Large mosaic copy number variations confer autism risk

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Although germline de novo copy number variants (CNVs) are known causes of autism spectrum disorder (ASD), the contribution of mosaic (early-developmental) copy number variants (mCNVs) has not been explored. In this study, we assessed the contribution of mCNVs to ASD by ascertaining mCNVs in genotype array intensity data from 12,077 probands with ASD and 5,500 unaffected siblings. We detected 46 mCNVs in probands and 19 mCNVs in siblings, affecting 2.8–73.8% of cells. Probands carried a significant burden of large (>4-Mb) mCNVs, which were detected in 25 probands but only one sibling (odds ratio = 11.4, 95% confidence interval = 1.5–84.2, $P = 7.4 \times 10^{-4}$). Event size positively correlated with severity of ASD symptoms ($P = 0.016$). Surprisingly, we did not observe mosaic analogues of the short de novo CNVs recurrently observed in ASD (eg, 16p11.2). We further experimentally validated two mCNVs in postmortem brain tissue from 59 additional probands. These results indicate that mCNVs contribute a previously unexplained component of ASD risk.

The genetic architecture of ASD is complex. Common variants, rare variants and germline de novo variants contribute substantially to risk[1–3]. Germline de novo CNVs (dnCNVs) play a central role, with such events observed in 5–10% of ASD probands[4–6]. Archetypal dnCNVs are recurrently observed in ASD probands, including duplications of 15q11–13, duplications and deletions of 16p11.2 and focal deletions of NRXN1 (ref. [6]). However, despite substantial progress understanding the genetic risk of ASD, a large portion of ASD susceptibility cannot be explained by known risk variants[7,8].

Early-developmental (mosaic) mutations have been proposed as a possible source of some unexplained ASD susceptibility[9]. Unlike de novo variants, which occur in parental germ cells and are, thus, present in all cells of the body, mosaic mutations arise after fertilization—sometimes during embryonic development[10]—and are present in only a fraction of cells. Nonetheless, both de novo and mosaic variants arise free from the reproductive pressures of natural selection, and, thus, the hypothesis that mosaic variants contribute to sporadic disease is an attractive one. Several studies have linked mosaic single-nucleotide variants to ASD[11–13] and causally implicated them in several other neurological disorders[14–16]. mCNVs have recently been linked to developmental disorders[17]; however, the contribution of mCNVs to ASD risk is currently unknown.

In this study, we systematically analyzed mCNVs (gains, losses and copy number neutral losses of heterozygosity (CNN-LOH)) in 11,457 ASD-affected families using genotype array data from the Simons Simplex Collection (SSC)[18] and the Simons Powering Autism Research for Knowledge (SPARK) datasets[19], drawing upon recent advances in statistical phasing[20] and the pedigree structure

of the data to sensitively detect mCNVs[21]. In both cohorts, we found a significant burden of mCNVs in probands relative to their unaffected siblings. This burden was driven by the presence of large (>4-Mb) mCNVs in probands, and increased event size significantly associated with increased severity of ASD symptoms. We additionally computationally detected and experimentally validated two mCNVs present in whole-genome sequencing (WGS) of brain tissue from an additional 59 probands. These results provide strong evidence that mCNVs contribute to ASD risk.

Results

Detection of mCNVs in ASD cohorts. We sought to characterize the contribution of mCNVs arising during early development to ASD risk. We analyzed blood-derived genotype array intensity data from 2,591 autism-affected families in the SSC cohort[18] and saliva-derived genotype intensity data from 8,866 autism-affected families in the SPARK cohort[19]. All SSC probands and siblings were 3–18 years old at enrollment; most SPARK probands and siblings were in or near the same age range, with a small fraction of older probands (1.2% between the ages of 30 and 40 and 0.3% over the age of 40; Supplementary Fig. 1a). After data quality control (Methods), 12,077 probands and 5,500 siblings remained (Table 1). On average, 900,935 genotyped variants remained in SSC samples and 579,300 in SPARK samples, due to differences in genotyping density between arrays.

We performed haplotype phasing using both a population reference panel and the pedigree structure of the data to obtain near-perfect long-range phase information in offspring. We leveraged the phase information to sensitively detect mCNVs in autosomes of

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Table 1 | Counts of samples carrying mCNVs

		Total samples	Samples with mCNVs (no. of events)	% occurrence	Samples with gain (no. of events)	Samples with loss (no. of events)	Samples with CNN-LOH (of events)
SSC	Probands	2,594	15 (16)	0.58	3 (3)	12 (13)	0 (0)[a]
	Siblings	2,424	13 (17)	0.54	9 (11)	4 (6)	0 (0)
SPARK	Probands	9,483	29 (29)	0.31	20 (20)	4 (4)	5 (5)
	Siblings	3,076	2 (2)	0.07	1 (1)	1 (1)	0 (0)

The modestly increased rate of detection in SSC is consistent with the higher density of genotyped variants in SSC relative to SPARK samples. No difference in rates was observed when restricting to mCNVs >4 Mb (Fig. 1). [a]The absence of CNN-LOH events in SSC was unsurprising given the smaller sample size of SSC compared to SPARK ($P = 0.33$, two-sided Fisher's exact test for comparing CNN-LOH frequency in SSC versus SPARK; $P = 0.59$, two-sided Fisher's exact test for a comparison restricted to probands).

probands and siblings using Mosaic Chromosomal Alterations caller (MoChA)[22] and checked parental genotypes to ensure that events were not germline (Methods; see URLs). We excluded sex chromosomes to avoid confounding from the imbalanced sex ratio between probands and siblings (9,776:2,301 males:females in probands versus 2,718:2,782 in siblings). Following previous studies[21,23], we filtered mCNV calls that exhibited evidence of DNA contamination, and we restricted our analysis to events for which copy number state could be confidently determined (Methods and Supplementary Fig. 2). We further excluded mCNVs frequently observed in age-related clonal hematopoiesis (specifically, focal deletions at *IGH* and *IGL* and low-cell-fraction CNN-LOH events[21,23–25]), which we expected to be present in a very small fraction of samples (<1%, given the young ages of participants) and unrelated to ASD status. We verified that genotyping intensity deviations within the remaining mCNVs were consistent with estimated mosaic cell fraction and copy number state (Supplementary Fig. 3).

We detected 64 mCNVs in 59 individuals (35 gains, 24 losses and five CNN-LOH in 0.34% of SSC and SPARK samples; Table 1 and Supplementary Table 1) ranging in cell fraction—ie, proportion of cells harboring a mosaic event—from 2.8% to 73.8% (median = 27.1%) and in size from 49.3 kb to 249.2 Mb (median = 2.5 Mb) (Fig. 1a). All but one carrier was younger than 28 years (oldest: 47 years; median: 12 years). Of the 64 detected mCNVs, 45 events were present in 44 unique probands (0.36%), and 19 events were present in 15 unique siblings (0.27%), with one sibling carrying five events on a single chromosome, reminiscent of chromothripsis (Supplementary Fig. 4 and Supplementary Note 1). Consistent with our filtering of age-related clonal hematopoiesis events, we did not observe a significant increase in mCNV detection rate with increasing age in SPARK samples (Supplementary Fig. 1b; individual age information was not available for SSC samples). We also did not observe a bias in the parental haplotype on which mCNVs were located (Supplementary Table 1, Supplementary Fig. 5 and Methods).

Due to the higher genotyping density in SSC, we had slightly greater power to detect short events in this cohort. To ensure that results were not driven by this sensitivity difference, we recalled events in SSC after randomly subsampling genotyped variants to the density of the SPARK arrays. We found that mCNV discovery was robust to genotype density, with perfect recall for mCNVs >1 Mb in size (Supplementary Fig. 6, Supplementary Table 2 and Supplementary Note 2).

ASD probands carry a burden of large mCNVs. We investigated whether mCNVs in probands had properties distinguishing them from mCNVs in siblings. The size distribution of mCNVs was markedly different between the two groups (Fig. 1a and Supplementary Fig. 7a): probands carried mCNVs that were an order of magnitude longer, on average, than those in siblings (median length = 7.8 Mb versus 0.59 Mb, $P = 1.6 \times 10^{-3}$, Mann–Whitney U-test; Fig. 1a,b), a trend apparent at the cohort level, consistent across copy number

states and robust to genotyping density and the exclusion of CNN-LOH events (Fig. 1b, Supplementary Figs. 7b and 8 and Supplementary Note 3). We did not observe a significant difference between mosaic cell fractions of mCNVs in probands and siblings (Supplementary Fig. 9), although this might reflect our limited power to detect mCNVs present in small proportions of cells (Supplementary Note 4 and Supplementary Fig. 10)

In both cohorts, we observed a significant burden in probands of mCNVs >4 Mb ($P = 0.043$ in SSC and $P = 6.6 \times 10^{-3}$ in SPARK, one-sided Fisher's exact test; Fig. 1c and Supplementary Fig. 7c), a conclusion further strengthened by meta-analysis of the two cohorts (Liptak's combined $P = 1.2 \times 10^{-3}$). We, thus, pooled events from both cohorts to maximize our statistical power[26].

