The phenotypic manifestations of rare genetic CNVs in autism spectrum disorder

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Significant evidence exists for the association between copy number variants (CNVs) and Autism Spectrum Disorder (ASD); however, most of this work has focused solely on the diagnosis of ASD. There is limited understanding of the impact of CNVs on the ‘sub-phenotypes’ of ASD. The objective of this paper is to evaluate associations between CNVs in differentially brain expressed (DBE) genes or genes previously implicated in ASD/intellectual disability (ASD/ID) and specific sub-phenotypes of ASD. The sample consisted of 1590 cases of European ancestry from the Autism Genome Project (AGP) with a diagnosis of an ASD and at least one rare CNV impacting any gene and a core set of phenotypic measures, including symptom severity, language impairments, seizures, gait disturbances, intelligence quotient (IQ) and adaptive function, as well as paternal and maternal age. Classification analyses using a non-parametric recursive partitioning method (random forests) were employed to define sets of phenotypic characteristics that best classify the CNV-defined groups. There was substantial variation in the classification accuracy of the two sets of genes. The best variables for classification were verbal IQ for the ASD/ID genes, paternal age at birth for the DBE genes and adaptive function for de novo CNVs. CNVs in the ASD/ID list were primarily associated with communication and language domains, whereas CNVs in DBE genes were related to broader manifestations of adaptive function. To our knowledge, this is the first study to examine the associations between sub-phenotypes and CNVs genome-wide in ASD. This work highlights the importance of examining the diverse sub-phenotypic manifestations of CNVs in ASD, including the specific features, comorbid conditions and clinical correlates of ASD that comprise underlying characteristics of the disorder.

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INTRODUCTION

Advances in our understanding of the genetics underlying Autism Spectrum Disorder (ASD) from linkage1–3 genome-wide association studies4–7 and next-generation sequencing studies8–10 have identified several potential genetic pathways to ASD including chromatin remodeling, metabolism, mRNA translation and synaptic function, and others.11 However, the bulk of the genetic research has focused on categorical classification of the presence or absence of an ASD diagnosis, which is characterized by a broad range of core and associated features and heterogeneity in its manifestations.12,13 Investigation of the role of susceptibility genes for specific sub-phenotypes of ASD is a critical next step in integrating this diverse research. The term ‘sub-phenotypes’ as used here describes the specific features, comorbid conditions or clinical correlates of ASD that comprise underlying phenotypic characteristics of the disorder.

Copy number variation (CNV), the most prevalent type of structural variation in the human genome,14,15 has been shown to contribute to genetic heterogeneity16 and may also provide an important tool to identify sources of phenotypic heterogeneity.17 Identification of specific components of the ASD phenotype or its correlates that are associated with CNVs could provide etiologic clues to the development of the ASD phenotype. There is growing evidence that certain recurrent CNVs, while predisposing toward ASD, also influence broader phenotypic manifestations, such as intellectual disability (ID), physical characteristics and seizures.18 This suggests that the impact of CNVs might extend beyond the current clinical diagnostic entities in neuropsychiatry, as demonstrated by the wide range of manifestations of well-characterized genetic disorders, such as the 22q deletion syndrome.19 Some CNVs might also act as modifiers or vulnerability factors for these phenotypes rather than influencing specific disease outcomes.20,21 Since multiple genes can be disrupted by a single CNV, one might expect greater evidence of syndromal presentations that transcend standard clinical features of the specific disorder with multi-systemic effects. To date, most of the CNV research in ASD has focused on disorder-specific CNV identification, and a few studies22–23 have systematically examined the impact of CNVs genome-wide on specific features, comorbid conditions or clinical correlates of ASD. With few exceptions (for example, refs. 1,5,24–28, there
has been limited investigation of sub-phenotypic features of ASD in linkage studies, genome-wide association studies or next-generation sequencing studies.

The aim of this work was to examine the phenotypic outcome of variation in genic CNVs in ASD, using a case-only design. The purpose of investigating genic CNVs was to narrow the analytic focus and target functional regions of the genome. We employed a data-driven approach (random forests) to determine whether there were more homogeneous subgroups within the sample that would be more amenable to genetic association analyses. Unlike parametric statistical approaches that are hypothesis driven, and assume a standard distribution of the data, the random forest approach does not assume a Normal distribution of the predictor data.29 We hypothesized that individuals carrying rare CNVs that impact genes implicated in either ASD or ID, or in differentially brain expressed (DBE) genes, would be more likely to present with general developmental anomalies, including non-verbal status, seizures, gait disturbances, lower IQ and adaptive function than those cases with CNVs impacting other genes.

