Mb; $p=1.1 \times 10^{-9}$) (Figure 3A). Considering deletions and duplications separately did not alter these findings. In summary, the burden of rare *de novo* CNVs is greater in probands with regard to number, size, and gene content.

***Strong association of rare, recurrent*, de novo *CNVs*—**Our interest in identifying specific regions of the genome contributing to ASD led us to next investigate whether multiple overlapping *de novo* events were present in probands and then to compare these findings to siblings. In total, 23 probands were found to carry recurrent *de novo* CNVs in 6 separate regions of the genome. Each of these intervals contains from 2 to 11 *de novo* CNVs in unrelated probands; no *de novo* CNVs overlapping these regions were found in siblings. In contrast only a single recurrent *de novo* event was observed in siblings (16p13.11 in 2 unrelated siblings) and one CNV overlapping the region was also found in a proband (Figure 4).

The 6 regions found in probands included 7 deletions and 4 duplications at chromosome 16p11.2, 4 duplications at 7q11.23 (the Williams-Beuren syndrome region), and 2 CNVs each at 1q21.1 (2 duplications), 15q13.2-q13.3 (1 deletion, 1 duplication), 16p13.2 (2 duplications), and disrupting the gene *Cadherin 13* (*CDH13*) at 16q23.3 (5Mb deletion and an overlapping 34kb exonic deletion).

The presence of multiple regions showing overlapping rare *de novo* CNVs restricted to probands, and the absence of similar findings in their sibling controls, is striking. However, in contrast to genome-wide common variant association studies, there is no widely accepted statistical approach or threshold to formally evaluate these results. Consequently, we set out to develop a rigorous method to assess the genome-wide significance of *de novo* events (methods). To do so, we determined the null expectation for recurrent rare *de novo* CNVs based on our data from unaffected siblings and then used this expectation to evaluate the p-value for finding multiple recurrences in probands.

Using this approach, the probability of finding 2 rare *de novo* CNVs at the same position in probands is 0.53. However, the observation of 4 recurrent *de novo* duplications at 7q11.23 ($p= 7 \times 10^{-6}$) and 11 recurrent *de novo* CNVs at 16p11.2 ($p= 6 \times 10^{-23}$) are both highly significant. In addition, we found that 16p11.2 deletions (N=7; $p=2 \times 10^{-14}$) and duplications (N=4; $p=7 \times 10^{-6}$) are strongly associated with ASD when considered independently (Figure S3).

Prior studies have often found a combination of rare transmitted and *de novo* CNVs at ASD risk regions. In our data, we observed 8 loci at which rare transmitted CNVs, present only in probands, overlapped one of the 51 regions in probands containing at least one rare *de novo* CNV. Conversely, in siblings we did not observe any cases in which a rare transmitted CNV, restricted to siblings, overlapped one of the 16 regions showing *de novo* events. Interestingly, the 8 regions in probands showing overlapping rare *de novo* and rare transmitted CNVs include 5 of the 6 intervals with recurrent rare *de novo* variants, 1q21.1, 15q13.3, 16p13.2, 16p11.2, and 16q23.3 (Figure 4), and 3 additional genomic segments with 1 rare *de novo* event: 2p15, 6p11.2, and 17q12.

While the use of matched sibling controls should preclude any confound of population stratification, we explored whether genotype data from the parents of probands with 16p11.2 or 7q11.23 CNVs suggested unusual ancestral clustering (Crossett et al., 2010; Lee et al., 2009) pointing to a particular haplotype that might increase the frequency of *de novo* events. We found no evidence for this. In addition, given the very large number of 16p11.2 CNVs in this study and the widespread attention afforded previous findings at this locus, we considered the possibility of ascertainment bias. A review of medical histories obtained at the time of recruitment revealed that parents had prior knowledge of a 16p11.2 CNV in 2 instances (1 *de novo* duplication, 1 transmitted deletion). Nonetheless, with these events removed, association of both deletions and duplications remains significant ($p=3 \times 10^{-19}$ all

de novo events (N=10); p=2 × 10^{-14} deletions (N=7); p=0.002 duplications (N=3)) (Figure S4).

***The distribution of* de novo CNVs in probands, supports marked locus heterogeneity—**The identification of multiple recurrent *de novo* events restricted to probands, and the absence of similar observations in siblings, led us to consider what these findings might indicate about the overall number of CNV-mediated ASD risk loci that are present in the genome. Consequently, we used the distribution of 67 *de novo* CNVs identified in SSC probands to calculate the number of regions likely to be contributing large rare *de novo* risk variants and estimated 130 loci (methods).

We then evaluated the implications of this likely genomic architecture for the planned second phase of genotyping and CNV analysis in the SSC, which is currently underway. We used the estimated number of predicted ASD loci to guide a simulation experiment (supplementary methods) and found that the most likely outcome of a second SSC cohort of similar composition and size to that reported here will be further confirmation of 7q11.23 and 16p11.2 and the identification of 2-3 additional regions of significant association. These were most likely to emerge at the intervals already identified containing recurrent *de novo* events in phase 1, namely 1q21.1, 15q13.2-13.3, 16p13.2, and the *CDH13* locus.

Genotype-phenotype analyses of probands carrying any rare de novo *CNV*—Given highly reliable phenotypic data and long standing interest in the role of sex in ASD risk and resilience, we investigated whether males or females carried quantitatively different types of rare *de novo* events and what impact rare *de novo* CNVs had on intellectual and social functioning in both groups.

We found little evidence for larger or more gene rich *de novo* CNVs in males versus females. By fitting a series of stepwise linear models, we evaluated whether the number of genes within a *de novo* CNV tended to differ after accounting for a critical covariate, CNV size. Neither sex (p=0.20) nor the interaction of size and sex (p=0.06) was a significant predictor of gene number. These results should to be viewed with some caution, given the trend toward significance and a relatively small sample size (Figure 3B).

In contrast, we found that male intellectual functioning was relatively more vulnerable to the effects of rare *de novo* CNVs. Again using a series of stepwise linear models we evaluated the relationship between intellectual functioning, sex, and the number of genes within rare *de novo* CNVs. For males, there was a significant relationship between IQ and number of genes (p=0.02), with the model predicting a decrease of 0.42 IQ points for each additional gene. In contrast, for females the estimated effect was ten-fold less and did not approach significance (Figure 3D).

To evaluate whether low IQ predicted if a proband carried a *de novo* CNV, we fit a logistic regression model with *de novo* CNV status for probands as the outcome and full-scale IQ as the predictor. We found the accuracy of prediction was quite low (Nagelkerke pseudo R^2=0.014). Overall, while the odds of carrying a *de novo* CNV varied three-fold for those with the lowest versus the highest IQ, the odds were never large (0.111 at IQ=30, 0.063 at IQ=80, and 0.036 at IQ=130). This relationship did not differ significantly by sex (interaction of IQ and sex, p=0.12).

Finally, we investigated the relationship between IQ, sex, and number of genes within rare *de novo* CNVs to determine if any of the models significantly predicted ASD severity (measured by the ADOS combined severity score (CSS)); of these only full-scale IQ predicted ASD severity (p=0.02).

Overall, the data show a strong effect of large rare genic *de novo* CNVs on affected status, but do not support either IQ or ASD severity as useful predictors for probands carrying large rare *de novo* risk variants in the SSC (Figure 3C). We did observe a trend toward more gene rich *de novo* CNVs in females and found females to be less vulnerable to the reduction in IQ associated with rare *de novo* CNVs.

Genotype-phenotype analyses of probands carrying 16p11.2 and 7q11.23 CNVs—We next investigate whether individuals with recurrent CNVs at 16p11.2 or 7q11.23 showed distinctive behavioral or cognitive profiles compared with probands who were not carrying rare *de novo* events. For each proband carrying a *de novo* CNV at 16p11.2 or 7q11.23, five other probands were selected as controls based on hierarchical matching criteria: first age, then sex, genetic distance, ascertainment site, and whether the sample was from a quartet or trio.

Our primary analysis focused on 4 variables: full-scale IQ, categorical diagnosis, severity of autism, and body mass index (BMI) (Table 2), with the latter motivated by multiple reports that 16p11.2 deletions (Bijlsma et al., 2009; Walters et al., 2010) contribute to obesity and the recent observation that duplications have the opposite impact on weight (Reymond et al., 2010). We then pursued a broader exploratory study of additional phenotypic variables, 10 of which are presented in Table 2 and the remainder in Table S5.

We found that probands carrying a 16p11.2 or 7q11.23 *de novo* CNV were indistinguishable from the larger group with regard to IQ, ASD severity, or categorical autism diagnosis (Table 2). However, we did find a relationship between body weight and 16p11.2 deletions and duplications. When we treated copy number as an ordinal variable (1, 2, and 3 copies), and used the matched controls as the diploid sample, BMI diminished as 16p11.2 copy number increased (estimated $\beta = -3.1 kg/m^2$ for each extra copy, $p=0.02$).

The extensive phenotypic data available on the SSC sample provides great potential to undertake fine-grained analyses of genotype-phenotype relationships. At present the limiting factor with regard to recurrent *de novo* CNVs is the small sample size, even for 16p11.2 duplications and deletions in this dataset. However, we undertook an exploratory analysis of a range of phenotypic features and found several that yielded significant p-values. While none would survive correction for multiple comparisons, we report them here (Table 2, Table S5) in the interest of generating hypotheses for future studies. For example, individuals with 16p11.2 duplications had higher hyperactivity scores, compared to matched control probands, while probands carrying 7q11.23 duplications showed significantly more behavioral problems (ABC total), but less severe social and communication impairment during ADOS administration.

Analysis of rare transmitted CNVs in the SSC

Rare, transmitted autosomal CNVs are equally represented in probands and siblings—Given the very strong association of rare *de novo* CNVs, we were somewhat surprised to find that rare transmitted CNVs were not present in greater numbers in affected individuals or in a greater proportion of probands versus siblings. As prior publications have shown an increased burden of specific subsets of CNVs in neuropsychiatric disorders including autism and schizophrenia, we considered multiple subcategories of rare transmitted events as well, including genic, exonic, brain-expressed, and ASD-related, and did not find a statistically significant result that survived correction for multiple comparisons (Figure 5).

These findings stood in contrast to a recent rigorous large-scale CNV study undertaken by the Autism Genome Project (AGP) (Pinto et al., 2010). Their sample included both simplex

and multiplex families and identified a significantly higher burden of genic and ASD-related CNVs in cases versus unrelated controls. Notably they did not differentiate between transmitted and *de novo* events in this analysis. We reanalyzed our data using the identical criteria detailed in their manuscript and found similar results (Table S6). However, when we again restricted the analysis of our sample to rare transmitted CNVs, by removing confirmed rare *de novo* events; there was no significant difference found between proband and siblings, suggesting that the excess burden in the SSC sample was entirely driven by rare *de novo* events.

We pursued this analysis further given strong evidence that certain rare transmitted CNVs carry ASD risk, as well as reports of particularly significant effects for maternal transmission of rare CNVs to male probands (Zhao et al., 2007). Consequently, we investigated whether mothers were more likely than fathers to transmit a rare CNV to an affected offspring. We also asked whether there were a greater number of maternally transmitted CNVs in probands versus their unaffected siblings. Neither analysis showed a significant result after correction for multiple comparisons despite considering combinations of the following variables: deletions, duplications, size, exonic, brain-expressed, and ASD-related. In addition, based on the possibility that risk might be confined to only the rarest transmitted events, presumably under the strongest purifying selection, we evaluated "singleton" CNVs, i.e. those observed in only one parent and transmitted to only one proband or sibling. In this case, we found a modest, non-significant excess of maternally transmitted CNVs in probands: 344 maternal autosomal singletons are transmitted to probands vs. 303 transmissions to siblings (OR= 1.14; p=0.059 one-sided; p=0.12 two-sided). For fathers, there was no similar trend (OR= 1.03; p=0.37 one-sided).

Rare transmitted X-linked CNVs are equally represented in probands and siblings—We asked similar questions regarding transmission of rare X-linked CNVs from mothers to male probands and obtained similar results. In a group of 353 male probands and 353 matched male siblings, we found, contrary to expectations, that more siblings carried maternally transmitted rare CNVs than probands (14% probands vs. 18% siblings, OR=0.76, p=0.11), though this difference was not significant. The result did not change when we evaluated the various subcategories of rare X-linked CNVs including exonic, deletions, duplications, size, brain-expressed, or ASD-associated.

Rare transmitted CNVs show greater biological coherence in probands versus siblings—We hypothesized that the absence of evidence of association for rare transmitted CNVs might be a consequence of the inability to differentiate functional from neutral variants. Consequently we looked to pathway analyses to help address this question, reasoning that if the specific genic content of CNVs contributed to disease risk, we would find a greater enrichment of biological pathways in probands versus siblings.