Of mCNVs >4 Mb long, 25 were carried by probands, and only one was found in a sibling. This significant burden in probands of mCNVs >4 Mb (odds ratio = 11.4, 95% confidence interval (CI) = 1.5–84.2, one-sided Fisher's exact test, $P = 7.4 \times 10^{-4}$) was robust to the exclusion of CNN-LOH events ($P = 4.0 \times 10^{-3}$); robust to the exclusion of carriers >20 years old ($P = 1.7 \times 10^{-3}$); unaffected by sensitivity differences to small CNVs between SSC and SPARK (Supplementary Fig. 7c); and robust to the choice of the 4-Mb length threshold ($P = 1.9 \times 10^{-3}$ after multiple hypothesis correction to adjust for considering all possible thresholds; Methods). The burden was technically significant for smaller choices of threshold as well (eg, events >1 Mb and >2 Mb, $P = 0.018$ and $P = 0.013$, respectively; Fig. 1d, Supplementary Fig. 7d and Supplementary Fig. 11). However, these results were driven almost exclusively by events >4 Mb in size (Supplementary Note 5). These results imply an excess of large mCNVs in ~0.2% of ASD cases (95% CI = 0.08–0.29%; Methods). Coupled with the observation that such CNVs appear to be extremely rare in unaffected individuals, this finding suggests that large mCNVs contribute substantial ASD risk to a small number of carriers.

We wondered whether some mCNVs <4 Mb in probands might contribute to ASD by altering dosages of specific genes previously implicated in autism susceptibility ('ASD genes'). We analyzed overlap of mCNVs with a curated set of 222 high-confidence ASD genes from the SFARI Gene database (Methods). Smaller (<4-Mb) mCNVs in probands overlapped ASD genes more often than expected by chance (Expected = 1.42, Observed = 4; $P = 0.044$), in contrast to smaller mCNVs in unaffected siblings (Expected = 1.69, Observed = 1; $P = 0.84$), suggesting that some smaller mCNVs might also contribute to the etiology of ASD. (This analysis was uninformative for large mCNVs, most of which are expected to overlap at least one ASD gene by chance.)

When possible, we verified that probands carrying an mCNV did not carry other high-risk germline genetic mutations. Of 15 SSC probands with mCNVs, four also carried previously reported dnCNVs[6]; only one was >1 Mb in size; and none overlapped ASD genes. One proband with an mCNV also carried a previously reported de novo loss-of-function variant in *AFM*[27], a gene with no known connection to ASD (Supplementary Table 3). Compared to other
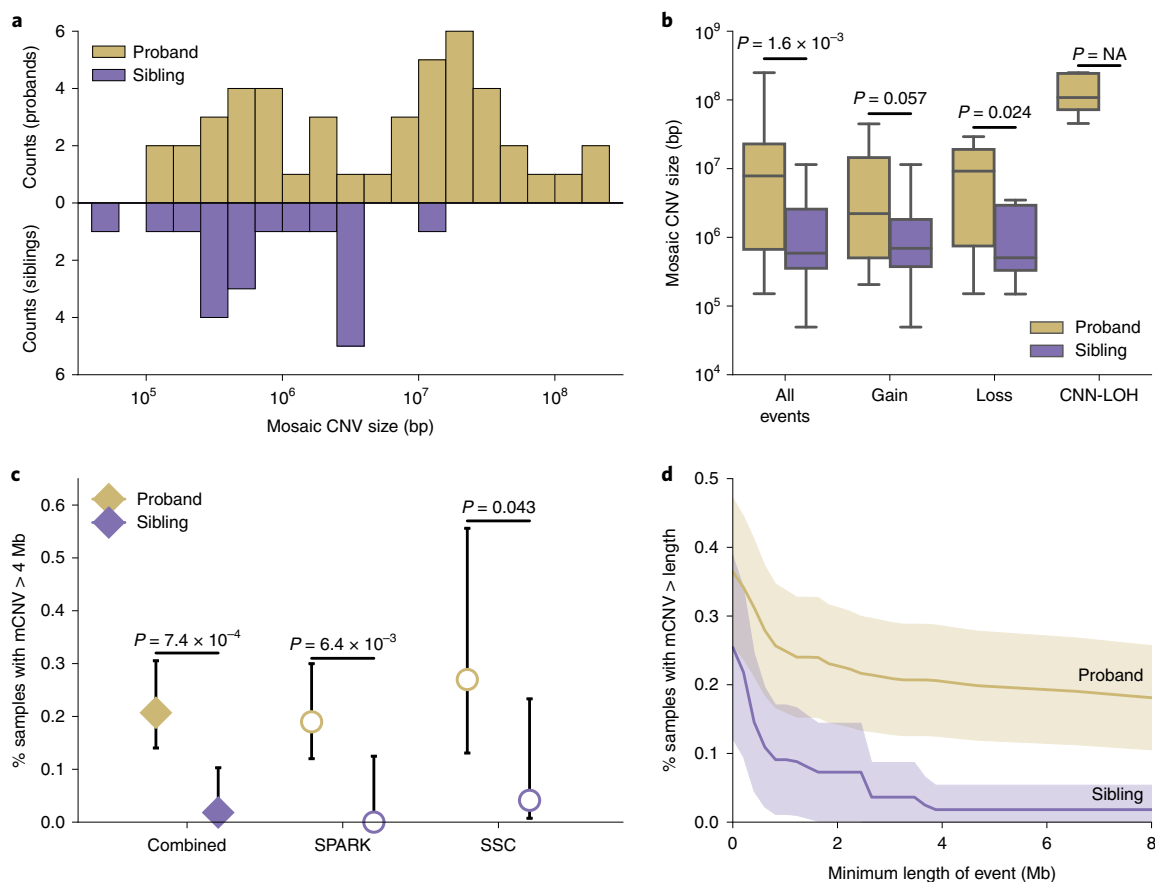
Fig. 1 | ASD probands carry a burden of large mCNVs. a, Histogram of mCNV sizes in probands (gold) and siblings (purple). **b**, Box-and-whisker plots of mCNV sizes in probands versus siblings across all events and stratified by copy number state (gain, loss or CNN-LOH); see Methods for box plot definitions. *P* values, one-sided Mann–Whitney U-test. No CNN-LOH events were detected in siblings. **c**, Percent of probands and siblings carrying an mCNV >4 Mb in size combined across cohorts (filled diamonds) and stratified by cohort (unfilled circles); data presented are rate ± 95% CI (Wilson score interval). **d**, Percent of probands and siblings carrying an mCNV of length at least *L*, with *L* varying from 0 Mb to 8 Mb; mean (solid lines) ± approximate 95% CI (shaded regions). The burden is robust to the choice of size threshold (Supplementary Fig. 11 and Supplementary Note 5). NA, not applicable.

probands in SSC, this group also did not carry excess risk from common variants significantly associated with ASD[28] ($P = 0.46$, Mann–Whitney U-test; Methods), although our power was limited. (We were unable to perform an equivalent analysis for SPARK probands because curated sets of de novo germline CNVs and loss-of-function variants are not yet available for this cohort). These results indicate that mCNVs comprise orthogonal genetic aberrations that independently contribute ASD risk.

Differences between germline and mosaic CNVs. Interestingly, mCNVs in probands had characteristics different from germline dnCNVs previously reported in SSC probands. mCNVs were significantly larger than dnCNVs (median length = 7.8 Mb versus 0.92 Mb, $P = 7.3 \times 10^{-5}$; Fig. 2a; we limited this comparison to dnCNVs >100 kb, the approximate detection threshold of our mCNV identification algorithm). This trend was consistent when mCNVs were compared to dnCNVs previously reported in the Autism Genome Project[29], and putative dnCNVs we identified in SPARK (Supplementary Fig. 12 and Supplementary Note 6). Moreover, mCNVs did not exhibit focal recurrence in any genomic location, although we did observe three events with breakpoints near *NTNG1* (encoding netrin G1), in which rare mutations have been identified in individuals with ASD[30] (Supplementary Fig. 13 and Supplementary Note 7). Moreover, mosaic versions of ASD-associated dnCNVs that have been recurrently observed in ASD probands[6] (ASD-dnCNVs;

eg, 16p11.2 deletion/duplication and 22q11.2 deletion/duplication) were notably absent from ASD probands compared to rates of ASD-dnCNVs (0 of 40 mosaic events versus 55 of 132 dnCNVs, as reported in Table 1 in Sanders et al.[6]) ($P = 4.2 \times 10^{-6}$, one-sided Fisher's exact test) (Fig. 2b and Supplementary Note 8).

We hypothesized that such mosaic analogues of ASD-dnCNVs 1) might be very rare or 2) might confer little or no ASD risk. To obtain further insight into both questions, we examined mosaic events previously detected in a population sample of 454,993 individuals of European ancestry in the UK Biobank[22]. Mosaic analogues of ASD-dnCNVs occurred much more rarely than their germline counterparts (Fig. 2b and Supplementary Table 4); of eight previously reported ASD-dnCNVs[6], only 16p11.2 deletions were detected recurrently in the mosaic state (in 73 UK Biobank samples comprising 0.016% of the cohort; Supplementary Note 9). Mosaic status was not associated with mental health conditions (Supplementary Table 5), although our power was very limited by the sparsity of reported mental health diagnoses.