MATERIALS AND METHODS

Subjects

This study is a secondary analysis of data from the Autism Genome Project (AGP), a collaborative data collection and genetics research initiative.30 The original AGP CNV study sample comprises 2705 parent-affected child trios (2384 of European ancestry) derived from a pooled sample, and a subset of the original AGP CNV study sample comprises 1590 cases of European ancestry with a diagnosis of an ASD with at least one rare CNV impacting any gene. Diagnosis inclusion was defined by DSM-IV criteria at all sites, assessed by the Autism Diagnostic Observation Schedule (ADOS)34 and the Autism Diagnostic Interview-Revised.35 Overall, the sample was 85.5% male, and the mean age at assessment was 8.8 years (105 months, s.d. 60.9 months, range 22–565 months). Ethical approval was granted at each participating AGP site. Both parents provided written informed consent, and consent was also obtained from subjects who were considered competent to provide it, based on the parents’ or caregivers’ assessment of competency. Ethically matched convenience controls used to estimate CNV frequencies were obtained from unrelated control subjects with no obvious psychiatric history from three additional studies (that is, Study of Addiction Genetics and Environment; 36 Ontario Colorectal Cancer cause–control study;37–39 and Health, Aging and Body Composition40). See Supplementary Materials Section 1.1 for additional information.

Clinical measures

These analyses were based on phenotypes of possible neurodevelopmental origin derived from the Autism Diagnostic Interview-Revised (ADIS) that were available across AGP sites including verbal status, age at first words and phrases (also collapsed into a composite language delay), gait disturbance and non-febrile seizures (did not require a diagnosis of epilepsy). Additionally, the ADOS severity score,41 Vineland Adaptive Behavior Scales (VABS);42 a selected composite intelligence quotient derived by an AGP sub-committee (IQ; verbal, performance, and full scale; see Supplementary Materials for additional information), maternal and paternal age at birth and family type were analyzed when they were available. Family type classification ascertained included simplex (no known affected individual among the first- to third-degree relatives), multiplex (at least one first- to third-degree relative of the proband with a validated, clinical ASD diagnosis) and unknown (where family history was not explicitly assessed). More detail on the clinical measures is included in the Supplementary Materials (Section 1.2), and phenotype missingness is reported in Supplementary Table S1.

Genotyping and CNV Calling

Genotyping procedures and CNV calling were thoroughly described in Pinto et al.43,44 Briefly, genotyping was completed on the Illumina Infinium 1 M or Human1M-Duo SNP microarray. CNV discovery included computational calling via QuantiSNP,45 GenoC emotional and PennCNV45 algorithms. After initial CNV calling, the sample was then limited to those individuals of European ancestry and to CNVs that occurred in <1% of cases and controls. There were a total of 1369 complete trios in whom the inheritance of their CNVs could be established (for example, where one or both the parents were not missing data due to QC filtering or the absence of parental DNA entirely, or at specific markers. N.B. this was not person-specific, but CNV-specific). The case and control CNV frequencies are provided in Pinto et al.44

Gene lists

Because individual CNV events were rare, we examined two candidate gene lists that were used to aggregate these rare CNV events for statistical analysis. The lists were (1) An ASD- and ID-implicated gene list ‘expert-curated’ via literature searches and database reviews through December 2009, and used in the original AGP CNV analyses (see Pinto et al.32 for review) and (2) A DBE list (see Raychaudhuri et al.46 for review). Briefly, the ASD-implicated gene list contains 36 genes strongly implicated in ASD, and the ID-implicated gene list contains 110 genes known to be implicated in ID but not yet in ASD. The ASD and ID-implicated lists were pooled for the analyses presented here, because the ASD list was too small for meaningful analysis. The 3268 DBE genes were defined by Raychaudhuri et al.46 using data from the Gene Expression Omnibus,47 to have specific, differential expression in the brain and central nervous system as compared with other healthy body tissues. A total of 42 genes overlap in the ASD/ID-implicated candidate list and DBE list. More details are provided in Supplementary Materials Section 1.3.

