Citation: Transl Psychiatry (2011) 1, e34, doi:10.1038/tp.2011.30

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Increased *de novo* copy number variants in the offspring of older males

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The offspring of older fathers have an increased risk of neurodevelopmental disorders, such as schizophrenia and autism. In light of the evidence implicating copy number variants (CNVs) with schizophrenia and autism, we used a mouse model to explore the hypothesis that the offspring of older males have an increased risk of *de novo* CNVs. C57BL/6J sires that were 3- and 12–16-months old were mated with 3-month-old dams to create control offspring and offspring of old sires, respectively. Applying genome-wide microarray screening technology, 7 distinct CNVs were identified in a set of 12 offspring and their parents. Competitive quantitative PCR confirmed these CNVs in the original set and also established their frequency in an independent set of 77 offspring and their parents. On the basis of the combined samples, six *de novo* CNVs were detected in the offspring of older sires, whereas none were detected in the control group. Two of the CNVs were associated with behavioral and/or neuroanatomical phenotypic features. One of the *de novo* CNVs involved *Auts2* (autism susceptibility candidate 2), and other CNVs included genes linked to schizophrenia, autism and brain development. This is the first experimental demonstration that the offspring of older males have an increased risk of *de novo* CNVs. Our results support the hypothesis that the offspring of older fathers have an increased risk of neurodevelopmental disorders such as schizophrenia and autism by generation of *de novo* CNVs in the male germline.
Translational Psychiatry (2011) **1,** e34; doi:10.1038/tp.2011.30; published online 30 August 2011

Introduction

The offspring of older fathers have an increased risk of a range of neuropsychiatric disorders, including autism,[1] schizophrenia,[2,3] bipolar disorder[4] and epilepsy.[5] The mechanisms underlying the increased risk of various neurodevelopmental disorders in the offspring of older fathers remain unclear; however, it has been proposed that *de novo* point mutations and copy number variants (CNVs) in the continually dividing spermatogonia in older males underlie this association.[2,6]

Copy number variants refer to regions of the genome with deletions, inversions or expansions of ~1 kb up to several 100 kb in size.[7] These may occur throughout the genome, but are enriched in regions flanked by segmental duplication in both humans[8,9] and mouse.[10,11] When CNVs encompass genes, they can give rise to an increase or a decrease in gene copy number, or they can contribute to the generation of pseudogenes. CNVs have been associated with neuropsychiatric disorders, including autism,[12–14] schizophrenia,[15–18] epilepsy[19,20] and mental retardation.[21,22] Carriers of such CNVs tend to present with a variable phenotype, and a number of these CNVs can occur in apparently healthy individuals.[23]

In light of the links between advanced paternal age (APA) and neurodevelopmental disorders such as schizophrenia and autism, various rodent-based models have been developed to explore the phenotypic correlates of APA.[24–26] On the basis of an APA C57BL/6J mouse model, we have previously reported that the offspring of older sires had subtle changes in anxiety-related outcomes and changes in cortical thickness.[27] To date, we have not explored our model with respect to *de novo* CNVs. In this preliminary study, we explored the feasibility of CNV detection in our APA model. We hypothesized that the offspring of older sires would have more *de novo* CNVs than would the offspring of control sires. As the offspring used in this study had also been assessed on selected behavioral tests and structural magnetic resonance imaging (MRI), we also had the opportunity to explore phenotypic correlates of identified CNVs as a secondary research question.

Materials and methods

Generation of control and APA families. Families with control sires and those with old sires were generated as described in detail elsewhere.[27] Virgin 3-month-old (control) and 12–16-month-old (APA) male C57BL/6J mice were selected to sire offspring; each was time mated with a 3-month-old female of the same strain. Offspring from 3-month-old males were used as controls, whereas offspring from males aged 12–16 months comprised the APA condition. Offspring were housed in groups of two to five with littermates where possible, but always with offspring from the same paternal condition. Mice were obtained from the University of Queensland C57BL6/J (JAX) mouse stock,

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Keywords: advanced paternal age; autism; behavior; copy number variation; C57BL/6J; schizophrenia
Received 23 June 2011; accepted 8 July 2011

and all procedures were performed with approval from the University of Queensland Animal Ethics Committee, under the National Health and Medical Research Council of Australia Guidelines. Further details are available in Supplementary Material S1.