We used two gene ontology and pathway analysis tools, MetaCore from GeneGo Inc. and DAVID (Dennis et al., 2003; Huang et al., 2009), to analyze 1,516 genes within CNVs exclusive to probands and 1,357 genes exclusive to siblings. The total number and size of rare, transmitted CNVs used to determine these gene sets were highly similar in probands and siblings (Figure 5). GeneGo Networks identified 22 pathways showing significant enrichment in probands versus only 4 enriched pathways among siblings. This difference was significant based on 100 permutations of the dataset (p=0.04). DAVID yielded consistent results with 59 pathways enriched in probands and 19 in siblings (p=0.01, permutation analysis) (Figure 6).

For the current analysis, we elected to restrict our evaluation of pathways to the general question described here. A manuscript describing a more extensive pathway analysis is in preparation focusing on both structural and gene expression data from the SSC.

Transmitted autosomal and X chromosome CNVs overlap with previously reported ASD loci—We next examined all rare CNVs in the SSC in light of previously reported findings, comparing our data to the list of ASD regions included in the recent AGP analysis (Pinto et al., 2010). We also considered recent common variant findings, including *SEMA5A* (Weiss et al., 2009), *MACROD2* (Anney et al., 2010), *CDH9* and *CDH10* (Wang et al., 2009), the *MET* oncogene (Campbell et al., 2006), *EN2* (Gharani et al., 2004), and selected schizophrenia loci (ISC, 2008; McCarthy et al., 2009; Millar et al., 2000; Stefansson et al., 2008; Walsh et al., 2008; Xu et al., 2008) (Table 3). We identified multiple regions in which rare transmitted and/or rare *de novo* events corresponded to previously characterized loci in both ASD and schizophrenia.

Rare transmitted CNVs do not show genome-wide association in the SSC—Finally, we looked for evidence of association for any other CNVs in the SSC sample, evaluating all high-confidence autosomal CNVs together with all confirmed *de novo* CNVs. In this instance, we did not use a frequency cutoff to define a set of rare transmitted events. A total of 3,667 recurrent regions were identified; 6 showed relative enrichment in probands and 5 in siblings. No result reaches significance after correction for multiple comparisons (Table S7, Figure 7C). The region showing the greatest difference in probands compared to siblings was 16p11.2 (p=0.001).

Expanded analysis of rare *de novo* CNVs across multiple ASD samples

***An analysis of* de novo CNVs in 3,816 probands from genome-wide studies of idiopathic ASD supports association of 6 genomic intervals—**Our approach to assessing the genome-wide significance of rare recurrent *de novo* CNVs allows for a statistical evaluation of events observed in cases without requiring additional matched control samples. Consequently, we were able to conduct a cumulative analysis across multiple studies in search of additional associated ASD loci. We included 4 other large-scale ASD CNV studies (Itsara et al., 2010; Marshall et al., 2008; Pinto et al., 2010; Sebat et al., 2007) meeting 4 criteria: standardized diagnosis, genome-wide detection, confirmed *de novo* structural variations, and sufficient information to permit the identification of duplicate samples.

These datasets catalogued 228 confirmed, rare *de novo* CNVs from a total of 3,816 individuals (Table S1). We found 6 regions that exceeded the threshold for significance (methods). Given prior evidence, and our own data, that reciprocal deletions and duplications at certain loci, both contribute to the ASD phenotype we evaluated significance for combined events at an interval, as well as calculating probabilities for deletions and duplications separately (Table 4, Figure S3).

The most frequent recurrent *de novo* CNV identified across all studies was 16p11.2 with 19 identified probands (14 deletions, 5 duplications) showing extremely strong evidence for association with ASD (2×10^{-55} combined; 5×10^{-29} for deletions; 2×10^{-5} for duplications). The proximal long arm of chromosome 15 showed two contiguous intervals: the first corresponds to the region 15q11.2-13.1 or BP2-BP3 (7 duplications; 4×10^{-9}) (Figure 7A), long cited as the most common cytogenetic abnormality identified in idiopathic ASD (Cook et al., 1997). We also found evidence of association for the interval mapping to 15q13.2-13.3 or BP4-BP5 (5 duplications and 1 deletion; 1×10^{-4} combined, 2×10^{-5} for duplications) (Figure 7B). Rare deletions and duplications in this region have previously

been associated with intellectual disability and ASD, and deletions with schizophrenia and epilepsy (Figure 7). It is important to note, however, that considering only events restricted to 15q13.2-13.3 (i.e. removing 3 overlapping isodicentric chromosome 15 events) we do not find significance (0.53 combined; 0.88 for duplications). This suggests either that the result is an incidental finding due to the proximity to a true ASD risk locus, or, alternatively, that the smaller 15q13.2-13.3 CNVs might point to a minimum region of overlap mapping to one or more ASD-related genes.

Recurrent *de novo* CNVs exceeding the significance threshold in the combined sample were also present at 7q11.23 (4 duplications; 0.003), 22q11.2 region (3 deletions and 2 duplications; 0.002 combined; 0.11 for deletions; 0.88 for duplications), and at the locus coding for the gene *NRXN1*. For *NRXN1* there were 5 *de novo* events: 1 intronic deletion, 3 exonic deletions, and 1 exonic duplication (0.002 combined, 0.004 for deletions).

Finally, we used the observed number and distribution of *de novo* CNVs in the combined proband data set to estimate the likely number of CNV regions contributing to ASD. From the total of 219 confirmed *de novo* events, we derived an estimate of 234 distinct genomic regions contributing to large ASD-related *de novo* structural variations (methods).

Discussion

Our results highlight the importance of rare CNVs for simplex ASD. We confirm an over-representation of rare *de novo* events in probands versus siblings with an odds ratio of 3.55 for all variants and 4.02 for rare *de novo* genic variants. Using a novel approach to assessing significance specifically for recurrent *de novo* CNVs, we find very strong evidence for the contribution of duplications at 7q11.23, showing, for the first time., genome-wide association in a case-control study. Moreover, we identify four additional rare recurrent *de novo* events restricted to probands. Two of these, 16p13.2 and the *CDH13* locus, are novel ASD loci and two, 1q21, 15q13.2-13.3, have been previously implicated in neuro-developmental disorders including ASD. Each of these four regions also show rare transmitted CNVs exclusive to probands. Finally, we find compelling evidence confirming the association of both 16p11.2 duplications and deletions.

It is striking that while we replicate findings of elevated rates of rare *de novo* CNVs in simplex families (5.8% of probands versus 1.7% in siblings), the percentage of the cohort carrying these events is the same magnitude as that seen previously. This is despite the intensive focus on the ascertainment of simplex quartets and the 10-fold increase in probe density since the earliest studies of ASD. We believe these results are best explained by the particular contribution of large genic *de novo* variants given the results of our analysis of gene number, CNV size, and affected status (Figure 3), and the observation of generally consistent results over time despite steadily increasing detection resolution.

While it may not seem surprising that large *de novo* events carry the greatest risk for developmental disorders, it is interesting to note that we did not find evidence that ASD diagnosis or severity was mediated by intellectual disability (ID). It has been argued that ASD in the presence of ID may reflect an epiphenomenon, in which a non-specific impairment of brain functioning unmasks and/or exacerbates limitations in an individual's capacity for social reciprocity (Skuse, 2007). It has also been widely held that the detection of large *de novo* CNVs will be enhanced by the ascertainment of ASD samples with greater intellectual disability. Our data shows that large *de novo* CNVs confer substantial risk for ASD in the SSC, but they are only modestly correlated with lower IQ and largely independent of ASD severity.

These data suggest both that this study has identified *bona fide* high-risk variants for autism spectrum disorders and that many of these loci also confer liability to a range of complex neurobehavioral phenotypes. They also suggest a more complex relationship of IQ and large *de novo* events than is often supposed: for example the relatively high rates of 16p11.2 and 7q11.23 CNVs and low rates of 15q11.2-13.1 duplications seen in this study compared to others may reflect particular subpopulations of rare *de novo* risk CNVs that are more readily ascertained in cohorts with higher mean IQ.

The results further show that the risk associated with large *de novo* events is related to their greater genic content, even after controlling for larger size. This observation points to two countervailing hypotheses: first, that the greater gene number is a surrogate for the increased chance of disrupting one particular gene or regulatory region due to the involvement of a larger segment of the coding genome; or second, that it is the contribution of multiple genes and/or regulatory regions simultaneously within these CNVs that increases risk.

Our data do not allow us to resolve this issue. Nonetheless we suspect that if many deletions or duplications encompassing small numbers of genes were as highly penetrant as multigenic events, we would have begun to show more evidence for this either in the form of an overall increased burden for smaller *de novo* variations and/or association of specific *de novo* events. However, it is important to note that despite higher resolution than some prior studies, we nonetheless have a clear ascertainment bias for detection of larger CNVs. It is likely that the combination of high-throughput sequencing, larger patient cohorts, and increasingly sophisticated approaches to evaluating combinations of risk variants will begin to shed light on this issue, with regard to both sequence and structural variation.

Our findings with regard to recurrent *de novo* events in the SSC sample point to 6 putative ASD loci: two of these, 7q11.23 and 16p11.2, show clear evidence for genome-wide association. Moreover, our simulation analysis suggests that the most likely outcome of the ongoing Phase 2 SSC study will be confirmation of 2-3 of the remaining 4 intervals already showing recurrent *de novo* events, namely 1q21.1, 15q13.2-13.3, 16p13.2, and 16q23.3 (*CDH13*).

Our findings at 7q11.23 point to extraordinary opportunities to illuminate the molecular mechanisms of social development. Duplications in this interval have previously been described in developmental disorders, including ASD (Berg et al., 2007; Van der Aa et al., 2009), though these have been restricted to case reports or series, with the attendant difficulties in controlling for ascertainment bias. The identification of clear association of duplications in this controlled study of ASD is particularly striking given that the reciprocal deletion results in a developmental syndrome characterized in part by an empathic, gregarious, and highly social personality (Pober, 2010). Moreover, several lines of evidence, including atypical deletions (Antonell et al., 2010), mouse models (Fujiwara et al., 2006; Hoogenraad et al., 2002; Meng et al., 2002; Sakurai et al., 2010), and gene expression x phenotype studies (Gao et al., 2010; Korenberg et al., 2000) have already identified *CAPGLY domain containing linker protein 2* (*CLIP2*), *LIM domain kinase 1* (*LIMK1*), *General transcription factor II, i* (*GTF2i*), and *Syntaxin 1A* (*STX1A*) as the leading candidates among the 22 genes within the region for involvement in the cognitive and social phenotypes. The characterization of this single region in which opposite changes in copy number contribute to contrasting social phenotypes promises to set the stage for a range of interesting studies of the role of gene dosage within this interval and the genesis of social mechanisms.

The strong replication of findings at 16p11.2 also highlights emerging opportunities for translational neuroscience. Firstly, the region is sufficiently circumscribed to interrogate

using molecular biological and model systems. Secondly, though we cannot quantify an odds ratio from our data, given the absence of events in siblings, there is clear evidence from this and prior studies (McCarthy et al., 2009) that 16p11.2 CNVs carry much larger effects than any common variant contributing to complex common disorders. Thirdly, the 1% allele frequency allows for prospective studies of natural history, neuroimaging, and treatment response, as, for example, in the recently launched Simons Variation in Individuals Project (https://sfari.org/simons-vip). Finally, the entirety of the data now strongly supports a role for both duplications and deletions of 16p11.2 in social disability. Together, these suggest that cross-disciplinary approaches can begin to address the means by which a single locus leads to a range of psychiatric outcomes previously conceptualized as distinct and to address the critical role that dosage sensitivity plays in the unfolding of these neuro-developmental phenotypes.

The notion that the 4 remaining recurrent *de novo* regions represent true ASD variants is supported by multiple lines of additional evidence. For example, they are among only 8 rare *de novo* CNVs that overlap with rare transmitted events restricted to probands. Moreover, 2 of the remaining 3 loci, 2p15 and 17q12, have been previously implicated in ASD (Liang et al., 2009; Moreno-De-Luca et al., 2010). In addition, rare 1q21.1 and 15q13.2-13.3 CNVs have been identified in developmental and neuropsychiatric syndromes, with deletions found in ASD (Miller et al., 2009; Shen et al., 2010), schizophrenia (ISC, 2008; Stefansson et al., 2008), idiopathic epilepsy (Helbig et al., 2009), and recurrent duplications reported here. *CDH13* has not previously been noted to be a risk variant, but the larger family of proteins has been implicated in ASD pathogenesis through CNV studies (Glessner et al., 2009), homozygosity mapping (Morrow et al., 2008), common variant findings (Wang et al., 2009), and our pathway analysis (Figure 6). The 16p13.2 region contains four genes, the most immediately notable of which are *C16orf72*, coding for a protein of unknown function, recently identified in a schizophrenia CNV study (Levinson et al., 2011), and *Ubiquitin Specific Peptidase 7* (*USP7*), which has been shown to have a role in oxidative stress response, histone modification and regulation of chromatin remodeling (Khoronenkova et al., 2010). Both would represent novel ASD risk genes, however for the latter, these biological processes, and the ubiquitin pathway in particular, have been previously implicated in ASD pathogenesis (Glessner et al., 2009).