Statistical analyses

In this case-only study, three analytic techniques were employed to explore these data: (1) Latent profile analysis (LPA) to examine the structure of the phenotype data and to determine whether there were more homogeneous subgroups among the highly correlated phenotypes; (2) Recursive partitioning via random forests to determine whether phenotypic subgroups could be predicted by CNV carrier status; and (3) Univariable and multivariable (that is, to control for potential confounders) association analyses via linear and logistic regression to validate the variables highlighted in the two data-driven analytic approaches.

The purpose of the LPA was to identify more homogeneous subgroups of patients based on the highly correlated phenotype data. The LPA was completed in the Mixplus software package,48 using the mixture model option, and model fit was evaluated for one through twelve class solutions. On the basis of the optimal Bayesian Information Criteron solution, there was no obvious solution for the sample as a whole, leading us to conclude that there were no obvious phenotypic subgroups within this sample. The results of this analysis are reported in the Supplementary Materials (Supplementary Figure S1).

Random forests, a type of classification and regression tree recursive partitioning, were employed to identify more homogeneous subsets of the heterogeneous clinical groups based on their CNV carrier status,29 and to address potential multicollinearity of the phenotypic variables. They also serve as a data reduction strategy, where the ‘important’ variables can be selected for additional analyses. Random forests can be used when there are missing data, with both continuous and categorical data, with non-normal data, in the presence of interactions, and do not require reduction of the predictor space before classification.29 Briefly, a classification tree is constructed by choosing an initial splitting variable (here, CNV carrier status) to determine the optimal splits of the data based on the randomly selected predictor variables (here, clinical phenotype variables). A collection of each of these trees is termed a forest.49,50 Here, a random forest analysis using the DOS command line version of the Willows software package51 was used to investigate the phenotypic differences between cases with and without CNVs impacting ASD/ID or DBE genes. Forests were created from 10 000 trees with a minimum terminal node size (smallest group of cases when the tree construction terminates) of 10 (0 terminal node size in the de novo analyses due to the rarity of de novo events). The number of variables chosen to split a node was selected by the program automatically (here it was four). Results presented here are the variable importance measure, an assessment of which variable is the best classifier of the data, and an out-of-bag (OOB) error measure. When constructing a tree, one-third of the cases are held out to test the tree; these are the OOB samples. Once the tree is constructed, the OOB sample is dropped through the tree, and each case is classified. The proportion of cases correctly classified is the accuracy, and 1 minus the accuracy is the error. The variable importance score is calculated by comparing...
classification accuracy when the case status in the original and OOB samples is randomly shuffled and then run through the tree. If the error rates do not increase when the case status is permuted, then the variable under examination is not a useful predictor of case status. This accuracy is averaged over all of the trees in the forest, and the overall classification accuracy of the forest is reflected by the reported OOB error. For reference, no variable importance score has a particular meaning; instead it is a relative ranking of the examined predictors. Therefore, the variable importance scores must be interpreted in reference to one another, and should not be compared across studies. Limitations of the method include a potential bias toward continuous variable types and highly correlated variables in the analyses.

The association analyses examined the relationship between rare CNVs impacting genes implicated in either the ASD/ID or DBE gene list and the phenotypes of interest, which serves to validate the random forest analyses. Typically one would follow-up the results of only the ‘important’ variables discovered in the random forest analysis; however, we completed association analyses on all variables since we were testing the random forest method. In the models presented here, CNV carrier status predicted the outcome phenotype, except in the case of parental age, where that predicted CNV status. Either linear or logistic regression was employed, depending upon the outcome variable. Preliminary analyses were performed univariable, unadjusted, and further analyses were multivariable, adjusted for relevant covariates (age at test, genotyping stage and age of the other parent for parental age). Full details of the statistical test and covariates used for each phenotype are described in Supplementary Table 2. Male-only analyses were completed, but without a change in the results, so only the combined sex analyses are shown here. The results presented here are not corrected for multiple comparisons, and all analyses were completed in SAS v9.2.

RESULTS

Frequency of CNVs

Table 1 shows the frequency of CNVs impacting genes in the ASD/ID gene list and the DBE gene list by CNV type among participants who carried at least one rare genic CNV. Deletions and duplications are not mutually exclusive; therefore, one individual can have both a deletion and a duplication in different portions of their genome. This results in overlapping, non-independent analyses for deletions, duplications and any CNVs. It is important to note that the results here only reflect a subset of those included in Pinto et al. where the rates of CNVs in cases and controls are presented.

