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Autism Res. Author manuscript; available in PMC 2018 June 15.

Published in final edited form as:

Autism Res. 2014 June ; 7(3): 355–362. doi:10.1002/aur.1378.

Modest impact on risk for autism spectrum disorder of rare copy number variants at 15q11.2, specifically breakpoints 1 to 2

Pauline Chaste[1,2,*], **Stephan J. Sanders**[3,4], **Kommu N. Mohan**[5,6], **Lambertus Klei**[1], **Youeun Song**[1], **Michael T. Murtha**[7], **Vanessa Hus**[8], **Jennifer K. Lowe**[9], **A. Jeremy Willsey**[3,4], **Daniel Moreno-De-Luca**[7,10], **Timothy W. Yu**[11], **Eric Fombonne**[12], **Daniel Geschwind**[9], **Dorothy E. Grice**[13], **David H. Ledbetter**[14], **Catherine Lord**[15], **Shrikant M. Mane**[16], **Donna M. Martin**[17], **Eric M. Morrow**[18,19], **Christopher A. Walsh**[20], **James S. Sutcliffe**[21], **Matthew W. State**[3,4], **Christa Lese Martin**[14], **Bernie Devlin**[1], **Arthur L. Beaudet**[6], **Edwin H. Cook Jr**[22], and **Soo-Jeong Kim**[23,*]

[1]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[2]FondaMental Foundation, Créteil, France

[3]Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA

[4]Department of Psychiatry, University of California at San Francisco, California, USA

[5]Department of Biological Sciences, BITS Pilani-Hyderabad Campus, Hyderabad, India

[6]Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas, USA

[7]Program on Neurogenetics, Yale University School of Medicine, New Haven, Connecticut, USA

[8]Department of Psychology, University of Michigan, Ann Arbor, MI, USA

[9]Neurogenetics Program, Department of Neurology and Center for Autism Research and Treatment, Semel Institute, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, USA

[10]Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut, USA

[11]Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts, USA

[12]Department of Psychiatry, Oregon Health & Science University, Portland, Oregon, USA

[13]Department of Psychiatry, Mount Sinai School of Medicine, New York, New York, USA

[*]Corresponding authors: Pauline Chaste, Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara St., Pittsburgh, PA 15213, USA. Phone: 412-246-6642 Fax: 412-246-6640 chastept@upmc.edu, Soo-Jeong Kim, Center for Integrative Brain Research, Seattle Children's Research Institute, 1900 9th Ave, Seattle, WA 98101, USA, kimsooj@uw.edu, Tel: +12068841275, Fax:+12068841210.

Conflict of Interest: Dr. Cathy Lord receives royalties for the scales of diagnostic instruments for which she's a coauthor; she donates all royalties to autism charities. Authors Mohan and Beaudet are faculty members of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from chromosomal microarray analysis offered in the Medical Genetics Laboratory. All other authors reported no biomedical financial interests or potential conflicts of interest.

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[14]Autism and Developmental Medicine Institute, Geisinger Health System, Danville, Pennsylvania, USA

[15]Center for Autism and the Developing Brain, Weill Cornell Medical College, White Plains, New York, USA

[16]Yale Center for Genome Analysis, Orange, Connecticut, USA

[17]Departments of Pediatrics and Human Genetics, University of Michigan Medical Center, Ann Arbor, Michigan, USA

[18]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island, USA

[19]Department of Psychiatry and Human Behavior, Brown University, Providence, Rhode Island, USA

[20]Howard Hughes Medical Institute and Division of Genetics, Children's Hospital Boston, and Neurology and Pediatrics, Harvard Medical School Center for Life Sciences, Boston, Massachusetts, USA

[21]Departments of Molecular Physiology & Biophysics and Psychiatry, Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN, USA

[22]Institute for Juvenile Research, Department of Psychiatry, University of Illinois at Chicago, Chicago, Illinois, USA

[23]Center for Integrative Brain Research, Seattle Children's Research Institute & Department of Psychiatry and Behavioral Science, University of Washington, Seattle, WA, USA