The fact that the family-based design used in our study played a key role in allowing us to identify association presents an important contrast to the prevailing wisdom with regard to genome-wide association studies of common variants, in which there is a tendency to rely on unrelated case-control designs, given the relative ease of generating very large sample sizes. It is notable that the statistical power afforded by the low probability of observing multiple recurrent rare *de novo* events by chance more than compensated for the comparatively small sample reported here. This is particularly striking with respect to 16p11.2. Based on a traditional case-control comparison, the most significant finding in this sample, 14 events in probands and 0 in siblings ($p=0.001$, Fisher's exact test), did not provide evidence sufficient to withstand correction for multiple comparisons, while the analysis based on the null expectation for *de novo* recurrence clearly detected association. It is certain that the SSC sample ascertainment process enhanced certain findings and attenuated others. There is little question that restricting the comparison group to matched siblings limited power to identify association of specific rare recurrent transmitted events; our assessment of significance for *de novo* CNVs was based on conservative assumptions and may have excluded true risk loci; the filtering for rare *de novo* CNVs and the small sample size curtailed the assessment of multi-hit hypotheses; the generally older parental age may have obscured the relationship between age and *de novo* variation (Figure S3) and, as noted, poor specificity at the lower bound of detection limited our assessment of small *de novo* structural variations.

However, despite these limitations, the manner in which the design mitigated important confounds, and preserved sufficient power to detect association of recurrent *de novo* events, yielded clear benefits, unambiguously replicating prior findings and identifying novel risk loci. Moreover, this report considers less than half of the Simons Simplex Collection: phase 2 of this study is currently underway, as is high-throughput sequencing of the collection, also focusing on *de novo* events. Together these endeavors promise to further illuminate the genomic architecture of simplex autism and to provide additional critical points of traction for efforts toward elaborating the molecular mechanisms and developmental neurobiology underlying ASD.

Experimental Procedures

Genotyping

All members of each family were analyzed on the same array version: either the Illumina 1Mv1 (334 families) or Illumina 1Mv3 Duo (840 families) Bead-array. These share 1,040,853 probes in common (representing 97% of probes on the IMv1 and 87% of probes on the 1Mv3). 824 or the 872 quartet families (94.5%) had all members hybridized and scanned simultaneously on the Illumina iScan in an effort to minimize batch effects and technical variation.

Identity quality control

Genotyped samples were analyzed using Plink (Purcell et al., 2007) to identify incorrect sex, Mendelian inconsistencies, and cryptic relatedness by assessing inheritance-by-descent (IBD); 11 families were removed as a result.

CNV detection

CNV detection was performed using three algorithms: 1) PennCNV Revision 220, 2) QuantiSNP v1.1, and 3) GNOSIS. PennCNV and QuantiSNP are based on the Hidden Markov Model (HMM). GNOSIS uses a continuous distribution function (CDF) to fit the intensity values from the HapMap data and determine thresholds for significant points in the tails of the distribution that are used to detect copy number changes. Analysis and merging of CNV predictions was performed with CNVision (www.CNVision.org), an in-house script.

CNV Quality Control

Specific genotyping and CNV parameters are detailed in the supplementary methods. 5% of the samples failed and were rerun; 39 families were removed due to repeated failures.

Criteria for a Rare CNVs

A CNV was classified as rare if ≤50% of its length overlapped regions present at >1% frequency in the Database of Genomic Variation (DGV) March 2010.

CNV burden

Burden analyses were performed on the matched set of 872 probands and siblings. Typically, three outcomes were assessed: proportion of individuals with ≥1 CNV matching the criteria (p-value calculated with Fisher's exact test); number of CNVs matching the criteria (p-value calculated with sign test); number of RefSeq genes within or overlapping CNVs matching the criteria (p-value calculated with Wilcoxon paired test). Where burden was assessed for unequal numbers of probands and siblings (e.g. by sex) the sign test and Wilcoxon paired test were replaced with the Wilcoxon test.

Statistical analysis of *de novo* recurrence

To determine the probability of finding multiple rare *de novo* CNVs at the same location in probands, we first estimated how many likely positions in the genome were contributing to the observed *de novo* CNVs in siblings. As there are widely varying mutation rates for structural variation across the genome (Fu et al., 2010), some positions are more likely to result in *de novo* CNVs observed in our sample than others. Consequently, the likely number of positions is much smaller than the total possible number of positions. We refer to the likely CNV regions as eCNVRs (effective copy number variable regions) and calculate their quantity "C" using the so-called "unseen species problem" which uses the frequency and number of observed CNV types (or species) to infer how many species are present in the population. Based on the observed *de novo* CNVs in the control sibling group, we apply the formula (Bunge and Fitzpatrick, 1993) $C = c/u + g^2*d*(1-u)/u$, in which c = the total number of distinct species observed; c_1= the number of singleton species; d = total number of CNVs observed; g = the coefficient of variation of the fractions of CNVs of each type, and u = 1 – c1/d. (In this calculation, due to the small number of observations, we assume that g equals 1.) For the *de novo* events in siblings, c_1=14, c=15, d=16 and C=232. This calculation is performed in the siblings because the observed rare *de novo* CNVs in this group are assumed to be predominantly non-risk variants and consequently represent the null distribution.

Next, we calculate the chance that two *de novo* events match at any one of "C" eCNVRs in probands, using methods from the classic "birthday problem" which assess the likelihood of seeing at least one pair of matching birthdays among a given number of people. Our interest was in seeing >2 matches (m) in probands under the null hypothesis of no association with ASD. This calculation is performed empirically by distributing "d" events at random among "C" eCNVRs and then counting the maximum number of CNVs falling in the same location. Repeating this experiment many times, we obtained an estimate of the probability of finding "≥m" counts for ≥1 eCNVR under the null hypothesis.

Given the importance of the estimate of eCNVRs in unaffected populations for the determination of significance, we re-calculated "C" based on a combined set of confirmed *de novo* CNVs in controls described in the literature and obtained a highly similar result (C=242) (supplementary materials). Moreover, we determined that the results reported here remain significant under the plausible range of estimates for "C" (supplementary materials).

Estimate of number of de novo *CNV regions contributing to ASD risk*

The unseen species problem was used to predict the total number of ASD risk-loci based on the distribution of *de novo* CNVs in probands. This required identification of the *de novo* CNVs that confer risk; to identify such CNVs we estimated that 75% of *de novo* CNVs in probands confer risk (67 *de novo* CNVs in probands – 16 *de novo* CNVs expected in siblings / 67 *de novo* CNVs in probands) and assumed that recurrent *de novo* CNVs were most likely to be associated with risk and should be included within this 75%. The remainder of the 75% is made up of 27 single occurrence *de novo* CNVs (though we do not identify which ones) leading to an estimate of the total number of risk conferring loci as 130 (c_1=27, c=33, d=51). A similar approach was applied to all *de novo* CNVs in 3,816 probands (count derived from the literature), leading to an estimate of 234 risk conferring loci (c_1=59, c=88, d=158).

Stepwise assessment of multiple variables

Predictors were examined in a logical order, e.g. to evaluate the relationship between gene number (G), CNV size (L), and affection status (A, proband vs. sibling), we fit a series of increasingly complex linear models in the following steps: (1) regress response G on predictor L, regress G on A; 2) if ≥1 term was significant, and assuming L had the best

predictive power, we regressed G on L and A; (3) assuming L and A were significant jointly, we regressed G on L, A and L*A (L interacting with A). The latter term permits the slope of the relationship between G and L to differ for probands vs. siblings. In each step, we determined if the newest term was significant, given the terms already in the model. We also fit the model using backward elimination, starting with the full model and simplifying it one term at a time.

Population structure of recurrent de novo *CNVs*

All parents were projected onto a five-dimensional ancestry map using eigenvector decomposition (Crossett et al., 2010; Lee et al., 2009). Euclidean distances were measured for the parent-of-origin. The mean and median distances between these pairs of parents were calculated and were evaluated relative to the remainder of the sample using a bootstrap procedure (supplementary methods).

Genotype-Phenotype analysis

For each sample with a 16p11.2 deletion (8 samples) or duplication (6 samples) or 7q11.23 duplication (4 samples) 5 control probands were selected based on a matching hierarchy: age (100% of control probands matched), sex (100%), genetic distance (91%, based on five-dimensional ancestry map), collecting site (46%), and quartet/trio family (34%). Probands with *de novo* CNVs or CNVs in regions previously associated with ASD were removed prior to matching; each control proband was only included once.

For continuous variables each stratum of a “case” proband matched to 5 “control” probands was treated as a block and the data analyzed as a randomized block design by using analysis of covariance. Thus mean values were allowed to vary across blocks and to be altered by case-control status. The difference due to the presence of the CNV of interest was assessed with an F-test with N, M degrees-of-freedom (N is the number of CNVs of interest and M is the residual degrees-of-freedom after accounting for model terms). Because IQ is known to affect many behavioral measures associated with ASD, it was treated as a covariate in models for outcomes besides itself and Body Mass Index (BMI). For diagnostic status, matching was taken into account by using a conditional logit model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acknowledgments

We are most grateful to all of the families at the participating SFARI Simplex Collection (SSC) sites. This work was supported by a grant from the Simons Foundation (SFARI 124827). C.A.W. and R.P.L. are Investigators of the Howard Hughes Medical Institute. We wish to thank: the SSC principal investigators: (A. Beaudet, R. Bernier, J. Constantino, E. Cook, E. Fombonne, D. Geschwind, D. Grice, A. Klin, D. Ledbetter, C. Lord, C. Martin, D. Martin, R. Maxim, J. Miles, O. Ousley, B. Peterson, J. Piggot, C. Saulnier, M. State, W. Stone, J. Sutcliffe, C.A. Walsh, E. Wijsman); the coordinators and staff at the SSC sites; the SFARI staff (M. Greenup and S. Johnson); R. Smith and Z. Galfayan at Microangelo Associates for bioinformatics support; Prometheus Research; the Yale Center of Genomic Analysis (YCGA) staff, in particular S. Umlauf and C. Castaldi; T. Brooks-Boone and M. Wojciechowski for their help in administering the project at Yale; and J. Krystal, G.D. Fischbach, A. Packer, J. Spiro, and M. Benedetti for their suggestions throughout and very helpful comments during the preparation of this manuscript. Approved researchers can obtain the SSC population dataset described in this study by applying at https://base.sfari.org.

References

Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science. 2008; 322:881–888. [PubMed: 18988837]

Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Sykes N, Pagnamenta AT, et al. A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet. 2010; 19:4072–4082. [PubMed: 20663923]

Antonell A, Del Campo M, Magano LF, Kaufmann L, de la Iglesia JM, Gallastegui F, Flores R, Schweigmann U, Fauth C, Kotzot D, Pérez-Jurado LA. Partial 7q11.23 deletions further implicate GTF2I and GTF2IRD1 as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. J Med Genet. 2010; 47:312–320. [PubMed: 19897463]

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med. 1995; 25:63–77. [PubMed: 7792363]

Berg JS, Brunetti-Pierri N, Peters SU, Kang SH, Fong CT, Salamone J, Freedenberg D, Hannig VL, Prock LA, Miller DT, et al. Speech delay and autism spectrum behaviors are frequently associated with duplication of the 7q11.23 Williams-Beuren syndrome region. Genet Med. 2007; 9:427–441. [PubMed: 17666889]

Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, Fransen van de Putte DE, Anderlid BM, Lundin J, Lapunzina P, Pérez Jurado LA, Delle Chiaie B, et al. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet. 2009; 52:77–87. [PubMed: 19306953]

Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, Saeed S, Hamilton-Shield J, Clayton-Smith J, O'Rahilly S, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature. 2010; 463:666–670. [PubMed: 19966786]

Bucan M, Abrahams BS, Wang K, Glessner JT, Herman EI, Sonnenblick LI, Alvarez Retuerto AI, Imielinski M, Hadley D, Bradfield JP, et al. Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. PLoS Genet. 2009; 5:e1000536. [PubMed: 19557195]

Bugge M, Bruun-Petersen G, Brøndum-Nielsen K, Friedrich U, Hansen J, Jensen G, Jensen PK, Kristoffersson U, Lundsteen C, Niebuhr E, et al. Disease associated balanced chromosome rearrangements: a resource for large scale genotype-phenotype delineation in man. J Med Genet. 2000; 37:858–865. [PubMed: 11073540]

Bunge J, Fitzpatrick M. Estimating the Number of Species: A Review. Journal of the American Statistical Association. 1993; 88:364–373.

Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S, Elia M, Schneider C, Melmed R, Sacco R, et al. A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci U S A. 2006; 103:16834–16839. [PubMed: 17053076]

Colella S, Yau C, Taylor JM, Mirza G, Butler H, Clouston P, Bassett AS, Seller A, Holmes CC, Ragoussis J. QuantiSNP: an Objective Bayes Hidden-Markov Model to detect and accurately map copy number variation using SNP genotyping data. Nucleic Acids Res. 2007; 35:2013–2025. [PubMed: 17341461]

Cook EH, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet. 1997; 60:928–934. [PubMed: 9106540]

Crossett A, Kent BP, Klei L, Ringquist S, Trucco M, Roeder K, Devlin B. Using ancestry matching to combine family-based and unrelated samples for genome-wide association studies. Stat Med. 2010; 29:2932–2945. [PubMed: 20862653]

Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003; 4:P3. [PubMed: 12734009]

Fischbach GD, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. Neuron. 2010; 68:192–195. [PubMed: 20955926]

Fu W, Zhang F, Wang Y, Gu X, Jin L. Identification of copy number variation hotspots in human populations. Am J Hum Genet. 2010; 87:494–504. [PubMed: 20920665]

Fujiwara T, Mishima T, Kofuji T, Chiba T, Tanaka K, Yamamoto A, Akagawa K. Analysis of knock-out mice to determine the role of HPC-1/syntaxin 1A in expressing synaptic plasticity. J Neurosci. 2006; 26:5767–5776. [PubMed: 16723534]

Gao MC, Bellugi U, Dai L, Mills DL, Sobel EM, Lange K, Korenberg JR. Intelligence in Williams Syndrome is related to STX1A, which encodes a component of the presynaptic SNARE complex. PLoS One. 2010; 5:e10292. [PubMed: 20422020]

Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH. Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. Mol Psychiatry. 2004; 9:474–484. [PubMed: 15024396]

Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature. 2009; 459:569–573. [PubMed: 19404257]

Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, Muhle H, de Kovel C, Baker C, von Spiczak S, et al. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. Nat Genet. 2009; 41:160–162. [PubMed: 19136953]

Hoogenraad CC, Koekkoek B, Akhmanova A, Krugers H, Dortland B, Miedema M, van Alphen A, Kistler WM, Jaegle M, Koutsourakis M, et al. Targeted mutation of Cyln2 in the Williams syndrome critical region links CLIP-115 haploinsufficiency to neurodevelopmental abnormalities in mice. Nat Genet. 2002; 32:116–127. [PubMed: 12195424]

Huang, d.W.; Sherman, BT.; Lempicki, RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4:44–57. [PubMed: 19131956]

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. Nat Genet. 2004; 36:949–951. [PubMed: 15286789]

ISC ISC. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008; 455:237–241. [PubMed: 18668038]

Itsara A, Wu H, Smith JD, Nickerson DA, Romieu I, London SJ, Eichler EE. De novo rates and selection of large copy number variation. Genome Res. 2010; 20:1469–1481. [PubMed: 20841430]

Khoronenkova SV, Dianova II, Parsons JL, Dianov GL. USP7/HAUSP stimulates repair of oxidative DNA lesions. Nucleic Acids Res. 2010

Klauck SM, Münstermann E, Bieber-Martig B, Rühl D, Lisch S, Schmötzer G, Poustka A, Poustka F. Molecular genetic analysis of the FMR-1 gene in a large collection of autistic patients. Hum Genet. 1997; 100:224–229. [PubMed: 9254854]

Korenberg JR, Chen XN, Hirota H, Lai Z, Bellugi U, Burian D, Roe B, Matsuoka R. VI. Genome structure and cognitive map of Williams syndrome. J Cogn Neurosci. 2000; 12(Suppl 1):89–107. [PubMed: 10953236]

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH, Dobyns WB, Christian SL. Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet. 2008; 17:628–638. [PubMed: 18156158]

Lee C, Abdool A, Huang CH. PCA-based population structure inference with generic clustering algorithms. BMC Bioinformatics 10 Suppl. 2009; 1:S73.

Levinson DF, Duan J, Oh S, Wang K, Sanders AR, Shi J, Zhang N, Mowry BJ, Olincy A, Amin F, et al. Copy Number Variants in Schizophrenia: Confirmation of Five Previous Findings and New Evidence for 3q29 Microdeletions and VIPR2 Duplications. Am J Psychiatry. 2011 appi.ajp.2010.10060876.

Levy D, Ronemus M, Yamrom B, Lee Y.-h. Leotta A, Kendall J, Marks S, Lakshmi B, Ye K, Buja A, et al. Rare de novo and transmitted copy number variation in autistic spectrum disorders. Neuron. 2011 (accepted).

Liang JS, Shimojima K, Ohno K, Sugiura C, Une Y, Yamamoto T. A newly recognised microdeletion syndrome of 2p15-16.1 manifesting moderate developmental delay, autistic behaviour, short stature, microcephaly, and dysmorphic features: a new patient with 3.2 Mb deletion. J Med Genet. 2009; 46:645–647. [PubMed: 19724011]

Lichtenstein P, Carlström E, Råstam M, Gillberg C, Anckarsäter H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry. 2010; 167:1357–1363. [PubMed: 20686188]

Lupski JR. Genomic rearrangements and sporadic disease. Nat Genet. 2007; 39:S43–47. [PubMed: 17597781]

Makoff AJ, Flomen RH. Detailed analysis of 15q11-q14 sequence corrects errors and gaps in the public access sequence to fully reveal large segmental duplications at breakpoints for Prader-Willi, Angelman, and inv dup(15) syndromes. Genome Biol. 2007; 8:R114. [PubMed: 17573966]

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, et al. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet. 2008; 82:477–488. [PubMed: 18252227]

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O, et al. Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet. 2009; 41:1223–1227. [PubMed: 19855392]

Mefford HC, Cooper GM, Zerr T, Smith JD, Baker C, Shafer N, Thorland EC, Skinner C, Schwartz CE, Nickerson DA, Eichler EE. A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease. Genome Res. 2009; 19:1579–1585. [PubMed: 19506092]

Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M, Lu WY, MacDonald JF, Wang JY, Falls DL, Jia Z. Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. Neuron. 2002; 35:121–133. [PubMed: 12123613]

Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, St Clair DM, Muir WJ, Blackwood DH, Porteous DJ. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet. 2000; 9:1415–1423. [PubMed: 10814723]

Miller DT, Shen Y, Weiss LA, Korn J, Anselm I, Bridgemohan C, Cox GF, Dickinson H, Gentile J, Harris DJ, et al. Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. J Med Genet. 2009; 46:242–248. [PubMed: 18805830]

Moreno-De-Luca D, Mulle JG, Kaminsky EB, Sanders SJ, Myers SM, Adam MP, Pakula AT, Eisenhauer NJ, Uhas K, Weik L, et al. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. Am J Hum Genet. 2010; 87:618–630. [PubMed: 21055719]

Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, Mukaddes NM, Balkhy S, Gascon G, Hashmi A, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science. 2008; 321:218–223. [PubMed: 18621663]

Noor A, Whibley A, Marshall CR, Gianakopoulos PJ, Piton A, Carson AR, Orlic-Milacic M, Lionel AC, Sato D, Pinto D, et al. Disruption at the PTCHD1 Locus on Xp22.11 in Autism spectrum disorder and intellectual disability. Sci Transl Med. 2010; 2:49ra68.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature. 2010; 466:368–372. [PubMed: 20531469]

Pober BR. Williams-Beuren syndrome. N Engl J Med. 2010; 362:239–252. [PubMed: 20089974]

Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res. 2007; 35:D61–65. [PubMed: 17130148]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

Reymond A, Zufferey F, Harewood L, Kutalik Z, Martinet D, Chrast J, Walters RG, Bouquillon S, Valsesia A, Hippolyte L, et al. Gene dosage at the 16p11.2 locus controls body mass index. American Society of Human Genetics (Washington DC). 2010

Risi S, Lord C, Gotham K, Corsello C, Chrysler C, Szatmari P, Cook EH, Leventhal BL, Pickles A. Combining information from multiple sources in the diagnosis of autism spectrum disorders. J Am Acad Child Adolesc Psychiatry. 2006; 45:1094–1103. [PubMed: 16926617]

Sakurai T, Dorr NP, Takahashi N, McInnes LA, Elder GA, Buxbaum JD. Haploinsufficiency of Gtf2i, a gene deleted in Williams Syndrome, leads to increases in social interactions. Autism Res. 2010

Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, et al. Strong association of de novo copy number mutations with autism. Science. 2007; 316:445–449. [PubMed: 17363630]

Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Månér S, Massa H, Walker M, Chi M, et al. Large-scale copy number polymorphism in the human genome. Science. 2004; 305:525–528. [PubMed: 15273396]

Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, Miller KJ, Frazier JA, Silverstein I, Picker J, et al. Clinical genetic testing for patients with autism spectrum disorders. Pediatrics. 2010; 125:e727–735. [PubMed: 20231187]

Skuse DH. Rethinking the nature of genetic vulnerability to autistic spectrum disorders. Trends Genet. 2007; 23:387–395. [PubMed: 17630015]

Smalley SL, Tanguay PE, Smith M, Gutierrez G. Autism and tuberous sclerosis. J Autism Dev Disord. 1992; 22:339–355. [PubMed: 1400103]

Stefansson H, Rujescu D, Cichon S, Pietiläinen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, et al. Large recurrent microdeletions associated with schizophrenia. Nature. 2008; 455:232–236. [PubMed: 18668039]

Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. N Engl J Med. 2006; 354:1370–1377. [PubMed: 16571880]

Van der Aa N, Rooms L, Vandeweyer G, van den Ende J, Reyniers E, Fichera M, Romano C, Delle Chiaie B, Mortier G, Menten B, et al. Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. Eur J Med Genet. 2009; 52:94–100. [PubMed: 19249392]

Veenstra-Vanderweele J, Christian SL, Cook EH. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet. 2004; 5:379–405. [PubMed: 15485354]

Vorstman JA, Morcus ME, Duijff SN, Klaassen PW, Heineman-de Boer JA, Beemer FA, Swaab H, Kahn RS, van Engeland H. The 22q11.2 deletion in children: high rate of autistic disorders and early onset of psychotic symptoms. J Am Acad Child Adolesc Psychiatry. 2006; 45:1104–1113. [PubMed: 16926618]

Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science. 2008; 320:539–543. [PubMed: 18369103]

Walters R, Jacquemont S, Valsesia A, de Smith A, Martinet D, Andersson J, Falchi M, Chen F, Andrieux J, Lobbens S, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature. 2010; 463:671–675. [PubMed: 20130649]

Wang K, Li M, Hadley D, Liu R, Glessner J, Grant SF, Hakonarson H, Bucan M. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res. 2007; 17:1665–1674. [PubMed: 17921354]

Wang K, Zhang H, Ma D, Bucan M, Glessner J, Abrahams B, Salyakina D, Imielinski M, Bradfield J, Sleiman P, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature. 2009; 459:528–533. [PubMed: 19404256]

Wassink TH, Piven J, Patil SR. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. Psychiatr Genet. 2001; 11:57–63. [PubMed: 11525418]

Weiss LA, Arking DE, Daly MJ, Chakravarti A, Consortium G.D.P.o.J.H.t.A. A genome-wide linkage and association scan reveals novel loci for autism. Nature. 2009; 461:802–808. [PubMed: 19812673]

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med. 2008; 358:667–675. [PubMed: 18184952]

Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M. Strong association of de novo copy number mutations with sporadic schizophrenia. Nat Genet. 2008; 40:880–885. [PubMed: 18511947]

Zhao X, Leotta A, Kustanovich V, Lajonchere C, Geschwind DH, Law K, Law P, Qiu S, Lord C, Sebat J, et al. A unified genetic theory for sporadic and inherited autism. Proc Natl Acad Sci U S A. 2007; 104:12831–12836. [PubMed: 17652511]

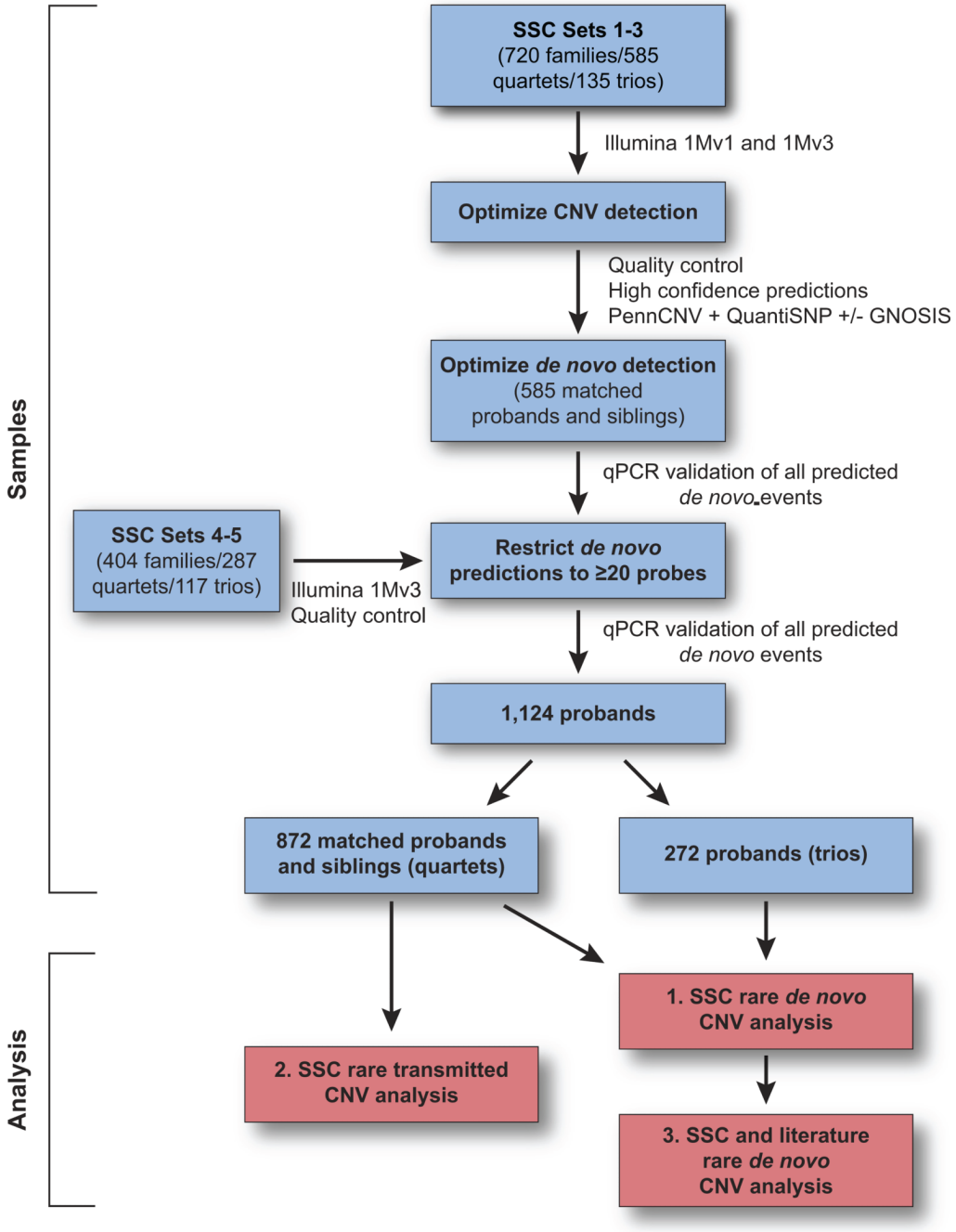


Figure 1. Flow chart of CNV detection and confirmation in the Simons Simplex Collection (SSC)