The frequency of cases with CNVs impacting the ASD/ID list genes was low, with 6.6% of the sample having a CNV that impacted a gene in this list. The frequency of cases with CNVs impacting DBE list genes was substantially higher than that of the ASD/ID list, with 51.32% of the sample having a CNV that impacted a gene (or genes) in this list. Overall, 26.73% of the sample had deletions that impacted a gene in the DBE list and 30.75% had duplications that impacted a gene in the list.

CNV inheritance information was only available for a subset of the 1590 rare, genic CNV carriers; 1369 cases had full trio data available for analysis (86% of the sample). Overall, 7.7% of these cases had a de novo CNV. Only 1.5% of the sample had a de novo CNV that impacted a gene in the ASD/ID gene list. The frequency of de novo CNVs impacting genes in the DBE list was higher, with 4.75% having a de novo CNV that impacted a gene in this list.

Recursive partitioning results

The results of the random forest exploratory analyses are presented in Table 2. Overall, there was great variability of the classification accuracy for the selected measures in this study. The ASD/ID list was a substantially better classifier than was the DBE list, with OOB error rates nearly tenfold lower for the ASD/ID list for all CNV types (3–7% in the ASD/ID list vs 27–52% in the DBE list over 10,000 permutations). The scale of the variable importance statistics was similar between both lists, but there were a greater number of variables that hindered classification (negative importance) in the DBE list than in the ASD/ID list. Among the ASD/ID forests, deletions showed better classification accuracy than did all CNVs and duplications. Verbal IQ was the best classifier for deletions and all CNVs, while VABS daily living skills were the best classifier for duplications. Conversely, sex and seizures were universally poor classifiers.

With respect to the DBE genes, based on the OOB error, deletions tended to be better classifiers than either duplications or all CNVs. Paternal age, performance IQ, VABS composite and daily living skills were the only phenotypes that were universally positive classifiers across CNV types, whereas others were quite variable. Notably, age at first words and phrases, full-scale IQ, maternal age and VABS socialization classification importance varied between positive and negative values depending on the CNV type. Seizures, family type, gait disturbance, language delay and the ADOS severity score hindered classification.

In the de novo random forest analyses, the best phenotypes for classifying de novo CNV carriers were the VABS scores, specifically the composite and daily living skills. ASD/ID de novo CNVs provided more robust classification values than the any de novo and DBE de novo CNVs.

Association analyses

Table 3 presents summaries of the associations between the presence or absence of CNVs impacting ASD/ID genes, DBE genes and the phenotypes described above. Table 4 shows the summary of associations between parental age and CNVs in the gene lists. In the tables, statistical significance was designated at the alpha = 0.05 level and 95% confidence limits are reported; there were nine statistical tests per phenotype.

Statistically significant associations between deletions impacting ASD/ID genes predicting the phenotypes under investigation were found for language delay (all ASD/ID CNVs and deletions, unadjusted and adjusted), verbal IQ (all ASD/ID CNVs and deletions, unadjusted and adjusted), VABS communication (duplications, unadjusted and adjusted), socialization (deletions, unadjusted and adjusted) and composite scores (deletions unadjusted, and duplications unadjusted and adjusted).

Statistically significant associations between CNVs impacting DBE genes were found for VABS communication (duplications, unadjusted and adjusted), socialization (duplications, unadjusted and adjusted), daily living skills (all DBE CNV and duplications,
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Table 2. Random forest variable importance scores and ‘Out of Bag’ (OOB) error rates