Abstract

Lay Abstract—Aside from the sex chromosome, all other locations in the human genome have the general expectation that two copies per location are inherited, one from mother and one from father. Yet, with finer and finer characterization of the genome we now know that this rule can be broken. When the rule is broken, we say the region has a copy number variant or CNV. These CNVs can arise *de novo* or can be inherited and they can be harmless or increase risk for disease. A particular region of chromosome 15, near the centromere on the long arm (i.e., 15q11.2-q13), is a hotspot for CNVs and it has several breakpoints (BP). Among the rearrangements observed in this region, CNVs from the interval between the common BP1 and BP2 have been reported to be associated with developmental disorders, including autism spectrum disorder (ASD). Yet its effect on risk for ASD, while likely to be small, is not fully characterized and thus poses a challenge to recurrence-risk counseling. We estimated its effect on risk and ASD-related phenotypes in a well-characterized ASD sample, the Simons Simplex Collection. We find that BP1-BP2 CNVs contribute only modestly to risk and have similarly small effect on traits related to ASD. To be consistent with the current American College of Medical Genetics guidelines for interpretation of postnatal CNV, the BP1-BP2 deletion and duplication CNVs would probably best be classified as variants of uncertain significance (VOUS): they appear to have an impact on risk, but one so modest that these CNVs do not merit pathogenic status.

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Scientific Abstract—The proximal region of chromosome 15 is one of the genomic hotspots for copy number variants (CNVs). Among the rearrangements observed in this region, CNVs from the interval between the common breakpoints 1 and 2 (BP1 and BP2) have been reported cosegregating with autism spectrum disorder (ASD). Although evidence supporting an association between BP1-BP2 CNVs and autism accumulates, the magnitude of the effect of BP1-BP2 CNVs remains elusive, posing a great challenge to recurrence-risk counseling. To gain further insight into their pathogenicity for ASD, we estimated the penetrance of the BP1-BP2 CNVs for ASD as well as their effects on ASD related phenotypes in a well-characterized ASD sample (n=2,525 families).

TDT revealed significant preferential transmission only for the duplicated chromosome in probands (20T:9NT). The penetrance of the BP1-BP2 CNVs for ASD was low, conferring additional risks of 0.3% (deletion) and 0.8% (duplication). Stepwise regression analyzes suggest a greater effect of the CNVs on ASD related phenotype in males and when maternally inherited.

Taken together, the results are consistent with BP1-BP2 CNVs as risk factors for autism. However their effect is modest, more akin to that seen for common variants. To be consistent with the current American College of Medical Genetics guidelines for interpretation of postnatal CNV, the BP1-BP2 deletion and duplication CNVs would probably best be classified as variants of uncertain significance (VOUS): they appear to have an impact on risk, but one so modest that these CNVs do not merit pathogenic status.

Keywords

15q11.2; deletion; duplication; penetrance; autism

Introduction

While converging results underscore the importance of rare de novo events of large effect on autism spectrum disorder (ASD) [Pinto *et al*. 2010; Sanders *et al*. 2011], it has proven challenging to estimate the contribution of inherited copy number variants (CNVs) [Bucan *et al*. 2009; Girirajan *et al*. 2013b; Krumm *et al*. 2013; Morrow *et al*. 2008; Pinto *et al*. 2010; Sanders *et al*. 2011]. There is no doubt, however, that some rare inherited CNVs substantially increase risk, e.g. deletion or duplication of loci affected by recurrent de novo CNVs, notably 16p11.2 [Sanders *et al*. 2011; Weiss *et al*. 2008]. Still, at the population level, the contribution to risk from individual inherited variants of large effect should be small, because the individual variants are subject to natural selection [Devlin *et al*. 2012], while rare inherited variants that modestly increase risk could play a substantial role in the genetic architecture of the disorder.