CNV detection was optimized by qPCR analysis of 115 predictions (Table S1, Figure S1). Quality control was performed to check for identity error and data quality (supplementary methods). *De novo* detection was optimized by qPCR analysis of 403 predictions (Figure S1) leading to the threshold of ≥20 probes and refinement of the prediction algorithm. All *de novo* CNVs reported in the study were confirmed using qPCR with absolute quantification.

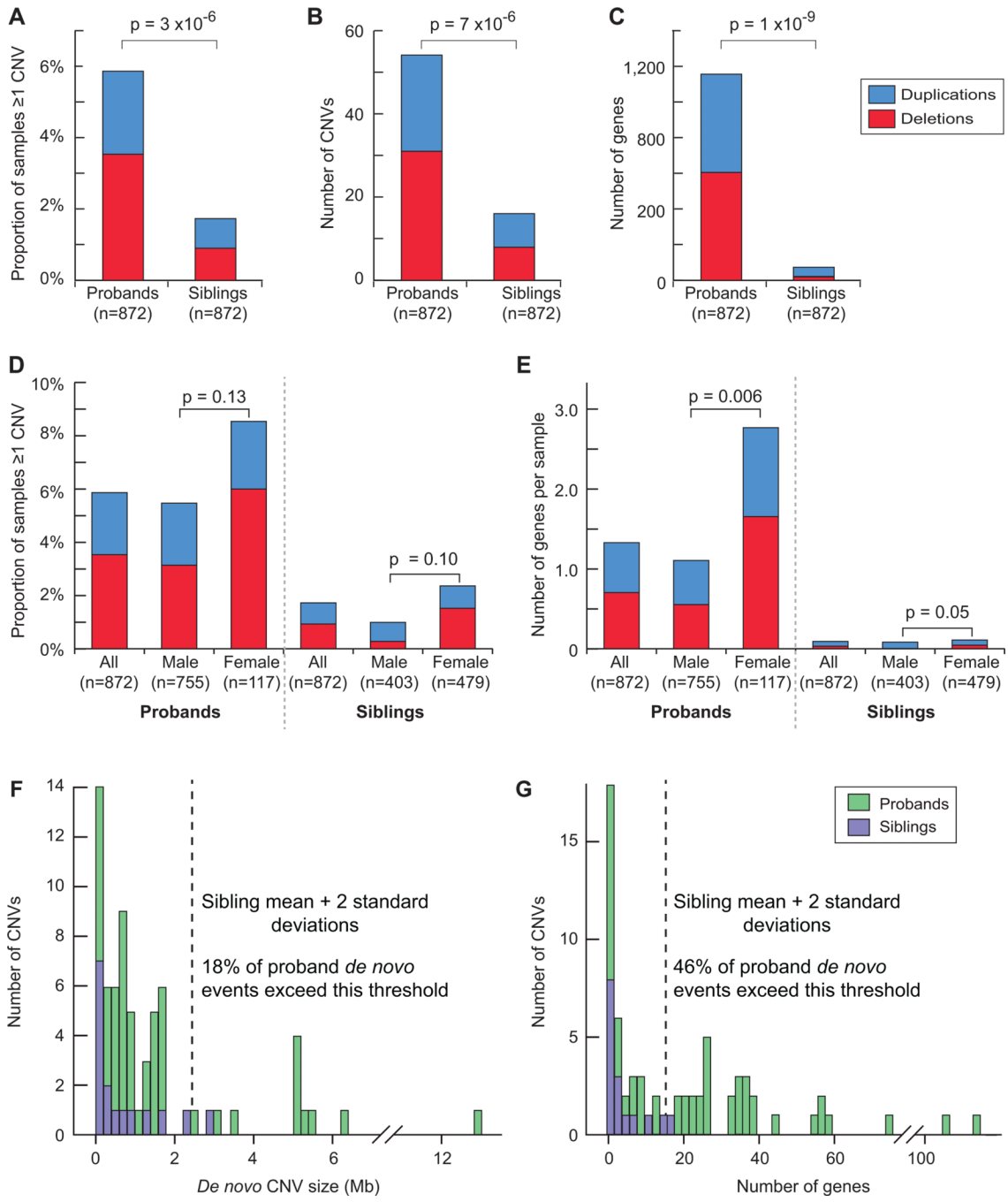


Figure 2. The burden of rare *de novo* CNVs and genes mapping within them in 872 probands and 872 matched siblings

A) % of individuals with ≥1 rare *de novo* CNV in probands vs. siblings. Red = deletions; blue = duplications for A to E. **B)** Total number of rare *de novo* CNVs in probands vs. siblings (2 probands and 1 sibling have more than one). **C)** Number of RefSeq genes (Pruitt et al., 2007) overlapping rare *de novo* CNVs in probands vs. siblings. **D)** % of individuals with ≥1 rare *de novo* CNV (as shown in A) split by sex. Specific comparisons and associated p-values are given. **E)** Number of RefSeq genes overlapping rare *de novo* CNVs (as shown in C) split by sex. **F)** The distribution of rare *de novo* CNVs by size in probands (green) and siblings (purple). The dashed vertical line represents the mean plus two standard deviations

of the sibling events. **G)** The distribution of rare *de novo* CNVs by number of RefSeq genes. Statistical significance was calculated using: Fisher's Exact test (A, D), Sign test (B), Wilcoxon paired test (C), and Wilcoxon test (E).

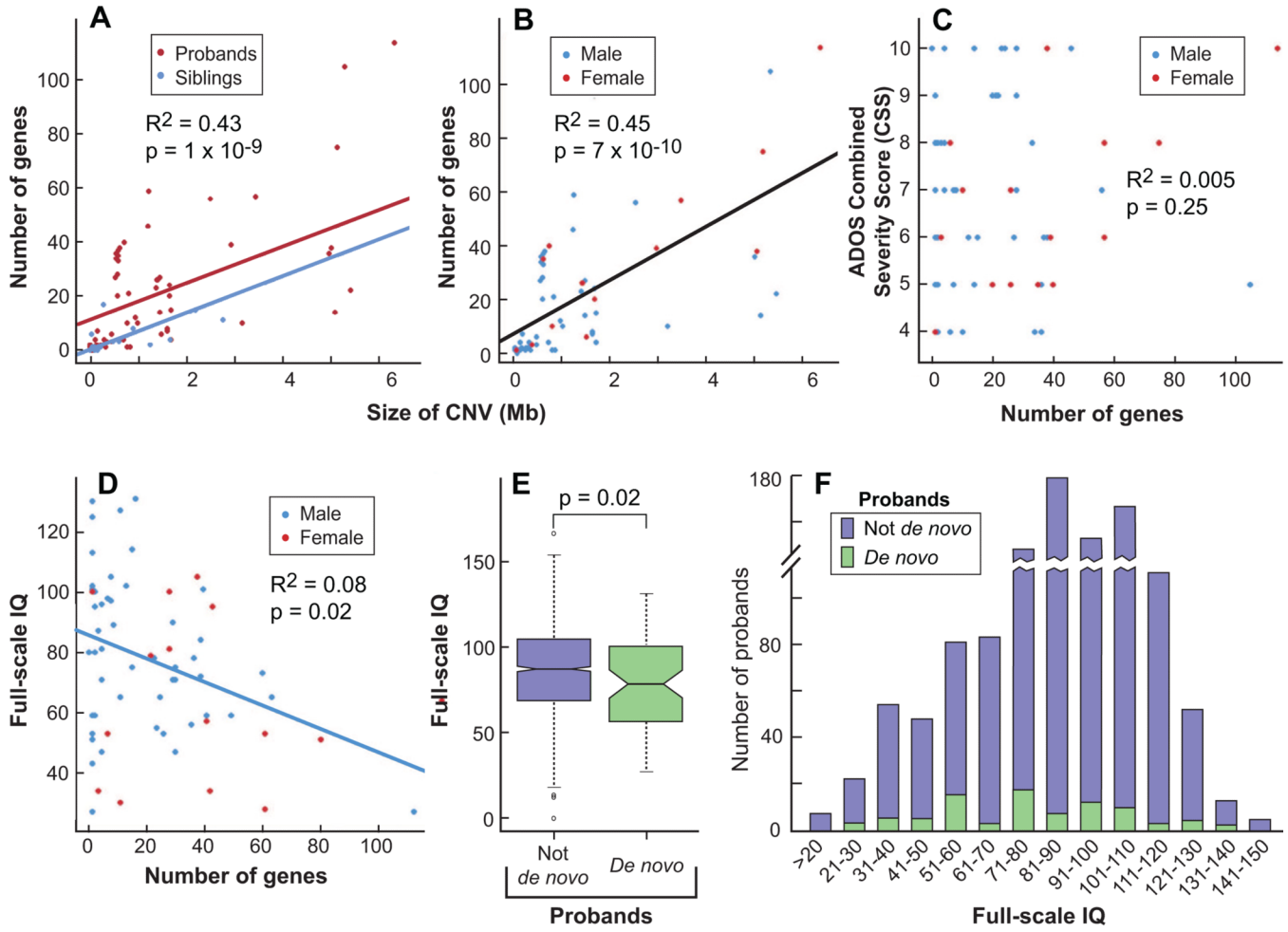
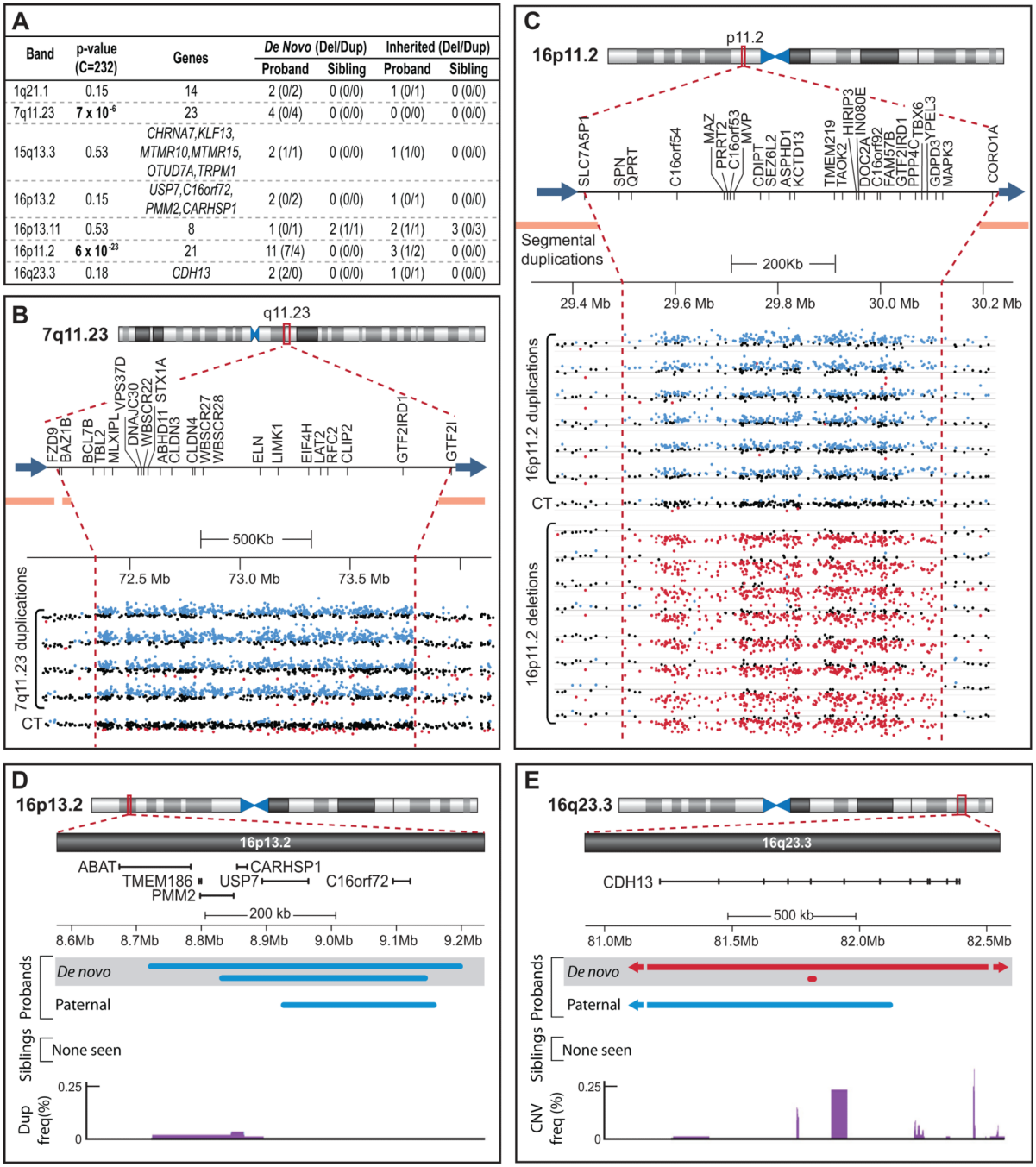