To better understand the phenotypic relationship between germline ASD-dnCNVs and mosaic analogues, we identified carriers of germline 16p11.2 deletions in the UK Biobank (Supplementary Fig. 14 and Methods) and compared their phenotypes to those of mosaic 16p11.2 deletion carriers. Although we were underpowered to directly measure ASD risk conferred by 16p11.2 deletions, we could compare the effects of germline and mosaic 16p11.2
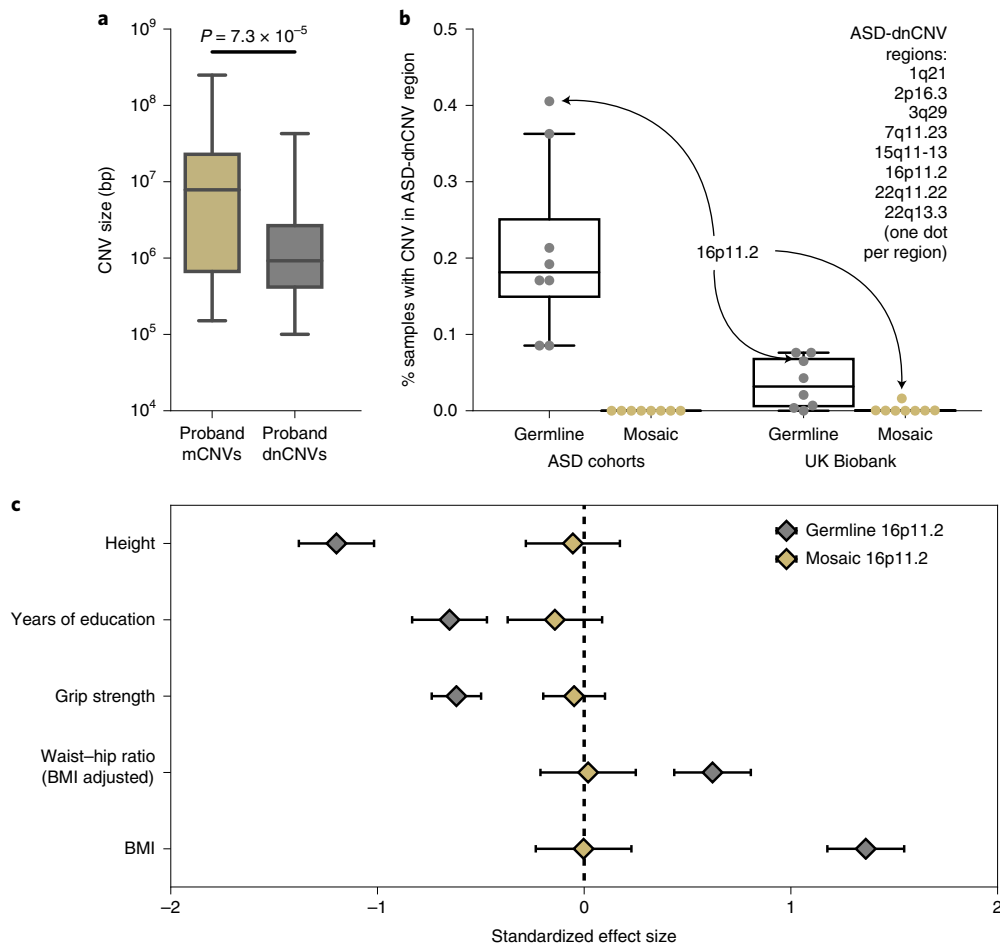
Fig. 2 | Mosaic and germline CNVs have different properties and effects. a, Sizes of mCNVs compared to sizes of dnCNVs identified by ref. [6] in SSC probands. dnCNVs <100 kb in size were removed to account for our limited sensitivity to detect mCNVs <100 kb in size; P value from one-sided Mann–Whitney U-test. **b**, Percent of samples carrying a germline or mosaic CNV (gain or loss) in each of eight ASD-dnCNV regions in ASD cohorts (SSC + Autism Genome Project for germline; SSC + SPARK for mosaic) or the UK Biobank. Each marker indicates the percent of carriers of a specific ASD-dnCNV; markers corresponding to *16p11.2* CNVs are indicated with callouts. **c**, Effects of germline ($n=111$) and mosaic ($n=71$) 16p11.2 deletions on phenotypes previously associated with 16p11.2 deletions (units, s.d.). Phenotypes were missing for some samples. See Supplementary Table 6 for exact sample sizes for each association. See Methods for box plot definitions. BMI, body mass index.

deletions on quantitative traits measured in the UK Biobank. Consistent with previous reports[31–33], germline 16p11.2 deletions were strongly associated with several traits, including fewer years of education, increased body mass index and decreased height. However, mosaic 16p11.2 deletions were not associated with any of these traits (Fig. 2c) even when restricting to events at high cell fractions (Supplementary Table 6). These data reinforce our observation that the burden of mCNVs in ASD probands was driven by large mCNVs that disrupted large swaths of the genome; smaller mCNVs might generally have limited phenotypic consequences, even when disrupting ASD-associated regions.

mCNV length associates with ASD phenotype severity. We next determined whether properties of mCNVs carried by probands were associated with ASD severity in these probands. ASD phenotypes were assessed with three measures common to both the SSC and SPARK cohorts, of which one measure—the Social Communication Questionnaire (SCQ)—was available for most proband mCNV carriers in both cohorts (13 of 17 SSC carriers and 20 of 29 SPARK carriers; Supplementary Table 1). The SCQ is a standardized evaluation form completed by a parent who rates an individual's symptomatic severity throughout his or her developmental history; higher scores

reflect a more severe ASD phenotype. Larger mCNV size significantly correlated with increased ASD severity as quantified by SCQ score (Fig. 3; Pearson correlation $r=0.43$, $P=0.016$). The longest mCNVs were CNN-LOH events; such events can both modify gene expression within imprinted regions and convert heterozygous gene-disrupting variants to the homozygous state (Supplementary Table 7 and Supplementary Note 10). These results further highlight the important role of size when considering the potential pathogenicity of a mosaic event: larger mCNVs appear to be more likely to both result in ASD and produce more severe phenotypes. We did not observe an association between mCNV cell fraction and phenotypic severity (Fig. 3 and Supplementary Fig. 15).

Identification of a complex mCNV in brain tissue. Although mCNVs are uncommon, they have been previously identified in subsets of single neurons in both normal and diseased brain tissue[34,35]. Their presence in a subset of cells presents the opportunity to identify essential cell types for a phenotype; thus, we sought to computationally identify and experimentally validate mCNVs directly in brain tissue, although we reasoned that the mCNVs we ascertained from blood- and saliva-derived DNA were likely present throughout the body given their moderate-to-high cell fractions[36]
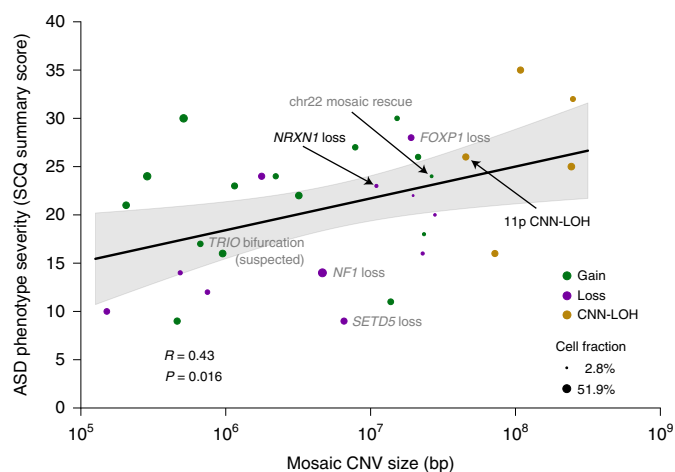
Fig. 3 | Mosaic CNV size positively correlates with ASD severity. ASD severity (quantified by the SCQ summary score) versus mCNV size (*n* = 31 probands with reported SCQ score). For probands with more than one mCNV, the longest event size is used. Marker color indicates mosaic copy number state; marker size indicates mosaic cell fraction. Events discussed in the main text are labeled with black text; events discussed in Supplementary Notes are labeled with gray text. *r*, Pearson correlation coefficient. Data are presented as regression mean (solid line) ± 95% CI (shaded region). The association was robust to the scale used for CNV size (Spearman rank correlation R_s = 0.42, *P* = 0.019).

and the young ages of carriers. We performed WGS of postmortem brain tissue from an additional 60 probands obtained through the National Institutes of Health Neurobiobank and Autism BrainNet (Supplementary Table 8). We genotyped germline variants using GATK HaplotypeCaller best practices[37] and identified mCNVs using MoChA (Methods).

We found two mosaic events (Supplementary Table 9): a mosaic 10.3-Mb gain of 2pcen-2q11.2 in sample AN09412 (Fig. 4a) and a mosaic loss of Y in ABN_XVTN. We also discovered nine germline CNVs overlapping ASD genes in other individuals, revealing potential causes of disease in several previously unresolved cases (Supplementary Table 10, Supplementary Fig. 16 and Supplementary Note 11).

The gain event on chromosome 2 in AN09412 was unique in that it appeared to exhibit three segments with varying degrees of mosaicism (Fig. 4a). Using phased allele fractions of germline heterozygous single-nucleotide polymorphisms (SNPs) and depth of coverage of sequencing reads, we estimated that the three segments were present in a ratio of 1:3:2 (Fig. 4b). Breakpoint analysis using split reads and discordantly mapped reads revealed three breakpoints (Supplementary Table 11): a tail-to-tail (T2T) inversion of 92.03–99.78 Mb, a tandem duplication (TD) of 99.87–101.94 Mb and a head-to-head (H2H) inversion located at 102.38 Mb, each of which corresponded to one of the three segments. Using this information, we reconstructed a parsimonious linear structure of the event (Fig. 4c and Methods) consistent with gain of a single complex rearrangement present in 26% of cells (Fig. 4b).