The 15q11.2-q13 region harbors five common breakpoints (BP1, BP2, BP3, BP4, and BP5), each breakpoint corresponding to a complex set of segmental duplications (SDs) [Christian *et al*. 1999]. While the large effect of BP1-BP3, BP2-BP3, and BP4-BP5 CNVs on neurodevelopment has been well-established [Cook *et al*. 1997; Girirajan *et al*. 2013a; Moreno-De-Luca *et al*. 2012; Shen *et al*. 2010], the picture is considerably less clear for the effects of BP1-BP2 CNVs. The BP1-BP2 genomic interval within band 15q11.2 (henceforth “BP1-BP2” and “15q11.2” are used synonymously.) encompasses four genes not known to

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be imprinted: *NIPA1, NIPA2, CYFIP1*, and *TUBGCP5*. Interestingly, while the BP2-BP3 region is considered as the critical region for Prader-Willi syndrome (PWS) and Angelman syndrome (AS), some have reported a correlation between the type of deletion (i.e., BP1-BP3 deletion vs. BP2-BP3 deletion) and the severity of the phenotype [Butler *et al*. 2004; Hartley *et al*. 2005; Sahoo *et al*. 2007]. Moreover, data accumulate to support the role of BP1-BP2 deletion, and to a lesser extent duplication, as risk factors for several neurodevelopmental disorders such as schizophrenia, developmental delay, intellectual disability with or without dysmorphism, epilepsy, and ASD [Abdelmoity *et al*. 2012; Burnside *et al*. 2011; de Kovel *et al*. 2010; Doornbos *et al*. 2009; Murthy *et al*. 2007; Sempere Perez *et al*. 2011; van der Zwaag *et al*. 2010; Vassos *et al*. 2010; von der Lippe *et al*. 2011]. The magnitude of their effect, however, remains uncertain.

A primary concern for genetic counseling is how much a BP1-BP2 CNV increases the risk of autism. Our goal in this research is to provide as much information as possible in this context. Estimating the penetrance of the CNV for autism is a key step in that process. Penetrance of BP1-BP2 CNVs is likely to be low, because the vast majority of BP1-BP2 CNVs identified in clinical cohorts are inherited from healthy parents and BP1-BP2 CNVs have been identified in control subjects. However, penetrance estimates vary widely across studies for the deletion (from 0.10 to 0.83) [Cooper *et al*. 2011; Rosenfeld *et al*. 2013] and are lacking for the duplication. Moreover, the contribution of BP1-BP2 CNVs to ASD risk, as opposed to developmental disorders more broadly, has never been examined separately.

Characterization of parent and sibling carriers can also inform our understanding of the nature of risk and has proven fruitful in several instances [Girirajan *et al*. 2010; Zufferey *et al*. 2012]. Given the great phenotypic heterogeneity of ASD, which is thought to reflect the extensive complexity of the architecture of genetic risk for autism, studying subtle autistic symptoms in relatives can disentangle the effect of variants on phenotype. Furthermore it is important to examine the relationship of CNV and phenotype in probands, such as sex specific expression and effect of parent-of-origin. To gain insight into the pathogenicity of the BP1-BP2 CNVs, we study a well-characterized ASD family sample (n=2,525 families) from the Simons Foundation Autism Research Initiative (SFARI). To our knowledge this is the first attempt to examine the pathogenicity of the BP1-BP2 CNVs specifically for ASD.

Methods

ASD families

Included were a total 2,525 ASD families with both phenotype and genotype data available. These consist of 2,482 families from the Simons Simplex Collection (SSC) (version 14.1), 31 families from the Simons Ancillary Collection (SAC), and 12 monozygotic twin families from the Simons Twin Collection (STC). The main properties of the families in the SSC have been described by Fischbach and Lord [Fischbach *et al.* 2010]. Additional information on the SAC and STC families is included in the Supplement. The ASD sample consists of 2,525 fathers, 2,525 mothers, 2,036 designated siblings (s1) and 2,525 designated probands (p1). All individuals in this sample were older than 4 years. Further information regarding inclusion/exclusion criteria for SSC, SAC and STC, as well as the complete list of

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instruments used to assess the phenotype of the families are available on the Simons Foundation Autism Research Initiative (SFARI) website (https://sfari.org/).