Figure 3. Genotype-phenotype analyses of probands carrying rare *de novo* CNVs

A) The number of RefSeq genes within rare *de novo* CNVs (*genes*) *vs.* CNV size (*size*), with proband (red) *vs.* sibling (blue). The slope of the lines shows the fitted significant ($p=1 \times 10^{-9}$) relationship between *genes* and *size* and the difference between the lines shows the fitted difference for probands and siblings (p=0.025): on average probands have more genes within a rare *de novo* CNV for any given size. **B)** *genes vs. size*, with sex of subject encoded by color as noted (*sex*). The slope of the line shows the fitted significant ($p=7 \times 10^{-10}$) relationship between *genes* and *size* while the presence of only one line reflects the lack of significant difference by *sex* (p=0.20). **C)** ADOS Combined Severity Score (CSS), a measure of autism severity, against *genes* and by *sex*. The lack of a line indicates the absence of a significant relationship. **D)** Full-scale IQ (*IQ*) against *genes* and by *sex*. The slope shows that IQ declines as a function of *genes* in males (p=0.02, Wilcoxon test); there is no significant relationship in females. **E)** Boxplot with 95% confidence intervals for *IQ* by presence (yellow) or absence (blue) of a detected rare *de novo* event in the probands. **F)** Distribution of *IQ* in probands with (yellow, N=63) rare *de novo* CNVs and without (blue, N=1,061).



Band	p-value (C=232)	Genes	*De Novo* (Del/Dup)		Inherited (Del/Dup)	
			Proband	Sibling	Proband	Sibling
1q21.1	0.15	14	2 (0/2)	0 (0/0)	1 (0/1)	0 (0/0)
7q11.23	7×10^{-6}	23	4 (0/4)	0 (0/0)	0 (0/0)	0 (0/0)
15q13.3	0.53	*CHRNA7,KLF13, MTMR10,MTMR15, OTUD7A,TRPM1*	2 (1/1)	0 (0/0)	1 (1/0)	0 (0/0)
16p13.2	0.15	*USP7,C16orf72, PMM2,CARHSP1*	2 (0/2)	0 (0/0)	1 (0/1)	0 (0/0)
16p13.11	0.53	8	1 (0/1)	2 (1/1)	2 (1/1)	3 (0/3)
16p11.2	6×10^{-23}	21	11 (7/4)	0 (0/0)	3 (1/2)	0 (0/0)
16q23.3	0.18	*CDH13*	2 (2/0)	0 (0/0)	1 (0/1)	0 (0/0)

Figure 4. Confirmed recurrent rare *de novo* CNVs

A) All recurrent *de novo* CNVs identified in 1,124 probands and 872 siblings. The gene count is given when >6 RefSeq genes map to an interval; a complete listing of genes is presented in Table S4. The total number of *de novo* and matching inherited CNVs in probands and siblings are shown for deletions (Del) and duplications (Dup) in parentheses. **B)** LogR data for 4 *de novo* duplications and 1 control with no CNV (CT) in the 7q11.23 interval. RefSeq genes within this region are noted below the ideogram; the orange bars represent flanking segmental duplications. NCBI 36 (hg18) genomic coordinates are shown with the scale indicated. The LogR for all probes within the region is shown; LogR values

greater than 0.15 are in blue (suggesting a duplication) while LogR values less than −0.15 are in red (suggesting a deletion). B allele frequency data is not shown but supports the presence of a corresponding CNV. The approximate boundaries of the CNVs are shown by the vertical dashed red line and blue arrows. **C)** LogR data for 6 duplications (4 *de novo*), 8 deletions (7 *de novo*), and 1 control with no CNV (CT) in the 16p11.2 interval. The ideogram and intensity plots are as in B. **D)** Overlapping rare *de novo* and rare inherited CNVs identified in the 16p13.2 interval. The brackets show the boundaries of RefSeq genes; 2 genes are in common between all 3 duplications: *USP7* and *C16orf72*. The frequency of duplications in the DGV is shown in purple: the majority of the recurrent *de novo* region is not present in the DGV. **E)** Overlapping rare *de novo* and rare inherited CNVs identified in the 16q23.3 interval. A 34kb deletion overlaps a 5Mb deletion over a *CDH13* exon (represented by ticks on the gene). The frequency of CNVs observed in the DGV is shown at the bottom in purple.

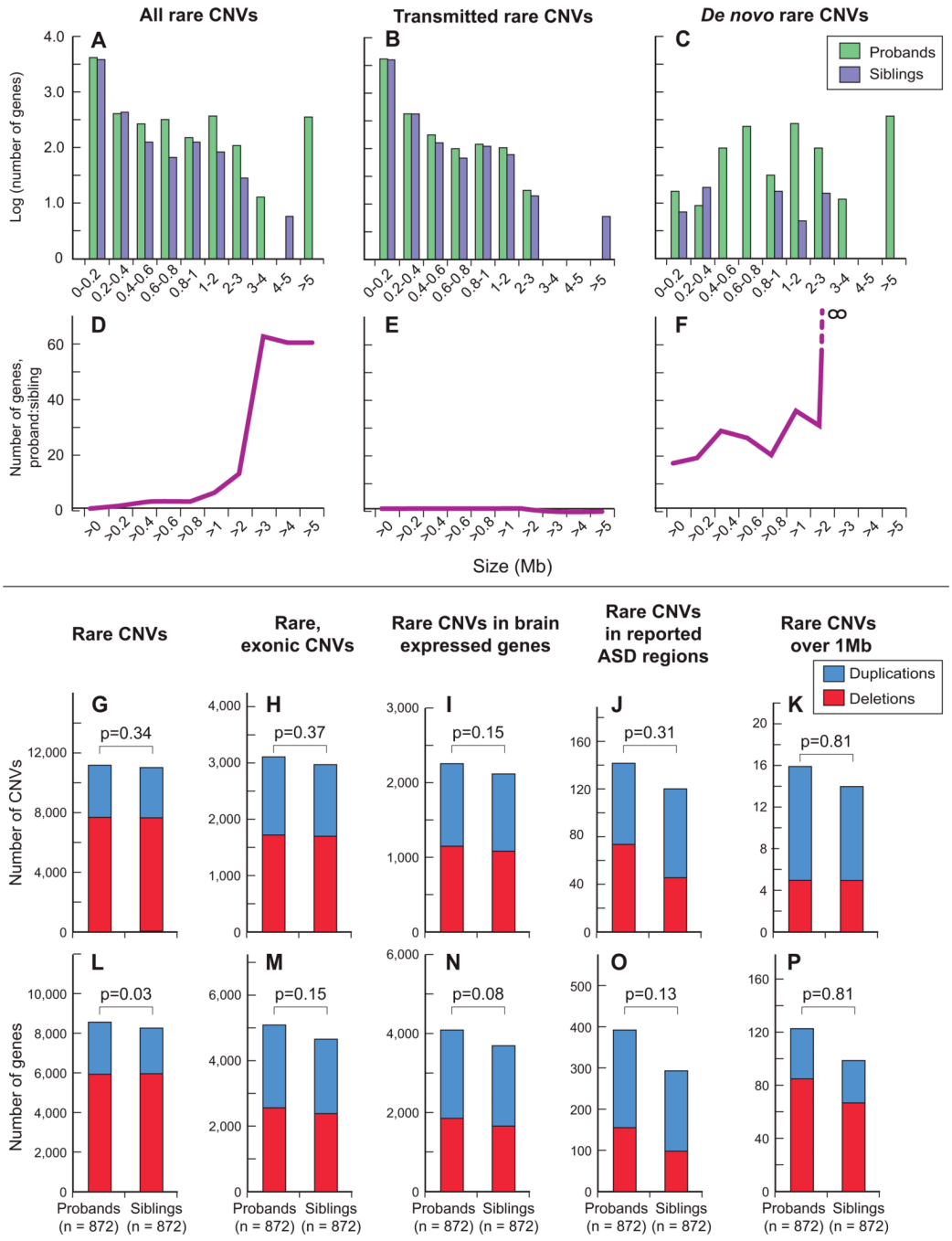


Figure 5. Burden of rare CNVs in 872 probands and 872 matched siblings

A) Bar graph showing the log (10) number of genes present in all rare CNVs binned by size (in Mb), with probands shown in green and siblings in purple. **B)** The data from A with confirmed *de novo* events excluded leaving only CNVs transmitted from a parent to offspring. **C)** Only confirmed *de novo* events are shown. **D-F)** The ratio (y-axis) of number of genes in probands *vs.* siblings for specific size thresholds (x-axis): **D)** all rare CNVs (transmitted and *de novo*); **E)** transmitted events; **F)** *de novo* events only. **G-K)** The total number of transmitted deletions (red) and duplications (blue) for probands and siblings for varying categories of CNV (shown above the graph). Definitions are described in

supplementary methods. P-values (noted above the bars) are calculated using the Sign test and are not corrected for multiple comparisons. **L-P)** As in G-K, with number of RefSeq genes within the CNVs (y-axis). P-values (noted above the bars) are estimated using a two-tailed paired t-test and are not corrected for approximately 3,000 comparisons.