Using quantitative digital droplet polymerse chain reaction (ddPCR), we confirmed that the three breakpoints were present in both neurons and non-neurons at a 26–36% mosaic cell fraction (Fig. 4d), indicating that the mCNV arose in a fetal progenitor that gave rise to both neurons and glial cells. (Non-brain tissue was not available for this sample, so we could not investigate the presence of the CNV elsewhere.) We further confirmed, using single-cell ddPCR (Fig. 4d), that all three breakpoints occurred within individual neurons and, using gel electrophoresis, that none

of the breakpoints were present in DNA from a control brain (Supplementary Fig. 17), suggesting that the CNV arose from a single event, likely at a very early stage of development. Although the clinical significance of this complex mosaic CNV is uncertain, it disrupts the same region as multiple pathogenic events reported in the DECIPHER database that are associated with intellectual and developmental disability[38,39] (Supplementary Fig. 18).

We also validated the mosaic loss of Y in ABN_XVTN (Supplementary Fig. 17) and determined that the loss was limited to non-neuronal cell populations. This finding was unsurprising given that the ABN_XVTN donor was 74 years old (the oldest in the cohort), and age-related loss of Y has been reported extensively in blood[40] and, more recently, in aging brain tissue[41].

These results complement our analyses of mCNVs in large ASD cohorts, in which we analyzed DNA derived from blood and saliva under the assumption that mCNVs detected at moderate-to-high cell fractions were likely present throughout the body. Our validation of an mCNV in post-mitotic neurons of AN09412 indicates that mCNVs can arise during early development and propagate to multiple cell lineages in the adult body.

Discussion

Here we demonstrate that large mCNVs contribute a modest but important component to ASD risk, at a rate about 20× lower than germline dnCNVs (~0.2% versus ~5% excess in probands), which are strongly associated with increased risk of ASD[4–6]. Whereas very large (>4-Mb) germline CNVs are rare in both affected and unaffected individuals[6,42], very large mCNVs accounted for a substantial proportion of mosaic chromosomal aberrations that we observed. Although the threshold of >4 Mb is larger than those generally used in clinical interpretation of germline CNVs[43], our power to assess a burden below this threshold was extremely limited (as we only observed five mCNVs of size 1–4 Mb in probands and four in siblings). We, thus, selected 4 Mb as the size threshold for our primary analyses.

Large mCNVs significantly increased ASD risk, and increasing mCNV size correlated with increasing ASD severity in affected individuals. In contrast, smaller, ASD-associated CNVs (such as 16p11.2 deletion) appeared to have limited phenotypic consequences in the mosaic state, suggesting that mosaic and germline CNVs might result in autism by fairly different mechanisms: the recurrent ASD CNVs (eg, 16p11.2 and 22q11.2) appear to be required in most cells to create disability, whereas the mosaic events are typically larger and, hence, likely more toxic but limited to a fraction of cells. We hypothesize that these events are not observed as germline ASD events because large mCNVs are more survivable than very large germline CNVs, which commonly cause spontaneous miscarriage[44].

Assessing the clinical significance of the identified mCNVs was challenging not only because of their large size and lack of analogous germline CNVs but also because of the phenotypic heterogeneity of ASD[45] and the limited phenotype data provided for each proband. Nonetheless, we observed several mCNVs with possible connections to the individual's phenotype (Supplementary Figs. 19–22 and Supplementary Notes 12–14). These included 1) an individual with a mosaic 18q distal deletion who had no verbal communication at 47 years of age, which is a common feature of germline 18q distal deletions[46]; 2) a proband with a germline–mosaic compound heterozygous knockout of *NRXN1*: the proband carried a mosaic *NRXN1* deletion on the paternal haplotype and an inherited rare start-lost germline variant on the maternal allele; and 3) a proband with an acquired paternal uniparental disomy (UPD) of 11p and reported growth delays reminiscent of germline disruption of the *11p15.5* imprinted region. These anecdotes hint at possible molecular mechanisms and clinical consequences of mCNVs, which are likely to be even more complex and heterogeneous. For example, we discovered an apparent partial mosaic rescue in which a mosaic duplication
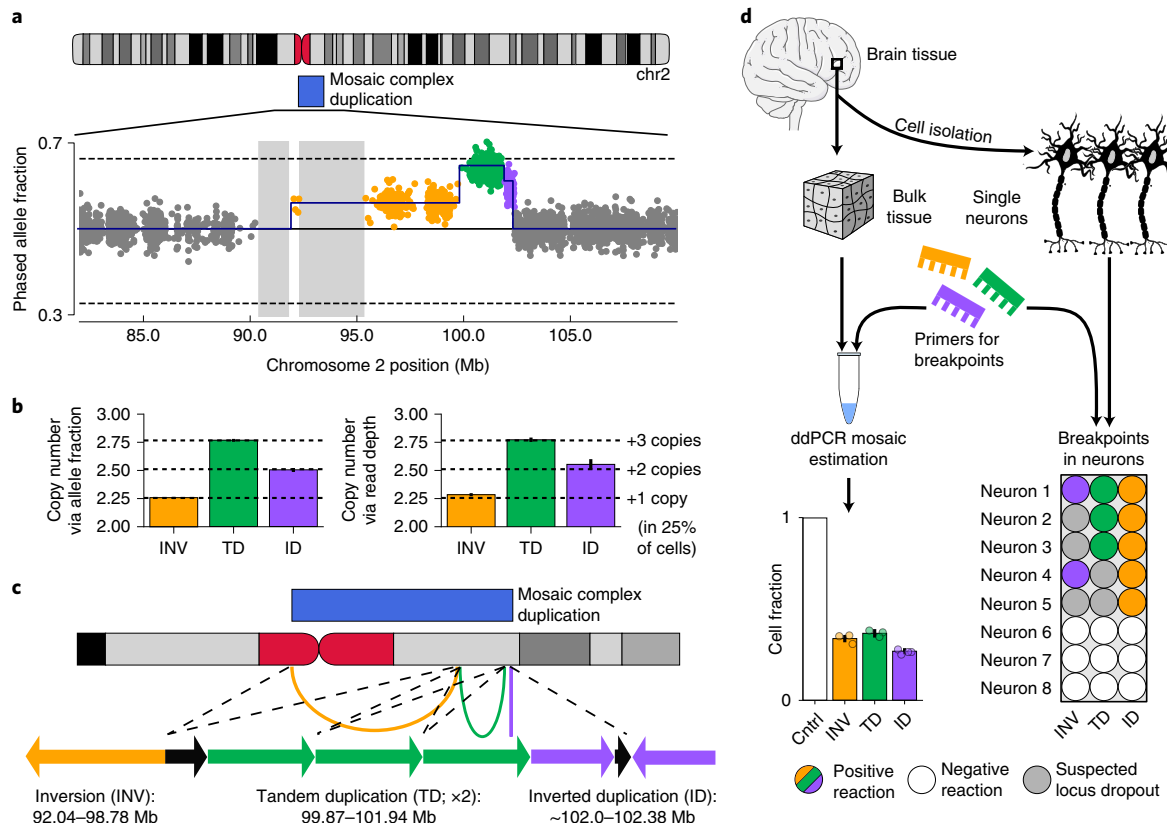
Fig. 4 | A complex mosaic chromosomal rearrangement present in neurons. a, Phased allele fraction at heterozygous SNPs on chromosome 2, binned into groups of four adjacent SNPs. SNPs within the mCNV are highlighted, with distinct copy number states indicated in different colors. Assembly gaps >1 Mb are shaded. **b**, Estimated mean copy number in each mCNV region as inferred from phased allele fractions (left) and sequencing read depths (right) at heterozygous SNPs; mean ± 95% CI (n INV = 876, n TD = 1,170, n ID = 375). CIs on allele fraction-based estimates are very narrow. **c**, Inferred structure of a complex duplication consistent with the observed data. Arcs on the ideogram indicate fusions supported by breakpoint analysis. Arrows are a schematic reconstruction of the event (not to scale); each arrow points in the 3′ direction relative to the GRCh37 reference genome. Black arrows indicate genomic regions with a single copy in the proper orientation within the duplicated region. The left breakpoint of the inverted duplication is approximate. **d**, Experimental validation of the three breakpoints, labeled according to their corresponding segment (INV, inversion; TD, tandem duplication; ID, inverted duplication). Left: fractions of cells containing each breakpoint estimated using ddPCR on DNA extracted from bulk brain tissue; mean ± approximate 95% CI (n of experimental replicates: INV = 3, TD = 3, ID = 4; replicates are shown as individual points). Right: validation of co-occurrence of breakpoints in single neurons. Observation of some, but not all, breakpoints in some neurons is probably explained by locus dropout, which is a common feature of single-cell whole-genome amplification[50].

appeared to revert an 8-Mb de novo germline deletion of distal 22q. We also observed mosaic UPD and CNN-LOH of chromosome 1 and 2 (two events on each chromosome), each of which converted heterozygous gene-disrupting variants to the homozygous state, but their clinical relevance was of unknown significance.