Phenotype measures

For parents, average score of the Broad Autism Phenotype Questionnaire (BAPQ) [Hurley *et al.* 2007] and total raw score of the Social Responsiveness Scale (SRS) [Constantino *et al.* 2005], adult version (SRS-A) were included in the analysis. For siblings, total T score of SRS-Parent version (SRS-P) was analyzed. The SRS-P score was not analyzed in the probands because the distribution of the scores showed a major ceiling effect in probands. Composite score of the Vineland Adaptive Behavior Scale-2nd edition (VABS-II) [Sparrow *et al.* 2005] was analyzed in both probands and siblings, separately. For probands only, effects of the CNVs on calibrated severity scales (CSS) [Gotham *et al.* 2009; Hus *et al.* 2012] of Social affect (SA-CSS) and restricted repetitive behavior (RRB-CSS) from the Autism Diagnostic Observation Schedule-WPS edition (ADOS-WPS) [Lord *et al.* 2006], and intellectual quotient (IQ) consisting of full scale IQ (FIQ), verbal IQ (VIQ) and nonverbal IQ (NVIQ) were modeled. IQ scores were derived from one or more of the following instruments: Differential Ability Scales, 2ndedition (DAS-II) [Elliott 2007], Wechsler Intelligence Scale for Children, 4thedition (WISC-IV) [Wechsler 2004], Mullen Scales of Early Learning [Mullen 1995], or the Raven's standard progressive matrices [Raven 1981]. When children had the Raven's, verbal IQ was estimated from the Peabody Picture Vocabulary Test-4th edition (PPVT-4) [Dunn *et al.* 2007].

Microarray analyses

The ASD families were genotyped on the Illumina Infinium® 1Mv1 (338 families), Infinium® 1Mv3 Duo (1,191 families) or the HumanOmni2.5-8 (996 families) microarrays (Illumina Inc, USA). All three microarray types had over 180 probes within the BP1-BP2 region. CNV prediction was performed by PennCNV (PN) [Wang *et al.* 2007], QuantiSNP (QT) [Colella *et al.* 2007], and Gnosis [Sanders *et al.* 2011], using CNVision [Sanders *et al.* 2011]. Detailed CNV detection protocol was described in [Sanders *et al.* 2011]. Sample identity within the family, including correct assignment of paternity and maternity, was confirmed using genetically-inferred identity by descent for all study participants including the de novo deletion of BP1-BP2 in a proband.

Statistical analysis

The distributions of SRS scores (SRS-A and SRS-P) in unaffected relatives were not symmetric (Figure 1S). Because extreme observations (out in the tail) can act as “influence points” for regression effects, we analyzed all data using the square root of SRS scores. The effects of BP1-BP2 CNVs in parents on ASD related phenotypes were examined using Generalized Estimating Equations (GEE) to take into account the correlation between the mother and father SRS scores and the mother and father BAPQ scores. Linear Regression was used to explore the results obtained with GEE further by analyzing the mothers and fathers separately. All analyzes were done using R ((http://cran.r-project.org). GEE was implemented using the gee package, with the identity variable being family, the correlation among family members was defined as fixed for the correlation between parents' scores (0.34 for SRS and 0.11 for BAPQ).

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All the models initially included sex and age as covariates, as well as sex* CNV interaction. Covariates were dropped when they were non-significant predictors, thereby identifying the most parsimonious model. For modeling the relationship between BP1-BP2 CNV and phenotype, we made the *a priori* assumption that both the deletion and duplication had a similar effect on phenotype: the relatively small number of both deletions and duplications would not permit distinction of subtle differences, even if they exist.

Transmission Disequilibrium Test (TDT) and Penetrance Calculations

The transmission equilibrium of inherited CNVs in our ASD family sample was examined to further evaluate pathogenicity of the BP1-BP2 CNVs, using a chi-statistics. Penetrance estimate was calculated as a conditional probability: **P(D|G)=P(G|D)*P(D)/[P(G|D)*P(D)+P(G|~D)*(1-P(D))]** [Vassos *et al.* 2010], in which **P** is probability, **D** means the subject is diagnosed with ASD, ~**D** means the subject is unaffected, **G** encodes carrier status for BP1-BP2 CNV, and **P(D|G)** is read as the probability of being diagnosed with ASD given the subject carries a BP1-BP2 CNV. The subtlety here is that this cohort was ascertained for affected probands and thus to solve for the penetrance **P(D|G)** we must reverse the conditional (e.g., **[P(G|D))** and use an estimate of the prevalence of ASD, **P(D)**, which we take to be equal to 0.01. The penetrance estimate was performed separately for deletion and duplication because published results from other cohorts suggest they differ somewhat in their penetrance for other developmental and neuropsychiatric outcomes [Cooper *et al.* 2011; Kirov *et al.* 2013]. We also estimated the penetrance for the deletion and duplication combined.