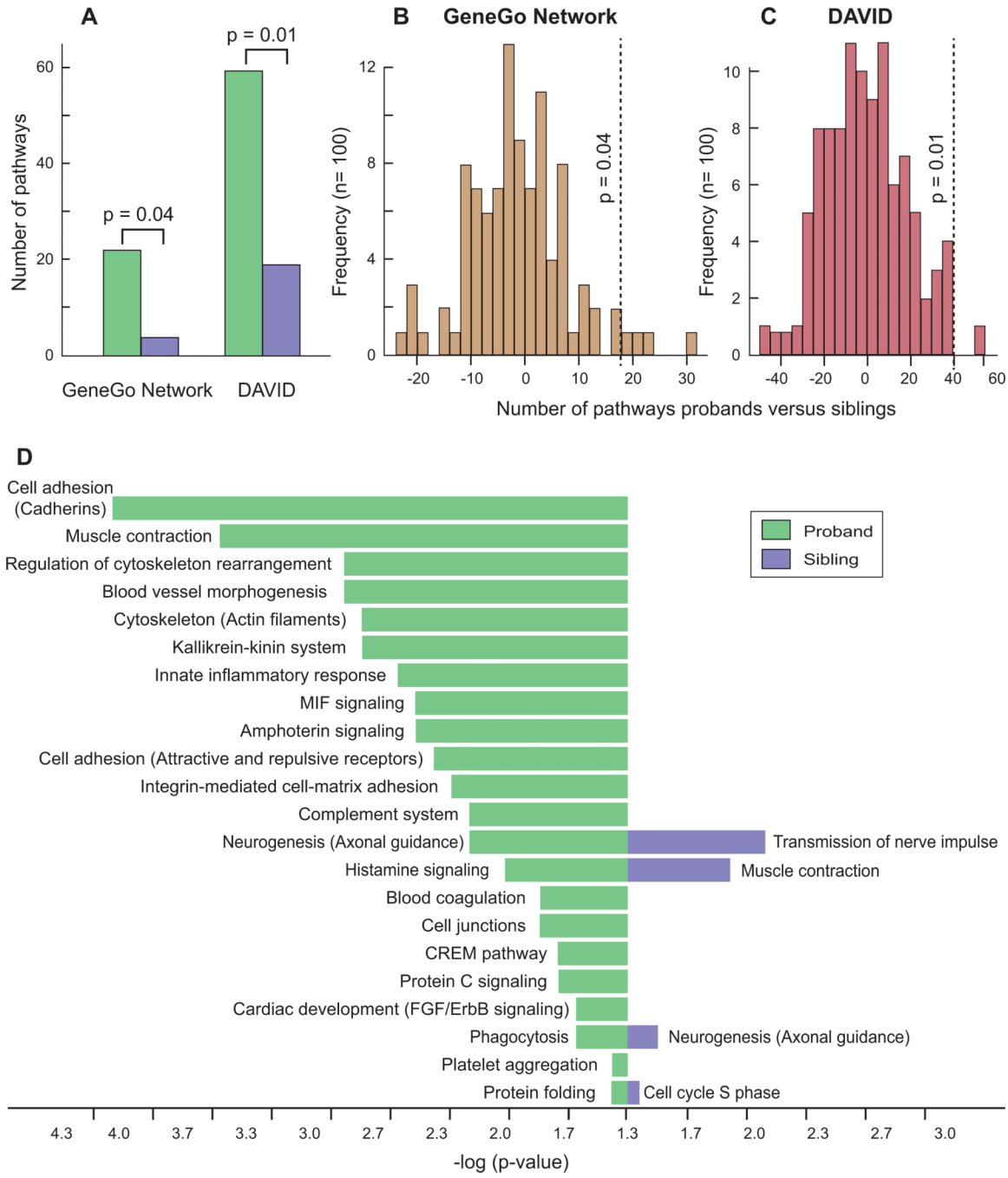


Figure 6. Pathway analysis of genes mapping within transmitted rare CNVs

A) The number of pathways with a corrected p-value ≤0.05 identified in probands (green) and siblings (purple) by the programs Metacore (GeneGo Networks) and DAVID (level 4 terms). The input consisted of 1,516 RefSeq genes found only in transmitted rare CNVs in probands and the 1,357 RefSeq genes found only in transmitted rare CNVs in siblings; p-values are from B and C. **B)** Permutation analysis to assess significance of the difference between probands and siblings. The 2,873 genes identified in probands or siblings were divided randomly between probands and siblings in the same initial proportions. The lists were submitted to GeneGo Networks and the difference between the number of pathways in probands and siblings was recorded. This process was performed 100 times and the image

shows the frequency of the results. Only 4 events showed a difference ≥18 (the difference seen in A, vertical dashed line) yielding a p-value of 0.04. **C)** Permutation analysis to calculate the significance value with DAVID (level 4 terms) using the same methods as in B. A single result was ≥40 (the difference seen in A, vertical dashed line) giving a p-value of 0.01. **D)** All pathways with a corrected p-value ≤0.05 identified by GeneGo Networks for probands (green) and siblings (purple). The length of the bar represents the significance value on a logarithmic scale.

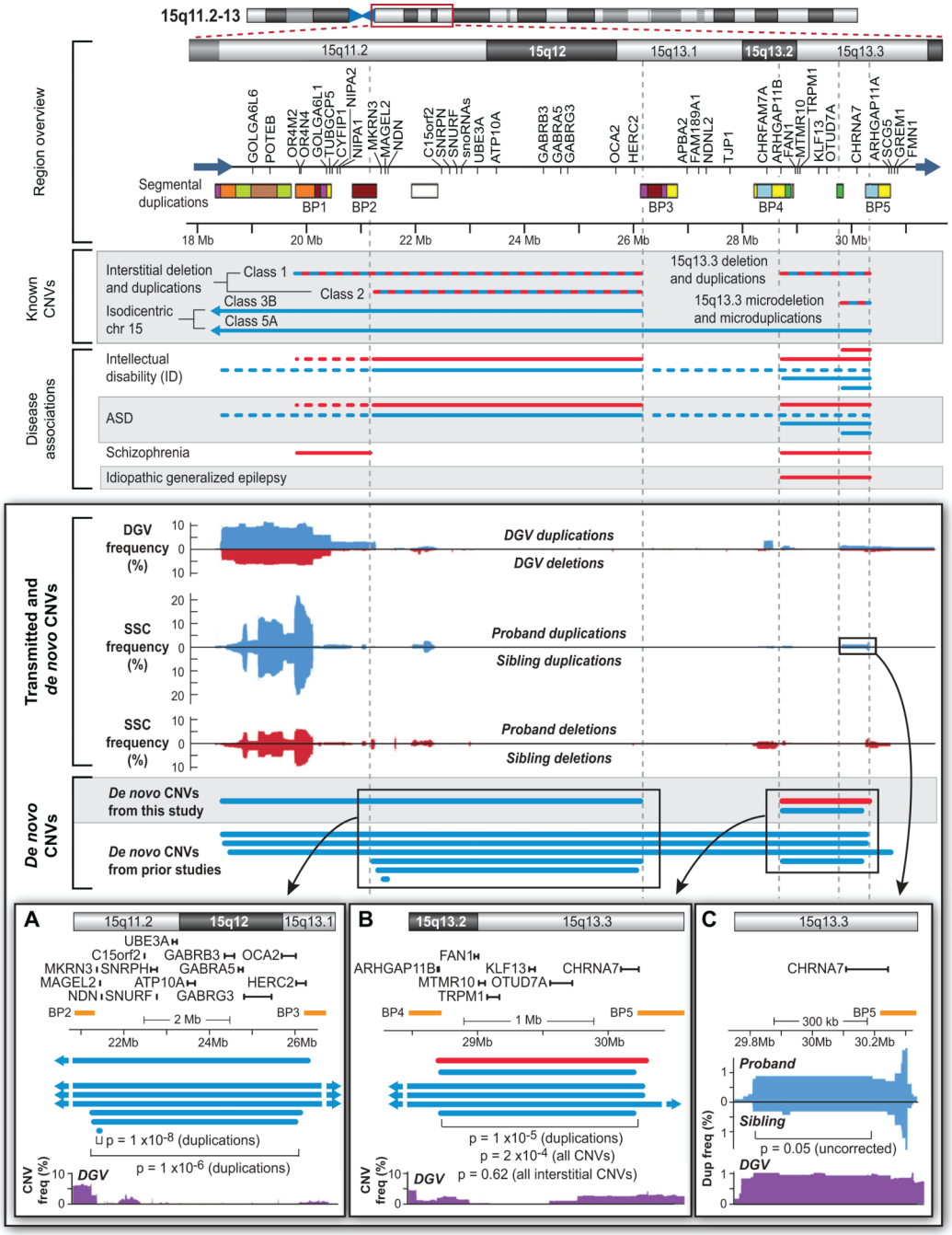


Figure 7. *De novo* and transmitted CNVs in 15q11.2-13

A 13Mb region is identified by the red box on the ideogram at the top. The **Region overview** identifies the RefSeq genes present within the interval and multiple segmental duplications (the colors identify regions of homology (Makoff and Flomen, 2007)). 5 of these segmental duplications are commonly referred to as BP1-BP5. **Known CNVs** identifies duplications (blue) and deletions (red) that have been reported in the literature; the alternating red and blue colors denote both deletions and duplications. **Disease associations** identifies regions with reported associations to four developmental and neuropsychiatric conditions (supplementary materials). Of note, BP2-BP3 deletions lead to Prader-Willi or

Angelman syndrome. **Transmitted and *de novo* CNVs** shows the frequency of duplications (blue) and deletions (red) in the DGV and SSC populations. While CNVs overlying the segmental duplications are common, CNVs between the breakpoints are generally rare. ***De novo* CNVs** shows confirmed *de novo* CNVs in single individuals identified in this study and prior ASD studies (Itsara et al., 2010; Marshall et al., 2008; Pinto et al., 2010; Sebat et al., 2007). **A)** An enlargement of BP2-3 showing the relationship of *de novo* CNVs, genes, and common regions in the DGV. A small atypical duplication includes the genes MAGEL2, MKRN3, NDN (Itsara et al., 2010). **B)** An enlargement of BP4-BP5 showing similar data and methods as A. Removing the three Class 5A isodicentric chr15 events results in a non-significant p-value (p=0.62). **C)** An enlargement of the *CHRNA7* region showing enrichment of duplications in probands (N=10) vs. siblings (N=3). The p-value is p=0.05 (Fisher's exact test); uncorrected for 3,667 comparisons; the rate of duplications in the DGV is similar to that seen in probands (Table S7).

Table 1

Burden of *de novo* CNVs in probands and siblings

Category	Analysis	All probands (N=1,124)	Matched probands (N=872)	Matched siblings (N=872)	Ratio (OR)	p-value[d]
***De novo* CNVs**	**CNVs**	67	54	16		
	Samples[a]	63	51	15		
	Proportion[b]	5.6%	5.8%	1.7%	3.4 (3.5)	**3×10^{-6}**
	Genes[c]	1,417	1,153	73	15.8	
***De novo* deletions**	**CNVs**	35	31	8		
	Samples	35	31	8		
	Proportion	3.1%	3.6%	0.9%	3.9 (4.0)	**1×10^{-4}**
	Genes	638	605	21	728.8	
***De novo* duplications**	**CNVs**	32	23	8		
	Samples	29	21	7		
	Proportion	2.6%	2.4%	0.8%	3.0 (3.0)	**0.006**
	Genes	779	548	52	10.5	
***De novo* genic CNVs**	**CNVs**	66	53	13		
	Samples	62	50	12		
	Proportion	5.5%	5.7%	1.4%	4.2 (4.4)	**4×16^{-7}**
	Genes	1,417	1,153	73	15.8	
***De novo* exonic CNVs**	**CNVs**	64	52	11		
	Samples	60	49	10		
	Proportion	5.3%	5.6%	1.1%	4.9 (5.1)	**9×10^{-8}**
	Genes	1,415	1,152	71	16.2	
***De novo* multigenic CNVs**	**CNVs**	53	44	9		
	Samples	52	43	8		
	Proportion	4.6%	4.9%	0.9%	5.4 (5.6)	**2×10^{-7}**
	Genes	1,404	1,144	69	16.6	
***De novo* autosomal CNVs**	**CNVs**	66	53	14		
	Samples	62	50	14		
	Proportion	5.5%	5.7%	1.6%	3.6 (3.7)	**2×10^{-6}**
	G^nes	1,416	1,152	67	17.2	
***De novo* chrX CNVs**	**CNVs**	1 (male deletion)	1 (male deletion)	2 (female duplications)		
	Samples	1 (male deletion)	1 (male deletion)	1 (female duplication)		
	Proportion	0.1%	0.1%	0.1%	1.0 (1.0)	0.75
	Genes	1	1	6	0.2	
Small *de novo* CNVs (<100kb)	**CNVs**	8	5	3		
	Samples	8	5	3		
	Proportion	0.7%	0.6%	0.3%	1.7 (1.7)	0.36
	Genes	8	5	7	0.7	
Medium *de novo* CNVs (100-1,000kb)	**CNVs**	32	26	9		
	Samples	30	25	8		

Category	Analysis	All probands (N=1,124)	Matched probands (N=872)	Matched siblings (N=872)	Ratio (OR)	p-value[d]
Large *de novo* CNVs (≥1,000kb)	**Proportion**	2.7%	2.9%	0.9%	3.1 (3.2)	**0.002**
	Genes	469	392	34	11.5	
	CNVs	27	23	4		
	Samples	26	22	4		
Single occurrence *de novo* CNVs	**Proportion**	2.3%	2.5%	0.5%	5.5 (5.6)	**2×10^{-4}**
	Genes	940	756	32	23.6	
	CNVs	44	37	14		
	Samples	40	34	13		
Double occurrence *de novo* CNVs	**Proportion**	3.6%	3.9%	1.5%	2.6 (2.7)	**0.001**
	Genes	862	754	54	14.0	
	CNVs	8	8	2		
	Samples	8	8	2		
≥3 occurrence *de novo* CNVs	**Proportion**	0.7%	0.9%	0.2%	4.0 (4.0)	0.05
	Genes	89	102	19	5.4	
	CNVs	15	9	0		
	Samples	15	9	0		
	Proportion	1.3%	1.0%	0.0%	NA (NA)	**0.002**
	Genes	466	297	0	NA	

[a] Four individuals have multiple *de novo* CNVs

[b] % of samples with ≥1 *de novo* CNV

[c] RefSeq genes within the CNV

[d] Fisher's exact test

Table 2

Phenotypic comparisons between subjects with 16p11.2 deletions, 16p11.2 duplications, and 7q11.23 duplications and matched probands.