Although our results provide strong evidence that large mCNVs confer ASD risk, our study does have limitations that suggest avenues for future exploration. The modest number of mCNVs that we detected precluded investigating properties of mCNVs such as burdens at smaller length scales (eg, 1–4 Mb), recurrence patterns, effects of mosaic cell fraction on phenotype and genetic or environmental factors that predispose an individual to mosaic copy number variation. These factors limited our ability to precisely estimate the ASD risk that mCNVs confer. As deeply phenotyped ASD case–control cohorts continue to expand, we think that these questions will become answerable, and risk estimates will be further refined.

Moreover, our analysis of mosaic analogues of ASD-associated dnCNVs in the UK Biobank provides useful, although incomplete, insight into the phenotypic consequences of mCNVs. As a population-level resource, the UK Biobank has some ascertainment bias for healthy individuals[47], and, thus, affected carriers might be

underrepresented. We think that this is unlikely to strongly bias our results because carriers of large-effect variants are not fully excluded, as verified by the presence of 121 carriers of 16p11.2 germline deletions with the expected phenotypes (eg, mean height reduced by 1.2 s.d.). In addition, the cell fraction of a mosaic event is likely associated with phenotypic outcome, although the nature of this relationship remains an open question. Although we did not observe significant effect sizes when restricting to carriers of high-cell-fraction 16p11.2 mosaic deletions, our statistical power was limited by the small number of carriers (n = 35). Indeed, distinguishing between germline CNVs and very-high-cell-fraction mCNVs is extremely difficult, and it is likely that germline analyses have inadvertently included some high-cell-fraction mCNVs and that our analysis might have inadvertently excluded some of these events.

Additionally, although we demonstrated the existence of mCNVs in a small set of postmortem brain tissue samples, our primary analyses relied on mCNVs computationally ascertained from blood and saliva genotyping available in large cohorts. We think that most of these mCNVs represent true early-developmental mutations present across tissues (based on high cell fractions, young ages of

participants and conservative filters to exclude clonal hematopoiesis events), but caution is nonetheless warranted in interpreting our results and similar analyses of peripheral tissues. As efforts to directly assay the genome of the brain expand[48,49], we expect the risk contribution and molecular mechanisms of mCNVs to be further refined for both ASD and other neurodevelopmental disorders.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-020-00766-5.

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Methods

Genotyping intensity data. Genotyping intensity data for probands, siblings and parents in SSC and SPARK were obtained from SFARI Base. For each genotyped position, the data included the genotype call, the B allele frequency (BAF; proportion of B allele) and log R ratio (LRR; total genotyping intensity of A and B alleles) as provided by SSC and SPARK. Further information is available in the Life Sciences Reporting Summary.

Three types of genotyping arrays were used for SSC samples: Illumina 1Mv1 ($n = 1,354$ individuals), Illumina 1Mv3 ($n = 4,626$ individuals) and Illumina Omni2.5 ($n = 4,240$ individuals). Details of data generation were previously described in Sanders et al.[6]. SPARK samples ($n = 27,376$ individuals) were genotyped on the Illumina Infinium Global Screening Array-24 v.1.0. Details were previously described in Feliciano et al.[19,51]. We did not analyze those SPARK samples that were previously genotyped on a different array as part of a pilot study ($n = 1,361$ individuals).

We defined probands to be individuals with a diagnosis of ASD. We defined 'unaffected siblings' as family members without an ASD diagnosis in the same generation as a proband (most of which were siblings). We defined parents as unaffected individuals with a proband as a biological child.

Converting Illumina Final Reports to BCF format. Genotyping intensity data for SSC were distributed in the Illumina Final Report format, with genotyped positions reported with respect to the hg18 human reference genome. Positions were lifted over to hg19 coordinates based on rsID number. Positions without an rsID were discarded. Final Reports were converted to the BCF format, and genotypes were converted from Illumina TOP-BOT format to dbSNP REF-ALT format using custom in-house scripts (positions for which TOP-BOT format could not be unambiguously converted to REF-ALT format were discarded). Samples from each of the three arrays were processed as separate batches.

Genotyping intensity data for SPARK were converted from PLINK PED format to BCF format using the recode option in plink1.9. Genotypes were converted from Illumina TOP-BOT format to dbSNP REF-ALT format using a modified version of the bcftools plugin fixref (URLs). Only single-nucleotide variants were retained for analysis.

LRR de-noising for SPARK samples. We observed genome-wide spatial autocorrelation 'wave' patterns[52] in many SPARK samples. Because the wave pattern was consistent across samples for each chromosome, we corrected the bias using the following algorithm based on principal components analysis (PCA):

1. Determine the mean LRR per chromosome per sample. For each sample, mean shift the LRR signal genome wide by the median of chromosome means for that sample.

2. For chromosome i:

a. Determine the cohort-wide LRR deviation for the chromosome i as the median of mean chromosome i LRR signal across samples. Mean shift each sample's chromosome i LRR signal by the cohort-wide LRR deviation.

i. To prevent confounding due to sex, this correction is performed independently for males and females.

3. For each chromosome i:

a. Project the LRR matrix (number of samples by number of genotyped positions on chromosome i) onto the space spanned by its top k principal components. Subtract the projected matrix from the full LRR matrix.

Steps 1 and 2 of the algorithm mean center the LRR signal genome wide across an individual and per chromosome across the cohort. This is necessary to prevent PCA from projecting away mean shifts due to large mCNVs. Step 3 removes the variance explained by the top k principal components. In practice, we found that $k = 10$ effectively removed the wave pattern (Supplementary Fig. 23).

PCA analysis was performed using the PCA method from the Python package sklearn[53], which implements efficient PCA using randomized singular-value decomposition. LRR values were extracted from BCF files using 'bcftools query', and corrected values were incorporated into BCF files using 'bcftools annotate'. One sample with >5% genotype missingness was excluded from the correction procedure. On average across autosomes, the top ten principal components explained 57.1% of LRR variance in the SPARK cohort.

Variant-level quality control. We excluded genotyped variants with high levels of genotype missingness (>2%), evidence of excess heterozygosity ($P < 1 \times 10^{-6}$, one-sided Hardy–Weinberg equilibrium test) and unexpected genotype correlation with sex ($P < 1 \times 10^{-6}$, Fisher's exact test comparing number of 0/0 genotypes versus number of 1/1 genotypes in males and females). We also excluded genotyped variants falling within segmental duplications with low divergence (<2%). Variant-level quality control was performed for each array independently. The number of genotyped variants and number of variants excluded by quality control are listed in Supplementary Table 12.

Sample-level quality control. We calculated two statistics to detect sample contamination: BAF concordance and BAF autocorrelation. Given that a heterozygous SNP has a BAF > 0.5 (<0.5), BAF concordance is the probability that the following heterozygous SNP has BAF >0.5 (<0.5). BAF autocorrelation is the correlation of the BAF at a heterozygous SNP with the BAF at the neighboring (downstream) heterozygous SNP. For each sample, we calculated the statistic for each chromosome independently and took the median across all chromosomes as the sample value.

Neighboring positions with heterozygous genotypes in the genome are expected to have uncorrelated genotype intensity measures on an array. BAF concordance and BAF autocorrelation significantly higher than, respectively, 0.5 and 0 could reflect sample contamination with DNA from another individual, because allelic intensities will be correlated at variants within haplotypes shared between the sample DNA and contaminating DNA. In SSC, we removed samples with a BAF concordance >0.51 or a BAF autocorrelation >0.03, resulting in the exclusion of 11 probands and nine siblings. We also excluded an additional proband (array ID: 7306256088_R02C01) with evidence of a large amplitude LRR wave pattern. In total, 2,594 probands and 2,424 siblings from SSC passed quality control (Supplementary Table 13).

In SPARK, we observed genome-wide evidence of BAF correlation between contiguous genotyped positions in high-quality samples. Thus, BAF concordance and BAF autocorrelation were not informative measures of contamination. Instead, we excluded samples with evidence of multiple very-low-cell-fraction CNN-LOH events (<10% of cells and LRR deviation from zero <0.2) because the probability of observing two or more true CNN-LOH events in a sample was exceedingly small given the young age of the individuals. We further removed any samples from individuals who had also participated in SSC ($n = 352$) and one additional proband (SP0072755) that had an uncorrected LRR wave pattern after LRR de-noising, resulting in exclusion of 622 probands and 54 siblings. Finally, we removed 37 siblings with a reported genetic diagnosis (of which one carried an mCNV; see main text). In total, 9,483 probands and 3,076 siblings from SPARK passed quality control (Supplementary Table 13).

Haplotype phasing. We used Eagle2 (ref. [20]) (default settings) and the Haplotype Reference Consortium[54] phasing panel to perform statistical haplotype phasing of SSC samples. We performed phasing for each genotyping array independently. For probands and siblings, we additionally used parental genotypes to correct phase-switch errors using the bcftools plugin trio-phase included with MoChA. Given the size of the SPARK cohort (>27,000 samples), we performed within-cohort statistical phasing using Eagle2. We additionally corrected proband and sibling phase estimates using parental genotyping data when available (at least one parent was also genotyped for the vast majority of probands and siblings). The combination of statistical haplotype phasing and pedigree-based phasing resulted in near-perfect long-range phase information without phase-switch errors.