Results

Estimate of frequency and penetrance of BP1-BP2 CNVs

89 subjects in 47 families carried a CNV involving the BP1-BP2 region of chromosome 15. Among those, four families were excluded from all analyses due to the presence of a larger 15q11.2-13 CNV involving the PWS/AS region (Table 1S); all four were de novo CNVs in probands. One father-proband pair carried a BP1-BP2 duplication involving only two genes (*TUBGCP5* and *CYFIP1*); this pair was included in the analyses. In the remaining 85 subjects in 43 families the BP1-BP2 deletion was observed in 6/2,525 fathers, 8/2,525 mothers, 5/2,036 siblings, and 8/2521 probands; whereas BP1-BP2 duplication was observed in 16/2,525 fathers, 13/2,525 mothers, 9/2,036 siblings, and 20/2521 probands. So the rates of deletion [duplication] were 0.28% [0.57%] in parents, 0.25% [0.44%] in unaffected siblings (s1), and 0.32% [0.79%]in probands, respectively (Table 1).

Only one CNV, a deletion, was de novo and it was transmitted by the father. Neither the frequency of the deletion nor the frequency of the duplication differed significantly between ASD probands and siblings (p=0.86 and p=0.19 respectively). The frequency of the deletion was comparable to published rates in controls (0.18% to 0.38%) [Cooper *et al.* 2011; Rosenfeld *et al.* 2013; Stefansson *et al.* 2008]and somewhat lower than in individuals with schizophrenia (0.59% [Kirov *et al.* 2013] to 0.62% [Vassos *et al.* 2010]), or in subjects with developmental delay (0.60% [Cooper *et al.* 2011] to 0.81% [Rosenfeld *et al.* 2013]). The frequency of the duplication in ASD probands was higher than the previously published

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rates in both cases (0.41% [Cooper *et al.* 2011]) and controls (0.34% [Stefansson *et al.* 2008] to 0.43% [Cooper *et al.* 2011]). No parent-of-origin-specific distortion of parental transmission was observed in this sample (Table 2S).

The estimate of penetrance was 0.013 for the deletion and 0.018 for the duplication (0.016 for either), given a prevalence of 0.01 for ASD [CDC 2012; CDC, Centers for Disease Control 2006], which means that the presence of the BP1-BP2 deletion/duplication would increase the risk of having autism by about 3 in a thousand for the deletion and 8 in a thousand for the duplication. However, the confidence interval of the odds ratio is quite large (deletion: OR= 1.3 CI95%=[0.42-3.96]; duplication OR= 1.8, CI95%=[0.82-3.97]).

Transmission analysis of BP1-BP2 CNVs

There was no preferential transmission of the deletion, either in probands or siblings in this sample, whereas the duplication was preferentially transmitted from parents to probands (20T vs. 9NT, X^2=4.2, one tailed p=0.041) but not to unaffected siblings (Table 3S and Figure 2S). Interestingly, preferential transmission of duplication was most evident in trio families (10T vs. 0NT) vs. quartet families (10T vs. 9NT). We explored further the transmission results for “stoppage effect (stop having children after the proband)” in relation to birth orders of the probands. We did not observe any meaningful pattern between trio and quartet families. Transmission to siblings and probands appears similar between fathers and mothers.

Effects of BP1-BP2 CNVs on autism related symptoms in relatives

In parents, the model including both sex and sex*CNV interaction revealed nominally significant CNV effect on SRS score in parents (Table 2). When the model was simplified, however, by dropping age (non-significant effect)and sex*CNV interaction (close to significance), the CNV effect on SRS score became not significant. Because these results suggest that there may be a modest difference between mothers and fathers in the effect of the CNV, data from mothers and data from fathers were analyzed separately. The results (Table 4S) were consistent with a greater effect of the CNV on SRS scores in fathers than mothers. GEE analysis for BAPQ score was not significant with or without sex and age included in the model (Table 2).