| Variable                          | ASD/ID (N = 1590) | DBE (N = 1590) | De Novo (N = 1369) |
|-----------------------------------|-------------------|----------------|-------------------|
|                                   | All   | Del  | Dup  | All   | Del  | Dup  | All   | ASD/ID | DBE  |
| ADOS severity score               | 0.05  | 0.06 | 0.08 | -0.16 | -0.25 | -0.27 | 0.00  | 0.13  | 0.15 |
| Family type                       | 0.04  | 0.03 | 0.02 | -0.37 | -0.11 | 0.00  | 0.23  | 0.15  | 0.46 |
| First phrases                     | 1.15  | 0.32 | 0.32 | -0.42 | 0.61  | -0.31 | 0.67  | 0.51  | 0.55 |
| First words                       | 0.20  | 0.45 | -0.04 | -0.71 | 0.57  | -0.74 | 0.00  | 0.42  | 0.67 |
| Full-scale IQ                     | 0.90  | 0.37 | 0.19 | -0.93 | 1.22  | 0.32  | 0.38  | 1.10  | 1.57 |
| Gait disturbance                  | 0.06  | 0.05 | 0.00 | -0.41 | -0.07 | -0.32 | -0.08 | -0.01 | 0.04 |
| Language delay                    | 0.94  | 0.54 | 0.05 | -0.09 | -0.13 | -0.06 | 0.22  | 0.29  | 0.39 |
| Maternal age                      | 0.83  | 0.32 | 0.48 | -0.47 | 1.29  | 0.39  | 0.91  | 0.43  | 0.33 |
| Paternal age                      | 0.99  | 0.42 | 0.43 | 0.34  | 2.69  | 0.58  | 1.27  | 0.31  | 0.41 |
| Performance IQ                    | 0.75  | 0.30 | 0.29 | -0.65 | 0.37  | 0.29  | 1.01  | 0.08  | 0.71 |
| Sex                               | -0.12 | -0.05 | -0.01 | -0.29 | -0.04 | -0.18 | 0.07  | -0.23 | -0.05 |
| VABS communication                | 0.80  | 0.23 | 0.48 | -0.21 | 0.14  | 0.99  | 3.72  | 1.03  | 3.15 |
| VABS composite                    | 0.59  | 0.62 | 0.53 | 0.29  | 1.14  | 0.52  | 6.29  | 1.79  | 6.34 |
| VABS daily living skills          | 0.95  | 0.61 | 0.56 | 0.04  | 0.44  | 0.35  | 4.30  | 1.51  | 3.22 |
| VABS socialization                | 0.82  | 0.34 | 0.35 | -0.04 | 0.51  | 1.54  | 3.37  | 0.84  | 2.70 |
| Verbal status                     | 0.77  | 0.22 | 0.11 | -0.21 | 0.21  | 0.36  | 0.28  | 0.14  | 0.10 |
| Verbal IQ                         | 2.01  | 0.87 | 0.36 | -0.23 | 1.89  | -0.20 | 1.46  | 0.40  | 1.40 |
| OOB error                         | 0.07  | 0.03 | 0.04 | 0.52  | 0.27  | 0.32  | 0.08  | 0.02  | 0.05 |

Abbreviations: ADOS, Autism Diagnostic Observation Schedule; ASD/ID, Autism Spectrum Disorder or Intellectual Disability; DBE, differentially brain expressed; Del, Deletion; Dup, Duplication; IQ, intelligence quotient; VABS, Vineland Adaptive Behavior Scales. The top three classifiers in each CNV type are in bold typeface. The OOB error rate reflects classification accuracy over 10,000 permutations. The variable importance score reflects the difference between the OOB error rates when the CNV status is and is not permuted over 10,000 iterations. If the error rates do not increase when the CNV status is permuted, then that variable is a poor classifier. Negative variable importance indicates that the variable hinders classification. Ultimately, variable importance demonstrates which variables have a major role in discriminating participants with and without CNVs.

Table 3. Summary of findings on significant associations of CNVs impacting Autism Spectrum Disorder or Intellectual Disability (ASD/ID) or differentially brain expressed (DBE) genes with clinical phenotypes

| Independent variable (gene list) | Dependent variable (phenotype) | N     | OR   | LCL  | UCL  | P     |
|----------------------------------|--------------------------------|-------|------|------|------|-------|
| ASD/ID All                       | Language delay                 | 1559  | 0.53 | 0.34 | 0.80 | 0.003 |
| Deletions in ASD/ID              | Language delay                 | 1559  | 0.43 | 0.24 | 0.77 | 0.005 |
| Duplications in ASD/ID           | VABS composite                 | 1247  | 0.48 | 0.25 | 0.90 | 0.022 |
| All CNVs in DBE                  | VABS communication             | 1261  | 0.45 | 0.24 | 0.83 | 0.010 |
| Deletions in DBE                 | VABS daily living skills       | 1254  | 0.73 | 0.57 | 0.93 | 0.012 |
| All De novo                      | VABS composite                 | 1247  | 0.63 | 0.49 | 0.82 | 0.001 |
| ASD/ID De novo                  | Deletions in DBE               | 1247  | 0.63 | 0.49 | 0.82 | <0.001 |
| ASD/ID/De Novo                  | Seizures                       | 1237  | 2.02 | 1.17 | 3.50 | 0.012 |
| DBE De novo                     | Family type (Simplex vs Multiplex) | 1168  | 2.50 | 1.22 | 5.11 | 0.012 |

Abbreviations: CNV, copy number variation; VABS, Vineland Adaptive Behavior Scales. Only statistically significant findings (α = 0.05) in adjusted models are shown, along with their associated odds ratio (OR), lower (LCL) and upper (UCL) 95% confidence limits and P-value (P).