Discovery of mCNVs. We applied MoChA to each genotyping array batch independently to detect mCNVs. The general statistical approach implemented in MoChA was previously described[21]. In brief, mCNVs result in allelic imbalance between the maternal and paternal haplotypes. Thus, the BAF of heterozygous SNPs within an mCNV will consistently deviate from the expected value of 0.5 toward either the paternal allele or the maternal allele. Such deviations can be sensitively detected even at low cell fractions using long-range phase information, provided that the event is long enough to contain multiple genotyped heterozygous SNPs. Formally, MoChA uses a hidden Markov model (HMM) to search for consistent deviations. Gains (losses) also result in an increase (decrease) of total LRR signal with magnitude proportional to the cell fraction of the event; an HMM can also be used to detect LRR deviations from zero. Incorporation of phase information particularly increases sensitivity to detect large, low-cell-fraction CNVs relative to previous models[21].

The details of MoChA differ from the previously described approach in two ways. First, MoChA uses two independent models to search for mCNVs: a haplotype phase model (BAF + phase) as described in Loh et al.[21] and an LRR and (unphased) BAF model (LRR + BAF) similar to previous models for the detection of germline CNVs[55]. A CNV is reported if it is discovered by either model. The introduction of the LRR + BAF model enables detection of germline (or very-high-cell-fraction mosaic) losses and germline duplications including more than two haplotypes. Second, MoChA uses the Viterbi algorithm to search for deviations in either the phased BAF signal or the LRR signal instead of computing total likelihoods and applying a likelihood ratio test. The Viterbi algorithm is more direct, but its calibration is less precise when detecting very-low-cell-fraction events. However, because we were interested in higher cell fraction mCNVs arising during early embryogenesis, such sensitivity was not necessary for this study.

Central to the sensitivity of MoChA is the quality of the long-range phase information. As discussed above, the combination of statistical haplotype phasing and pedigree phasing using parental genotypes resulted in near-perfect long-range phase information without phase-switch errors.

Classification of mosaic copy number state. We needed to sensitively distinguish age-related and early-developmental mCNVs in a way that was robust to LRR signal noise due to, for example, guanine–cytosine (GC) content. Previous work on mCNVs did not typically distinguish between age-related and early-developmental

events. Thus, we developed a new statistical method to classify events as gains, losses, CNN-LOH or unknown using an expectation–maximization (EM) algorithm similar to k-means clustering where each cluster is defined by a line instead of a centroid. Let $X = |\Delta BAF|$ be the absolute deviation from 0.5 of phased BAF estimated across an event; let $Y = |\Delta LRR|$ be the absolute deviation from zero for LRR estimated across an event; and let $C \in$ {Gain, Loss, CNN-LOH} denote the copy number state of the mosaic mutation. Then, for gains, X and Y will linearly increase according to $Y = X\beta_{Gain} + \epsilon$, where $\beta_{Gain} > 0$; for losses, Y will linearly decrease as X increases according to $Y = X\beta_{Loss} + \epsilon$, where $\beta_{Loss} < 0$; and for CNN-LOH, $Y = \epsilon$, where $\epsilon \sim N(0, \sigma^2)$ is Gaussian noise in the estimation of X and Y.

Given a set of events, the parameters of the linear models and the copy number state C_i for each event i are unknown. We, thus, iteratively apply the following custom EM algorithm:

1. Randomly initialize $\beta_{Gain} \in (0,3]$ and $\beta_{Loss} \in [-3,0)$ d set $\beta_{CNN-LOH} = 0$
2. Assign each event i a copy number state C_i using least-squares classification: $C_i = \text{argmax}_C \|Y_i - X_i\beta_c^2\|$.
3. Estimate the linear model parameters β_C for $C \in$ {Gain,Loss} using univariate linear regression without an intercept term applied to all events assigned to class C in Step 2:

$$\beta_c = \frac{\sum_{(i|C_i=C)} X_i Y_i}{\sum_{(i|C_i=C)} X_i^2}$$

   a. Because $\beta_{CNN-LOH} = 0$ is known, it is not re-estimated.

4. Repeat Steps 2 and 3 until convergence.
5. Estimate σ_{Gain} and σ_{Loss} using univariate linear regression on the events classified as gains and losses, respectively.

To classify mCNV copy number states in probands and siblings, the model was first trained on mCNVs in parents (after removal of germline CNVs). Events in probands and siblings were then classified using the linear model parameters estimated from the parents. The method implicitly accounts for errors in LRR and BAF measures and, thus, is robust to noise in these signals.

We applied an additional step to improve classification of events extending to telomeres, given that CNN-LOH events generally arise due to mitotic recombination and, therefore, terminate at a telomere. To ensure that apparent gains and losses terminating at a telomere were not misclassifications, we calculated the Bayes factor to compare the likelihood that the event arose under the Gain or Loss model against the likelihood under the CNN-LOH model:

$$B = \frac{\exp\left(-(Y - X\beta_C)^2/2\sigma_C^2\right)}{\exp\left(-Y^2/2\sigma_C^2\right)}$$

where $C \in$ {Gain,Loss} and σ_C is the s.d. estimated from fitting the model on parental data. If $B < 10$ for a putative gain or loss terminating at a telomere, the copy number state was reclassified as unknown.

Filtration of mCNV calls. *In probands and siblings.* Following Sanders et al.[5], we required all potential mCNVs to overlap at least 20 heterozygous SNP sites. We then excluded germline events and events likely to arise due to age-related clonal hematopoiesis. To remove germline events, we filtered all events designated as a 'copy number polymorphism' by MoChA. Given a panel of known CNV polymorphisms (1000 Genomes Project in this case), for each sample and each segment in the list of polymorphisms, MoChA checks for evidence of 1) germline copy number alteration within the segment and 2) diploid copy number in the regions on either side of the segment. A segment within a sample satisfying both conditions is classified as a copy number polymorphism.

We additionally excluded any event that reciprocally overlapped an event found in an individual's biological parents by >85% or reciprocally overlapped any CNV reported in the 1000 Genomes Project[42] by >75%. When calculating overlap, we accounted for copy number state: overlaps between gains and losses were not considered. Finally, we removed any event with an estimated cell fraction >1. For gains, we additionally removed any events with $|\Delta BAF| > 0.11$ to ensure that germline gains were not misclassified as mosaic, following previous work[21,23].

To filter mCNVs likely to have arisen due to clonal hematopoiesis, we excluded mCNVs contained within loci commonly altered within the immune system, specifically *IGH* (chr14:105,000,000–108,000,000) and *IGL* (chr22:22,000,000–24,000,000). We also excluded CNVs within the extended major histocompatibility complex region (chr6:19,000,000–40,000,000) due to the known propensity to call false-positive mosaic CNN-LOH events within this locus[21]. We also removed events whose copy number state could not be determined and, following Vattathil et al.[23], we classified and removed CNN-LOH events in less than 20% of cells (ie, $|\Delta BAF| < 0.1$) as likely clonal hematopoiesis. The filtration of low-cell-fraction CNN-LOH removed 73 calls in probands (34 in SSC and 39 in SPARK) and 48 calls in siblings (28 in SSC and 20 in SPARK). The rate of low-cell-fraction CNN-LOH (<1% in probands and siblings) is consistent with rates observed in individuals <45 years old in the UK Biobank[21]. We further excluded one CNN-LOH event in a proband >20 years old because his age (43 years) increased the probability that the event could have arisen due to clonal hematopoiesis.

In parents. We also called mCNVs in parents for the purpose of fitting the EM model (described above) that we subsequently used to infer copy number state of mCNVs in probands and siblings. Before fitting the EM model on events called in parents, we filtered events labeled as copy number polymorphisms by MoChA, reciprocally overlapping 1000 Genomes Project CNVs by >75%, reciprocally overlapping events in other adults by >80% or reciprocally overlapping events in non-biological children by >80%.

Determination of haplotype of origin. For mosaic gains and losses, the parental haplotype of origin was defined to be the haplotype carrying the mCNV. For CNN-LOH, the parental haplotype of origin was defined to be the haplotype that was duplicated. To assign haplotype of origin, we calculated the average ALT allele frequency of heterozygous SNPs at which the ALT allele was unambiguously inherited from the father and the average ALT allele frequency of heterozygous SNPs at which the ALT allele was unambiguously inherited from the mother. For losses, the haplotype of origin was paternal if the average allele fraction of paternal SNPs was less than that of maternal SNPs; otherwise, the haplotype of origin was maternal. For gains and CNN-LOH, the haplotype of origin was paternal if the average allele fraction of paternal SNPs was greater than that of maternal SNPs; otherwise, the haplotype of origin was maternal.

Burden analysis. The statistical significance of the hypothesis that probands carry more mCNVs >4Mb than their unaffected siblings was quantified using a one-sided Fisher's exact test. Using Wilson's score interval, 95% CIs for the percent of samples carrying an mCNV were calculated. To adjust the burden P value for multiple possible choices of the size threshold for defining 'large mCNVs', we performed the following permutation analysis: proband and sibling labels of mCNVs were randomly permuted based on the total number of probands and siblings in our study. We then determined the P value of the most significant burden across all size thresholds for the permutation. This procedure was repeated 100,000 times. We calculated the threshold-adjusted P value as

$$P_{adj} = \frac{\sum_i 1_{(P_i \geq P_{obs})}}{100,000}$$

where P_{obs} is the uncorrected P value from the observed data, P_i is the maximum burden P value from permutation i and 1 is the indicator function.