In siblings (Table 4S) a CNV effect on SRS score was not observed either overall or in male siblings only (Estimate -0.08946, p value 0.67); however, there was a significant effect of “Parent-of-Origin” (PoO) on SRS score (Table 4S), specifically CNVs from mothers had a greater impact than those from fathers. Thus there appeared to be modest heterogeneity by PoO, but it is important to also note the small sample size of 13 siblings (one did not have SRS data). Lastly, while the analysis of the effect of the CNV on composite score of VABS in siblings did not reveal a significant CNV effect, the results of the model including PoO, sex and an interaction between PoO and sex, suggested a trend toward a greater effect in males who received the CNV from their mother.

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The effects of BP1-BP2 CNVs in Probands

Before analyses, two probands were excluded from the phenotype/genotype analyzes because they carried a large de novo CNV (3.5 Mb and 5.4 Mb respectively) in another chromosome (Table 1S). Performing the same stepwise analyses described earlier, neither the analysis of the effect of CNV nor the analysis of PoO supported a large effect of the CNV on any of the analyzed phenotypes (Table 5S), and there was no consistency between the results of the different models.

Discussion

ASD is a group of heterogeneous neurodevelopmental disorders causing significant social, communication, and behavioral deficits and challenges [APA 2000]. While genome-wide genotyping and sequencing are beginning to elucidate the complex architecture of autism risk, the contribution of inherited rare variation to genetic risk for autism remains elusive [Bucan *et al.* 2009; Girirajan *et al.* 2013b; Krumm *et al.* 2013; Morrow *et al.* 2008; Pinto *et al.* 2010; Sanders *et al.* 2011; Sanders *et al.* 2012]. Although this contribution is likely to be low in terms of proportion of phenotype explained in a sample (i.e., heritability related to rare inherited variation), it is an important issue at the individual level because of the consequences for genetic counseling. Moreover, the discovery of some inherited CNVs occurring at loci affected by de novo CNVs shows that some rare inherited variation carries risk for ASD. To gain further insight into the pathogenicity of the BP1-BP2 CNVs, which have been proposed as risk factors for ASD, we have estimated the penetrance of the CNV and effects on ASD phenotype in a well-defined ASD family sample.

Intriguingly, the results obtained in this sample are different than the results from previous studies on broader neurodevelopmental phenotypes [Cooper *et al.* 2011]. Indeed, there was no evidence of association between the deletion and ASD in this sample, whereas a significant over-transmission of the duplication to probands was observed. Moreover, the estimated penetrance was lower for the deletion than for the duplication (0.013 vs. 0.018). This could be the consequence of an ascertainment bias. For this sample parents were selected so that they did not present with even a mild autism phenotype and they would thus be different from the unscreened parental population. A relevant observation is that the frequency of BP1-BP2 deletion in both unaffected relatives and probands was similar to published rates in controls [Cooper *et al.* 2011; Rosenfeld *et al.* 2013;Stefansson *et al.* 2008]. While it is possible that differences in CNV detection confound interpretation of these data, another plausible explanation for these differences is that the recruitment modalities of the SFARI lead to the selection of families less likely to carry rare, inherited ASD risk variation, such as BP1-BP2 CNVs. Certainly this was the purpose of the strict family assessment of the SFARI and its impact has been documented for common variants affecting risk for ASD [Klei *et al.* 2012]. While in this study the penetrance of the duplication was estimated to be slightly higher than the penetrance of the deletion, this could also be a result of ascertainment bias: families with the duplication being less likely to have been excluded because the duplication has smaller phenotypic effect; while the smaller sample size of parents carrying deletions translates to greater variance in transmission than that for duplications. The sole BP1-BP2 de novo event, a deletion, lends some credence to this

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possibility. Thus it is possible, given these results, that the impact of BP1-BP2 on risk is somewhat underestimated by our study. Nonetheless, there can be no doubt that its impact is modest and its penetrance is low.

All other results are consistent with a very modest effect. Interestingly, the results of the regression analysis of the effect of the CNVs on SRS scores suggested that males might be more sensitive to the effect of the CNV, with a greater effect of CNVs in fathers than in mothers. Still there is no effect of presence of the CNV on the score for the BAPQ for any model. It is tempting to say that these results show that the impact of the CNV is principally on sociability unrelated to broader autism phenotypes, but that interpretation must be tempered by the ascertainment used to recruit parents for the study, namely that they show few if any traits of the broader autism phenotype. This confounding limits our ability to interpret negative findings, but bolsters our confidence in positive results, which move contrary to expectations based on the ascertainment of parents for this study.