Primary	16p Deletion (N=8) Mean[a] (SD)	Deletion Matches (N=40) Mean (SD)	16p Duplication (N=6) Mean (SD)	Duplication Matches (N=30) Mean (SD)	Duplications (N=4) Mean (SD)	Duplication Matches (N=20) Mean (SD)
CPEA Diagnosis						
• Autism	*75%*	*98%*	83%	97%	100%	85%
• Autism Spectrum Disorder	*13%*	*3%*	0%	0%	0%	10%
• Asperger Syndrome	*13%*	*0%*	17%	3%	0%	5%
ADOS Combined Severity Score (CSS)	6.5	7.0	7.2	7.4	*7.0*	*7.6*
	(1.6)	(1.7)	(2.1)	(1.5)	*(2.2)*	*(1.6)*
Full-Scale IQ	76.9	82.5	75.7	81.0	84.0	81.3
	(17.6)	(27.8)	(23.2)	(26)	(14.9)	(30.5)
Body Mass Index (BMI)	23.2	20.9	**17.1**	**19.3**	23.1	21.6
	(6.4)	(5.3)	**(1.4)**	**(5.2)**	(5.9)	(6.4)
Exploratory						
ADI-R Social Interaction Total	*17.9*	*21.3*	21.8	19.1	20.0	19.9
	(6.7)	*(5.5)*	(3.5)	(5.6)	(5.5)	(7.0)
ADOS Social Affect Total	9.9	9.5	*10.8*	*10.7*	11.0	11.7
	(4.0)	(4.2)	*(5.3)*	*(3.2)*	(4.8)	(3.3)
ADOS Social and Communication Total	12.4	11.5	*14.2*	*12.7*	**11.8**	**13.7**
	(3.9)	(4.2)	*(6.0)*	*(3.1)*	**(5.7)**	**(3.6)**
ADI-R Restricted and Repetitive Behavior (RRB) Total	5.4	6.8	8.5	6.3	7.3	6.3
	(2.6)	(2.3)	(1.9)	(2.1)	(1.7)	(2.6)
ADOS RRB Total	*2.0*	*3.7*	4.3	3.6	*2.0*	*4.1*
	(1.3)	*(1.9)*	(2.6)	(1.7)	*(1.4)*	*(2.0)*
Aberrant Behavior Checklist (ABC) Total	46.8	42.0	*68.7*	*42.5*	**66.0**	**47.2**
	(24.3)	(28.6)	*(21.5)*	*(17.9)*	**(26.7)**	**(21.0)**
ABC Irritability	14.9	9.6	19.5	10.5	**20.0**	**12.0**
	(9.3)	(9.1)	(6.4)	(7.8)	**(13.0)**	**(7.6)**
ABC Hyperactivity	16.5	13.9	**26.7**	**14.0**	20.3	18.3
	(9.1)	(10.3)	**(6.6)**	**(7.9)**	(9.7)	(9.1)
ABC Lethargy/Social Withdrawal	9.5	10.0	11.5	10.3	*13.8*	*9.8*
	(6.6)	(7.7)	(8.6)	(7.2)	*(8.4)*	*(6.2)*
Age First Concern	**2.8**	**1.7**	1.7	2.0	1.8	2.1
	(1.5)	**(0.9)**	(0.9)	(0.9)	(1.0)	(1.3)

[a] Significance is shown in bold ($p \leq 0.05$) and italics ($0.05 < p \leq 0.1$)

Table 3

CNVs in genes and regions previously associated with ASD.

Gene/Region[a]	Location (NCBI 36/hg18)	All (*de novo*)[b]		Deletions (*de novo*)		Duplications (*de novo*)	
		Proband	Sibling	Proband	Sibling	Proband	Sibling
NRXN1	chr2:50,000,991-51,113,178	3	1	3	1	0	0
CDH10	chr5:24,522,967-24,680,668	0	1	0	1	0	0
MET	chr7:116,099,695-116,225,676	1	0	0	0	1	0
VPS13B	chr8:100,094,669-100,958,984	1	0	1	0	0	0
CACNA1C	chr12:2,032,676-2,677,376	1	1	0	0	1	1
UBE3A	chr15:23,133,488-23,235,221	1 (1)	0	0	0	1 (1)	0
NF1	chr17:26,446,120-26,728,821	1	0	1	0	0	0
MACROD2	chr20:13,924,146-15,981,841	0	1	0	1	0	0
TBX1	chr22:18,124,225-18,151,112	1 (1)	0	1 (1)	0	0	0
ADSL	chr22:39,072,449-39,092,521	2 (1)	2 (0)	1 (1)	1 (0)	1 (0)	1 (0)
NLGN4X	chrX:5,818,082-6,156,706	1M	0	0	0	1M	0
DMD	chrX:31,047,265-33,267,647	1F	1M,5F	1F	1M,5F	0	0
NLGN3	chrX:70,281,435-70,307,776	1M (1M)	0	1M (1M)	0	0	0
ATRX	chrX:76,647,011-76,928,375	0	1F	0	0	0	1F
FMR1	chrX:146,801,200-146,840,333	1F	1F	1F	1F	0	0
RPL10	chrX:153,279,911-153,285,232	1M	0	0	0	1M	0
1q21.1	chr1:144,022,893-147,496,468	3 (2)	0	0	0	3 (2)	0
3q29	chr3:197,244,288-198,830,238	1 (1)	0	1 (1)	0	0	0
4p16.3	chr4:1-2,043,468	1 (1)	0	0	0	1 (1)	0
7q11.23	chr7:71,970,679-74,254,837	4 (4)	0	0	0	4 (4)	0
15q11.2-13.1	chr15:20,768,955-26,230,781	1 (1)	0	0	0	1 (1)	0
15q13.2-13.3	chr15:28,698,632-30,234,007	3 (2)	0	2 (1)	0	1 (1)	0
16p13.11	chr16:15,421,876-16,200,195	3 (1)	5 (2)	1 (0)	1 (1)	2 (1)	4 (1)
16p11.2	chr16:29,474,810-30,235,818	14 (11)	0	8 (7)	0	6 (4)	0
17q12	chr17:31,893,783-33,277,865	2 (1)	0	2 (1)	0	0	0
22q11.21 typical	chr22:17,412,646-19,797,314	1 (1)	0	1 (1)	0	0	0
22q11.21 distal	chr22:22,028,923-23,368,015	1	0	0	0	1	0

[a] For genes a CNV was included if it overlapped ≥1 exon; for regions CNVs spanning ≥50% of the region and ≥1 exon and included.

[b] *De novo* CNV count in parentheses; for chromosome X sex is indicated by M for male and F for female.

Table 4

Recurrent (≥2) *de novo* regions across this and other studies.

Type	Band	Location (NCBI 36/hg18)	Size (kb)	Recurrence (del/dup)	Frequency (N=3,816)	p-value[a] (C=232)	Studies[b]	Genes[c] (RefSeq)
Deletions	2p16.3	chr2:51,002,576-51,157,742	155	2	0.05%	0.94	3	*NRXN1* [d]
	2q24.2	chr2:162,212,720-162,311,972	99	2	0.05%	0.94	5	*SLC4A10* (intronic)
	2q37.3	chr2: 238,217,066-242,701,103	4,484	2	0.05%	0.94	5	41
	3p14.1	chr3:65,674,445-65,725,692	51	2	0.05%	0.94	2,4	*MAGI1* (intronic)
	3p14.1	chr3:67,223,272-70,633,200	3,410	2	0.05%	0.94	1,4	10
	5p15.2	chr5:11,403,359-11,491,117	87	3	0.08%	0.11	1,4	*CTNND2*
	7q31.1-31.31	chr7:113,335,000-119,223,887	5,889	2	0.05%	0.94	4	20
	7q36.2	chr7:153,380,710-154,316,928	936	2	0.05%	0.94	3,4	DPP6
	9p24.3	chr9:98,998-334,508	235	2	0.05%	0.94	3	*C9orf66, CBWD1, DOCK8, FOXD4*
	11q13.3	chr11:70,154,458-70,187,872	33	2	0.05%	0.94	3	*SHANK2*
	14q32.12	chr14:92,476,815-92,496,373	19	2	0.05%	0.94	2	*ITPK1*
	15q23-24.1	chr15:69,601,300-71,944,199	2,343	2	0.05%	0.94	1,4	22
	16p11.2	chr16:29,578,715-30,001,681	422	14	0.37%	**5×10^{-29}**	1,2,3,4,5	26
	16q23.3	chr16:81,796,275-81,830,296	34	2	0.05%	0.94	1	*CDH13*
	16q23.3	chr16:82,557,318-82,683,859	126	2	0.05%	0.94	1,2	*MBTPS1, NECAB2, OSGIN1, SLC38A8*
	18q22.1	chr18:64,812,093-6,484,6196	34	2	0.05%	0.94	2,4	*CCDC102B*
	20p12.1	chr20:14,616,243-14,751,454	135	2	0.05%	0.94	1,3	*MACROD2* (intronic)
	22q11.21	chr22:17,257,787-19,786,200	2,528	3	0.08%	0.11	1,3,4	56
	22q13.33	chr22:49,243,247-49,465,883	222	3	0.08%	0.11	4,5	16
Duplications	1q21.1	chr1:144,838,175-146,324,832	1,487	2	0.05%	0.88	1	14
	2p25.3	chr2:143,279-196,704	53	2	0.05%	0.88	2	0
	3q21.2	chr3:125,966,642-127,254,388	1,288	2	0.05%	0.88	2	11
	7q11.23	chr7:72,411,506-73,782,113	1,371	4	0.09%	**0.003**	1	22
	8p23.3	chr8:710,491-1,501,580	791	2	0.05%	0.88	3,4	*DLGAP2*
	10q11.23-21.1	chr10:52,699,516-54,408,816	1,709	2	0.05%	0.88	1,2,5	*CSTF2T, DKK1, MBL2, PRKG1*
	12q24.31	chr12:120,628,928-120,862,589	233	2	0.05%	0.88	2,4	7
	15q11.2	chr15:21,343,866-21,505,342	161	7	0.18%	**4×10^{-9}**	1,2,3,4,5	*MAGEL2, MKRN3, NDN*

Type	Band	Location (NCBI 36/hg18)	Size (kb)	Recurrence (del/dup)	Frequency (N=3,816)	p-value[a] (C=232)	Studies[b]	Genes[c] (RefSeq)
Combined[f]	15q11.2-13.1	chr15:21,240,037-26,095,621	4,856	6	0.16%	**4×10^{-4}**	1,2,3,4,5	12
	15q13.2-13.3	chr15:28,723,577-30,231,488	1,508	5[e]	0.13%	**2×10^{-5}**	1,2,4,5	*CHRNA7, KLF13, MTMR10, MTMR15, OTUD7A, TRPM1*
	16p13.2	chr16:8,828,382-9,147,487	319	2	0.05%	0.88	1	*PMM2, CARHSP1, USP7, C16orf72*
	16p11.2	chr16:29,563,365-30,085,308	521	5	0.13%	**2×10^{-5}**	1,3,4	26
	20q13.33	chr20:60,949,339-61,220,552	271	2	0.05%	0.88	2,4	7
	22q11.21	chr22:17,265,500-19,791,274	2,526	2	0.05%	0.88	2,4	56
	1q21.1	chr1:145,013,719-146,293,282	1,280	3 (1/2)	0.08%	0.53	1,2	14
	2p16.3	chr2:50,539,877-50,677,835	138	2 (1/1)	0.05%	1.00	3	*NRXN1* [d]
	7q31.1	chr7:108,242,570-108,393,666	151	2 (1/1)	0.05%	1.00	2,4	*C7orf66*
	7q31.1	chr7:111,065,681-111,454,179	388	2 (1/1)	0.05%	1.00	2,4	*DOCK4*
	9p24.3	chr9:175,632-334,508	159	3 (2/1)	0.08%	0.53	1,3	*C9orf66, DOCK8*
	15q13.2-13.3	chr15:28,723,577-30,231,488	1,508	6 (1/5)[e]	0.16%	**1×10^{-4}**	1,2,4,5	*CHRNA7, KLF13, MTMR10, MTMR15, OTUD7A, TRPM1*
	16p11.2	chr16:29,578,715-30,001,681	521	19 (14/5)	0.50%	**2×10^{-55}**	1,2,3,4,5	26
	16q22.3	chr16:69,987,425-70,647,241	660	2 (1/1)	0.05%	1.00	1,2	13
	20q13.33	chr20:61,056,624-61,076,763	20	3 (1/2)	0.08%	0.53	2,4	*SLC17A9*
	22q11.21	chr22:17,265,500-19,786,200	2,521	5 (3/2)	0.13%	**0.002**	1,2,3,4	56

[a] p-values are calculated as described in the methods.

[b] 1. This study; 2. Itsara et al., 2010; 3. Pinto et al., 2010; 4: Marshall et al., 2008; 5. Sebat et al., 2007.

[c] Counts are given for CNVs with >6 RefSeq genes mapping to the interval. A complete listing of genes is in Table S4. All genes shown represent exonic overlap unless otherwise indicated.

[d] While only 2 *de novo* CNVs overlap within *NRXN1*, there are 5 *de novo* events overlapping a section of the gene: 1 intronic deletion, 3 exonic deletions, and 1 exonic duplication (p=0.004 combined, p=0.007 for deletions).

[e] 3 of the duplications contributing to 15q13.2-13.3 are isodicentric chr15 events; since there is a longstanding associated with ASD and isodicentric chr15, this region is also considered without these events. For interstitial CNVs alone there 2 duplications and 1 deletion (p=0.62 combined; p=0.92 duplications) (Figure 7).

[f] Regions are only listed in the combined category if there are a combination of deletions and duplications resulting in a different p-value when the two types of CNVs are considered together.