The excess burden of large (> 4-Mb) mCNVs in ASD probands was estimated as the difference between the percent of probands carrying a large mCNV and the percent of siblings carrying a large mCNV. The 95% CI between proportions was estimated using Wilson's score interval as modified by Newcombe[56].

Overlap of mCNVs with ASD genes. We downloaded all genes included in the SFARI Gene database of genes implicated in ASD. We restricted the list to the 222 genes that are classified as 'Category 1' (high confidence), 'Category 2' (strong candidate) or 'S' (syndromic). We refer to this restricted list of genes as 'ASD genes'. We determined whether mCNVs overlapped ASD genes by annotating their overlap with all genes in the RefSeq database and intersecting the name of the RefSeq genes with the ASD gene list.

To determine whether a set of mCNVs overlapped ASD genes more often than expected by chance, we randomly permuted the mCNVs in probands around the genome K times, excluding assembly gaps >1Mb in size in the hg19 reference. After each permutation, we determined the number of segments overlapping an ASD gene. Let N_{obs} be the number of mCNVs overlapping ASD genes in the observed data. Let N_i be the number of permuted segments overlapping ASD genes in permutation i. The P value of observing N_{obs} or more overlaps by chance is $P = \frac{\sum_i 1_{(N_i \geq N_{obs})}}{K}$, where 1 is the indicator function. When testing ASD gene overlap for short events (<4Mb), we used $K = 10,000$. For long events, we used $K = 1,000$ for computational efficiency. We excluded CNN-LOH events when testing long events because they were too large to be randomly permuted.

Risk from common ASD-associated variants. We obtained variant effect sizes for common variants significantly associated with ASD at the genome-wide level ($P < 5 \times 10^{-8}$) from Table 1 of Grove et al.[28], which is the largest ASD genome-wide association study published to date. We obtained genotypes for SSC samples from WGS, available for most of the cohort, and we calculated each individual's risk as a linear combination of genotypes weighted by variant effects. We excluded one variant (rs71190156) because it had >50% missingness across individuals, and we excluded any individual with missing genotypes for any other variant. In total, we examined risk from 11 variants in 2,310 probands and 1,868 siblings. Of these, ten probands and six siblings carried mCNVs, so our statistical power to compare between groups was very limited.

Counts of germline CNVs. Counts of germline ASD-associated CNVs in ASD cohorts were obtained from Table 2 of Sanders et al.[6], which included samples from SSC and the Autism Genome Project. Counts of germline ASD-associated CNVs in UK Biobank individuals were obtained from Crawford et al.[32].

Identification of 16p11.2 germline deletion carriers in the UK Biobank. We extracted LRR and genotype calls from the 16p11.2 ASD-associated region listed in

Table 2 of Sanders et al.[6] for individuals in the UK Biobank. Germline carriers of 16p11.2 deletions were defined as individuals with average LRR < −0.5 and <5 heterozygous SNP calls across the region (Supplementary Fig. 10).

Phenotype associations of germline and mCNVs in ASD-associated regions. We defined high-confidence ASD-associated CNV regions as those listed in Tables 1 and 2 of Sanders et al.[6] expanded by ~1.5 Mb on either side (Supplementary Table 4 lists the exact expanded regions). We identified carriers of mCNVs in the UK Biobank reported by Loh et al.[22] falling within the ASD regions. We refer to these individuals as ASD-dnCNV-analogue carriers. We used self-reported responses to the UK Biobank Mental Health Questionnaire to count the number of ASD-dnCNV-analogue carriers with a diagnosis of ASD, schizophrenia, bipolar affective disorder, depression or anxiety.

Following Owens et al.[31], we quantified the association between carrier status of germline or mosaic 16p11.2 deletions and phenotypes using the following linear regression model for continuous phenotypes:

$$y_i = x_{c,i}\beta_c + x_{age,i}\beta_{age} + x_{sex,i}\beta_{sex} + x_{array,i}\beta_{array} + \sum_{j=1}^{15} x_{PC_j,i}\beta_{PC_j} + \epsilon_i$$

where y_i is the phenotype of individual i; $x_{c,i}$ is the 16p11.2 CNV carrier status of individual i; $x_{age,i}$ is the age of individual i; $x_{sex,i}$ is the sex of individual i; $x_{array,i}$ is the array used to genotype individual i; $x_{PC_j,i}$ is the jth genetic principal component of individual i; βs are the corresponding effect sizes; and $\epsilon_i \sim N(0, \sigma^2)$ is the remaining phenotypic variance. For binary phenotypes, we applied logistic regression with the same covariates. Continuous phenotypes were inverse-normal transformed within sex strata after adjusting for relevant covariates before analysis[57]. We restricted to individuals passing quality control filters from ref.[22] and of self-reported European ancestry.

We identified a set of quantitative traits and medical outcomes previously associated with 16p11.2 germline deletions[31–33,58]. The association results for mosaic 16p11.2 deletions, high-cell-fraction mosaic 16p11.2 deletions (CF > 0.3) and germline 16p11.2 deletions for all tested traits are reported in Supplementary Table 5. Medical phenotypes were coded as binarized versions of the following data fields from the UK Biobank Data Showcase: renal failure: 132030, 132032 and 132034; obesity: 130792; and heart failure: 131354.

Determining carriers of high-risk germline de novo variants. Curated germline dnCNVs and loss-of-function variants in SSC individuals[6,27,59] were obtained from ref.[6]. We cross-referenced our list of mCNV carriers with carriers of dnCNVs and loss-of-function variants. For any mCNV carriers who also carried a dnCNV, we determined whether the dnCNV overlapped an ASD gene as described above. The list of high-confidence germline dnCNVs was also used to estimate the size distribution of dnCNVs in Fig. 2a. We removed dnCNVs <100 kb in size to account for our limited sensitivity to detect mCNVs below that size threshold.

Genotype–phenotype associations. We obtained phenotype data for individuals in SSC and SPARK from SFARI Base (SSC version 15 and SPARK version 2). Of the three ASD severity measures shared between SSC and SPARK (the Development Coordination Disorder Questionnaire, the Repetitive Behavior Scale-Revised and the SCQ), only the SCQ was missing in fewer than 50% of SSC and SPARK samples. We measured association between SCQ score and mCNV properties (size and cell fraction) using both Pearson and Spearman rank correlation. z normalizing SCQ scores independently in SSC and SPARK before association did not qualitatively change the results.

Identification of putative damaging variants within mCNVs in SPARK individuals. We obtained from SFARI Base exonic SNPs and indels detected in WGS data of SPARK individuals. In carriers of mosaic losses and CNN-LOH, we identified rare, putative damaging variants within the mCNV, defined as 1) variants with cohort variant allele frequency <1% and 2) annotated as 'High Impact' (start-lost, stop-lost, stop-gain, frameshift, splice-acceptor and splice-donor) or annotated as missense with Combined Annotation-Dependent Depletion >20 (ref. [60]) by Variant Effect Predictor[61].

Analysis of brain tissue. *Human tissue.* Postmortem human brain specimens were obtained from the Lieber Institute for Brain Development, the Oxford Brain Bank and the University of Maryland Brain and Tissue Bank through the National Institutes of Health Neurobiobank and from Autism BrainNet. All specimens were de-identified, and all research was approved by the institutional review board of Boston Children's Hospital.

DNA extraction and sequencing. DNA was extracted from prefrontal cortex where available (or generic cortex in a minority of cases) using lysis buffer from the QIAamp DNA Mini Kit (Qiagen) followed by phenol chloroform extraction and isopropanol clean-up. Samples UMB4334, UMB4899, UMB4999, UMB5027, UMB5115, UMB5176, UMB5297, UMB5302, UMB1638, UMB4671 and UMB797 were processed at the New York Genome Center using TruSeq Nano DNA library preparation (Illumina) followed by Illumina HiSeq X Ten sequencing to a minimum 200× depth. All remaining samples were processed at Macrogen using TruSeq DNA PCR-Free library preparation (Illumina) followed by minimum 30× sequencing of seven libraries on the Illumina HiSeq X Ten sequencer, for a total minimum coverage of 210× per sample. All paired-end FASTQ files were aligned using BWA-MEM version 0.7.8 to the GRCh37 reference genome, including the hs37d5 decoy sequence from the Broad Institute[62].

Structural variant validation. For germline events with known breakpoints, standard PCR was designed with primers spanning the breakpoint. For mosaic events with known breakpoints, custom Taqman assays (Thermo Fisher Scientific) were designed to span the breakpoint and subsequently used in ddPCR with RNAseP as a reference. For events without known breakpoints, pre-designed Taqman copy number assays for the region of interest were ordered and optimized with known positive and negative controls when possible. ddPCR was performed according to the manufacturer's instructions (Bio-Rad).

Single-cell sorting. Nuclear preparation and sorting were performed as previously described[63]. Single NeuN+ cells, as well as pools of 100 NeuN+ (neuronal) and NeuN− (non-neuronal) cells, were collected and amplified using GenomePlex DOP-PCR WGA according to a published protocol[64], and samples were purified using a QIAquick PCR Purification Kit (Qiagen) before ddPCR analysis. Locus dropout is a common feature of whole-genome amplification with GenomePlex DOP-PCR WGA.

Detection of mCNVs. mCNVs were detected using MoChA. When running on WGS data, MoChA explicitly models read counts of the ALT allele and the REF allele using a beta-binomial distribution, where the expected counts are a function of the total sequencing depth and the allele balance of the hidden state.