When we evaluated the effects of the BP1-BP2 CNVs on the severity of autism phenotype in probands, we did not observe any effect of the CNV in a positive or negative direction. This is consistent with the low penetrance and low impact on risk, suggesting this CNV and other risk factors, perhaps many, are required for a carrier to be diagnosed with ASD. The results yield a glimpse of the great challenge of identifying rare inherited variants that increase risk for autism.

To our knowledge, this is the first systematic study to investigate the effect of the BP1-BP2 CNVs on ASD risk. The results suggest that BP1-BP2 CNVs confer very little additional risk for ASD. One intriguing observation is that the effect of maternally inherited CNVs effect could be slightly higher than attributable to paternally inherited CNVs. However these results should be considered as exploratory and larger samples will be required to understand if this effect is confirmed or simply a stochastic effect due to small sample size and multiple testing. The main limitation of this study is the relatively small sample size of carriers, consistent with the rarity of the BP1-BP2 CNVs in the population in general. To replicate our results on penetrance with good power (80%, with α=0.05), for example, a very large sample size will be required (20027 probands and controls for the deletion, 3466 probands controls for the duplication). However, a strength of this study is that the family based analyses avoid population structure bias, which is likely to be important in studies of rare variants [Liu *et al.* 2013]. In any case the results of this study strongly suggest that the effect on ASD risk is small. Following the current American College of Medical Genetics guidelines for interpretation of postnatal CNV [Kearney *et al.* 2011], these CNVs remain classified as variants of unknown significance (VOUS)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to all of the families at the participating Simons Simplex Collection (SSC) sites, as well as the principal investigators (A. Beaudet, R. Bernier, J. Constantino, E. Cook, E. Fombonne, D. Geschwind, R. Goin-

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Kochel, E. Hanson, D. Grice, A. Klin, D. Ledbetter, C. Lord, C. Martin, D. Martin, R. Maxim, J. Miles, O. Ousley, K. Pelphrey, B. Peterson, J. Piggot, C. Saulnier, M. State, W. Stone, J. Sutcliffe, C. Walsh, Z. Warren, E. Wijsman). We appreciate obtaining access to phenotypic data on the Simons Foundation Autism Research Initiative (SFARI) Base. Approved researchers can obtain the SSC population dataset described in this study (http://sfari.org/resources/sfari-base), by applying at https://base.sfari.org. This work was supported by a grant from the Simons Foundation (SFARI 124827 to the investigators of the SSC Genetic Consortium); NIH K23MH082883 (SJK); NIH R01HD065272 (CL); NIH R01MH089390 (CL); CIHR (Doctoral Research Award to AJW); HHMI (International Student Research Fellowship to SJS), and Fondamental Foundation (PC).

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Table 1
BP1-BP2 CNV frequency in 2,525 ASD families

15q11.2 CNV	father	mother	s1	p1
BP1-BP2 Deletion	6	8	5	8
BP1-BP2 Duplication	16	13	9	20
No CNV	2503	2504	2022	2493
CNV overlapping the PWS/AS CR	0	0	0	4
Sum	2525	2525	2036	2525

PWS/AS CR – Prader-Willi syndrome/Angelman Critical Region (BP2-BP3); s1 – designated sibling; p1 – designated proband

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Table 2

Effect of CNV on SRS (square root) and BAPQ scores in parents using Generalized Estimating Equations

Clinical Measure	Predictors	Estimate	Naïve SE	Naïve z	Robust S.E.	Robust z	P value
SRS	CNV	1.04661980	0.41466441	2.524016	0.53327662	1.962621	**0.0497**
	sex	0.05556904	0.05480882	1.013870	0.05473152	1.015302	0.31
	sex*CNV	-1.09070467	0.59330134	-1.838365	0.65829129	-1.656872	**0.0975**
BAPQ	CNV	0.1122471	0.09024223	1.243842	0.09343174	1.20138	0.23
	sex	-0.2915663	0.01665541	-17.505799	0.01665045	-17.51102	$1.18e^{-68}$

SRS: Social Responsiveness Scale; BAPQ: Broad Autism Phenotype Questionnaire; sex*CNV: interaction between sex and CNV