Table 4. Summary of findings on significant associations of parental age with CNVs impacting Autism Spectrum Disorder or Intellectual Disability (ASD/ID) or differentially brain expressed (DBE) genes

| Independent variable (parental age) | Dependent variable (gene list) | N     | OR   | LCL  | UCL  | P     |
|-------------------------------------|--------------------------------|-------|------|------|------|-------|
| Maternal age                        | All CNVs in DBE                | 1292  | 0.969| 0.939| 0.999| 0.046 |
| Paternal age                        | Deletions in DBE               | 1292  | 1.034| 1.004| 1.064| 0.225 |

Abbreviation: CNV, copy number variation. Only statistically significant findings (α = 0.05) in adjusted models are shown, along with their associated odds ratio (OR), lower (LCL) and upper (UCL) 95% confidence limits and P-value (P).
unadjusted and adjusted) and composite scores (all CNV and duplications, unadjusted and adjusted) with the CNV effect being for a less severe presentation with better adaptive function than those without these CNVs. Associations were also found for maternal (any CNV, adjusted) and paternal age (deletions, adjusted).

Among de novo CNVs, significant associations emerged for verbal status, seizures and family type. Interestingly, these measures were not associated in the ASD/ID and DBE univariable analyses. Non-febrile seizures were associated with all of the investigated de novo CNV types, with the exception of the DBE CNVs in the adjusted model. A statistically significant association between family type and de novo CNVs was found only in the adjusted model. However, a statistically significant association between verbal status and de novo ASD/ID CNVs was found only in the adjusted model.

Full details of the means or proportions for each phenotype by CNV group are detailed in Supplementary Tables 3–5.

DISCUSSION

CNVs in the ASD/ID list were primarily associated with communication and language domains, whereas CNVs in DBE genes were related to broader manifestations of adaptive function. There was substantial variation in the classification accuracy of the two sets of genes in the random forest analysis demonstrating specific versus broad impact. The novel use of random forests (validated by additional standard association analyses) highlights the importance of defining homogenous subgroups for genotype–phenotype correlation research, and the promise of their utility as a data reduction strategy in future research.

The greater classification accuracy of the ASD/ID gene list than the DBE list in the random forests suggests that CNVs impacting genes on the ASD/ID list are more closely associated with the sub-phenotypes of ASD. Further, the contribution of the language-related variables in these analyses supports the role of genes in the ASD/ID list in language and communication. By contrast, the statistically significant univariable associations between the clinical sub-phenotypes of interest and CNVs in the DBE gene list occurred consistently for duplications that influenced adaptive function. Therefore, the CNVs in the DBE gene list appear to have impacted on broad manifestations of neurodevelopmental disorders, rather than on specific features of ASD, thereby suggesting a more generalized role in function. Duplications tended to be associated with a less severe presentation than deletions, confirming earlier work that demonstrates duplications are less deleterious than deletions. A possible explanation for this finding is that the absence of a protein product cannot be easily corrected downstream, while the over production of a protein product can be regulated (for example, 17p11.2, where deletions, con...
moderators, highlights the importance of careful dissection of phenotypes and genotypes in elucidating the etiology of ASD. This work documents the importance of investigating the core features and correlates of ASD phenotypes in elucidating the sources of heterogeneity in the genetic architecture of ASD and other neurodevelopmental disorders. It also documents the need for inclusion of standardized measures of the specific aspects of neurodevelopment in future genetic studies. In spite of the difficulty in establishing direct clinical consequences of CNVs, deeper studies of CNVs to investigate their function could inform our understanding of mechanisms underlying disorders, syndromes, sub-phenotypes or clinical correlates (for example, McLysaght et al.).

Although this work focuses solely on CNVs, it illustrates the complexity of the genetic architecture of ASD. The influence of environmental risk factors may have differential influence on those who harbor either rare or common susceptibility variants for ASD, and CNVs may also combine with other genetic risk factors to influence the expression of ASD. Our findings suggest that CNVs may have a role both in specific components of ASD and in their severity. These findings highlight the need for future research that integrates different genetic pathways to ASD, as well as environmental exposures that may either have additive or interactive influences on neurodevelopmental disorders.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)