Mosaic copy number estimation. For each segment of the mosaic complex duplication, we estimated mosaic copy number from allelic sequencing read fractions using the following relationship. Let $|\Delta BAF|$ be the average absolute deviation from 0.5 of phased allele frequency estimated across a segment. Then, for a gain, the estimated mosaic cell fraction in the bulk sample is:

$$\widehat{CF} = \frac{2|\Delta BAF|}{0.5 - |\Delta BAF|}$$

This corresponds to a mosaic copy number of $2 + \widehat{CF}$ in a diploid genome.

Let $\overline{DP_s}$ be the average read depth (or LRR) at SNPs within a segment, and let $\overline{DP_G}$ be the average read depth (LRR) at SNPs genome wide. Then, the estimated average copy number in the bulk sample is:

$$\widetilde{CN} = \frac{\overline{DP_s}}{0.5 * \overline{DP_G}}.$$

When estimating the read depth-based copy number of the complex mosaic duplication, we estimated the genome-wide copy read depth using the average read depth across all SNP sites on chromosome 1. To account for read depth biases (eg, GC content), we inferred the segment's copy number in each of the other 59 postmortem brain samples. We then estimated the copy number bias as the average deviation from CN = 2 and subtracted this estimate from $\widetilde{CN}$ to get a corrected copy number estimate, $\widehat{CN}$. These are the values shown in Fig. 4b. Estimator variance is the sum of the estimated variance of $\widetilde{CN}$ and the estimated variance of the bias estimate.

Inferred structure of a complex duplication. We inferred a linear structure of the complex duplication consistent with the following observations: three segments with relative abundance of +1 copy, +3 copies and +2 copies; a T2T inversion fusing 92.04 Mb to 98.78 Mb; a TD of 99.87–101.94 Mb; and an H2H inversion fusing 102.382 Mb to 102.383 Mb. We first observed that each breakpoint corresponded to a segment with unique copy state: T2T inversion corresponded to a +1 copy state, TD to a +3 copy state and H2H to a +2 copy state. We, thus, concluded that the TD must result in an additional three copies of 99.87–101.94 Mb, and the H2H inversion is likely the result of an inverted duplication resulting in two copies of ~102.0–102.382 Mb separated by a 1-kb segment (102.382–102.383 Mb) in the proper orientation (where the left breakpoint at ~102.0 Mb is approximate because it is estimated based on discontinuity in allele fraction and read depth estimates rather than direct observation). We estimated via read depth that the segment 102.382–102.383 Mb is present in a +1 copy state. We further concluded that the duplication carries one copy of 92.04–98.78 in an inverted 3′–5′ orientation and one copy of 99.78–99.87 Mb in the proper 5′–3′ orientation.

Plotting mCNV events. mCNV events with ideograms and gene/region annotations were plotted using a modified version of pyGenomeTracks[65].

Description of box plots. All box plots have the following properties: center line is the median, box limits are upper and lower quartiles and whiskers are 1.5× interquartile range. Outliers are not included in Fig. 2a for clarity.

Statistical analysis. We did not predetermine sample size but, rather, obtained all samples currently available from SSC, SPARK and the UK Biobank; the resulting sample sizes were similar to or larger than those reported in previous publications[11,12,17,21,31–33]. Data were collected by SSC and SPARK without input from the authors. We did not perform randomization beyond that performed by SSC and SPARK during sample collection. Because data were received as curated by SSC and SPARK, we were not blinded to covariates included with the data. Burden and association analyses were performed as described above. Comparisons of CNV sizes were performed using Mann–Whitney U-tests. Data met the assumptions for all statistical tests.

Accession codes. Accession number for WGS data of postmortem brain from the National Institute of Mental Health Data Archive: 1503337.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data on individuals with ASD and their families were collected by the Simons Foundation as part of the Simons Simplex Collection and the Simons Powering Autism Research for Knowledge cohort. Mosaic event calls are available in the Supplementary Data. Genotype array data and phenotype information for the SSC and SPARK cohorts are available from SFARI Base (https://base.sfari.org) for approved researchers. Access to the UK Biobank Resource is available via application (http://www.ukbiobank.ac.uk/). Data from the DECIPHER database are available from https://decipher.sanger.ac.uk/. WGS data of postmortem brain tissue are available from the National Institute of Mental Health Data Archive under accession number 1503337. Source data are provided for gels shown in Supplementary Figs. 16c and 17a.

Code availability

MoChA and custom BCFtools plugins are available on Github via URLs listed below. Custom analysis scripts are available from the authors upon reasonable request.

URLs:

MOsaic CHromosomal Alterations (MoChA) caller: https://github.com/freeseek/mocha

BCFtools: https://samtools.github.io/bcftools/bcftools.html

Custom BCFtools plugins: https://github.com/freeseek/gtc2vcf

Eagle2 software: https://data.broadinstitute.org/alkesgroup/Eagle/

PLINK: https://www.cog-genomics.org/plink/1.9/

pyGenomeTracks: https://github.com/deeptools/pyGenomeTracks

1000 Genomes dataset: http://www.1000genomes.org/

Haplotype Reference Consortium: http://www.haplotype-reference-consortium.org/.

UK Biobank: http://www.ukbiobank.ac.uk/

SFARI Gene database: https://gene.sfari.org/

SFARI Base: https://base.sfari.org

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Acknowledgements

Author contributions

M.A.S., P.J.P., C.A.W. and P.-R.L. conceived and designed the study. M.A.S., G.G. and P.-R.L. designed and implemented the statistical methods. M.A.S. performed computational analyses. C.D. curated phenotype data. R.E.R. performed WGS and experimental validation in postmortem brain tissue. A.R.B., R.E.M. and B.B. provided comments and guidance throughout. All authors wrote and edited the manuscript.

Ethics statement

The first part of this study used existing and publicly available genomic datasets of families with ASD from the Simons Simplex Collection (SSC) and Simons Powering Autism Research for Knowledge (SPARK). Collection of SSC samples was approved and monitored by the institutional review board of Columbia University Medical Center. SPARK samples were collected under a centralized review board protocol (Western IRB Protocol no. 20151664). The second part of the study generated and analyzed genomic data on de-identified postmortem human specimens obtained from brain tissue banks, including the AutismBrainNet, the Lieber Institute for Brain Development, the Oxford Brain Bank and the University of Maryland Brain and Tissue Bank through the National Institutes of Health Neurobiobank. This study did not engage human subjects or collect their identifiable data; rather, the individual tissue banks have their own approval and consent process. Our study was approved by the institutional review board of Boston Children's Hospital.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41593-020-00766-5.

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Software and code

Policy information about availability of computer code

Data collection	All data pertaining to SSC and SPARK were obtained from SFARI base after obtaining approval. No software was used for this download. WGS data were generated by the New York Genome Center or Macrogen and delivered to us on secure hard-drives.
Data analysis	Data cleaning was performed using PLINK v1.9. Haplotype phasing was performed using Eagle2 v2.4. CNV analysis was performed using MoChA v2018-09-12, bcftools v1.9 and htslib v1.9. Post-hoc analysis and filtration were performed using custom scripts coded in python 3.5.2. CNV events were plotted using a customized version of pybedtools v3.0. Whole-genome sequencing data from the 60 post-mortem brain tissue samples were aligned using BWA-MEM v0.7.8. Digital droplet PCR was measured using QuantaSoft Analysis Pro v1.0. MoChA is available at https://github.com/freeseek/mocha Custom BCFtools plugins are available at https://github.com/freeseek/gtc2vcf

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Data on individuals with Autism Spectrum Disorder and their families were collected by the Simons Foundation as part of the Simons Simplex Collection and Simons Mosaic events calls are available in Supplementary Data. Powering Autism Research for Knowledge cohort. Genotype array data and phenotype information for SSC

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Sample size	Sample size was set by the total number of samples in the Simons Simplex Collection and Simons Powering Autism Research for Knowledge cohort. We excluded a small number of samples which failed to pass quality control checks. While no statistical method was used to predetermine sample size, the sample sizes were equivalent to or larger than similar studies (see Methods for additional information). The 60 post-mortem brain samples represented the totality of post-mortem brain samples available for individuals with ASD from the Lieber Institute for Brain Development, the Oxford Brain Bank, and the University of Maryland Brain and Tissue Bank through the NIH Neurobiobank, and from Autism BrainNet. These samples were not used for statistical analysis but to find individual examples of mosaic CNVs in brain tissue.
Data exclusions	Samples with evidence of contamination with other DNA were excluded from analysis because contamination can manifest as mosaic CNVs under the haplotype phase model. This exclusion is well-established in the literature and is described extensively in Methods.
Replication	We used digital-droplet PCR to confirm the presence of two mosaic CNVs in post-mortem brain tissue discovered via the computational pipeline. Both events were successfully confirmed via quantitative PCR. Each ddPCR reaction was replicated at least three independent times. Gels shown in supplementary Fig. 16c and 17a were replicated three independent times.
Randomization	Samples were allocated into groups via the Simons Foundation. We analyzed SSC and SPARK cohorts separately to control for the distinct experimental procedures used to produce the data. We additionally analyzed the three sub-cohorts of the SSC data separately, again to control for differences in experimental procedure.
Blinding	Blinding was not used because the data had previously been allocated into groups by the Simons Foundation. This allocation was done prior to the conception of this study and the authors had no control over the allocation.

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