Array hybridization and detection of CNVs. Two arrays were used in combination to identify CNVs. For genome-wide screening, a commercially available microarray-based Comparative Genomic Hybridization (aCGH) (Mouse Genome CGH 244A Oligo Microarray, Agilent Technologies Australia, Forest Hill, Victoria, Australia) was used, which contained 236 000 mapped 60-mer probes tiling the entire mouse genome at an average distance of ~11 kb. CNVs occur with higher frequency in the vicinity of segmental duplication, and the genomic structure in these regions is often complex in nature.[10] Furthermore, it becomes more difficult to distinguish a one-copy change in a target region already present in multiple copies in the general population, as the expected log(2) intensity ratio derived from a two-color array decreases as a function of increasing reference copy number. To address these considerations, a custom array targeting CNVs identified recently by She *et al.*[28] in a comprehensive comparison of different strains with C57BL/6J was used. Designed by Dr S. Chong and printed by Agilent, this array contained 11 800 60-mer probes spread over the genome at random and 15 580 targeted probes printed in duplicate. Selecting CNVs that could be targeted with at least two pre-designed and computationally validated probes, the custom array achieved 40% coverage of the variant intervals mapped by She *et al.*[28] The resulting probe spacing at CNV loci varied but averaged at 1.5 kb.

Using these two arrays, we examined CNVs in two APA and two control families (three offspring in each of the families and equal number of sexes within each experimental group; total offspring $n = 12$). To control for sex chromosome loading, each female test sample was competitively hybridized against a common female reference sample, and each male test sample against a common male reference sample. A comparison of the references allowed detection of any bias in autosomal and pseudoautosomal regions where aberrations were identified on the arrays. Preparation and labeling of genomic DNA, array hybridization, scanning and feature extraction were performed in accordance with the manufacturer's recommended protocols. Arrays were analyzed further using the Agilent DNA Analytics v4.0.76 software to assure hybridization quality and to detect aberrations. Detected aberrations were subjected to further filtering to select distinct amplifications and deletions for further inspection. These procedures are described in detail in Supplementary Material S1.

Validation and examination of candidate CNVs in an independent sample. The candidate CNVs identified by arrays were validated with Sequenom (Sequenom, QLD, Australia), which combined competitive quantitative PCR and matrix-assisted laser desorption/ionization-time of flight mass spectrometry[29,30] (see Supplementary Material S1). In brief, assays were designed targeting regions of interest (seven CNVs in total and four control regions without genomic aberrations). CNV2 to CNV7 and control regions were targeted by one assay each. CNV1 was targeted by three assays. Using these same methods, we also evaluated the prevalence of the selected CNVs in an independent sample of 77 offspring. These additional offspring, which were generated from 10 APA and 10 control breeding pairs, included 18 APA female and 16 APA male offspring, as well as 24 control female and 19 control male offspring. On the basis of the combined samples, all families were carefully evaluated for *de novo* CNV aberrations in offspring. A CNV that was detected in an offspring but was not detected in either parent was classified as '*de novo*'.

Behavioral and neuroanatomical phenotyping. Behavioral phenotyping was conducted when the offspring were 10 weeks of age and on separate and consecutive days in the following order: elevated plus maze, hole board, light/dark emergence, 2-day forced swim test and 2-day novelty-suppressed feeding. The order of testing was such that the tests most sensitive to handling were performed first and those most stressful performed last. After 1 week of free feeding, animals were tested on a 3-day active avoidance and extinction protocol, tests for nociception and prepulse inhibition of the acoustic startle response. All behavioral observations were made blind to CNV status and recorded from a central overhead camera, which was attached to computerized tracking and event-recording software, EthoVision version 3.1 (Noldus, Wageningen, The Netherlands). Mice were acclimated to the testing room for 1 h before testing and all arenas and apparatus were cleaned between trials with 20% ethanol. After completion of behavioral testing, animals were killed with pentobarbitone and perfused transcardially with 4% paraformaldehyde containing 1% of a separate MRI contrast-enhancement agent, Magnevist (gadopentetate dimeglumine; Schering AG, Berlin, Germany) to optimize gray/white matter boundaries). Immediately before imaging, the brains were suspended in fomblin. Animals were imaged in the 16.4-T microimaging facility (Centre for Advanced Imaging, University of Queensland). Using a 15-mm diameter solenoid coil, fast-low action shot images were obtained in three dimensions with 50-μm voxel resolution. Regions-of-interest volumetric estimates were derived using the OsiriX software package (Rosset; GNU General Public License) with boundaries determined from a mouse brain atlas.[31] Brain regions assessed included the hippocampus, striatum, septum, corpus callosum and anterior commissure. All measurements were made blind to CNV status and analyzed as proportions of total brain volume. Further details of these measures are described in a related publication.[27]

Statistics and data analysis. We hypothesized that the offspring of older sires would have significantly more *de novo* CNVs than would the offspring of control sires. On the basis of the number of *de novo* CNVs in the offspring of the APA and control groups, the odds ratio and 95% confidence interval (two-tailed) were calculated. As no CNVs were called in the control offspring, we used the Haldane correction by adding 0.5 to each cell in the table, to generate a finite odds ratio. For phenotype assessments, all offspring (regardless of paternal age) were assessed with either (1) *t*-tests, in which we could clearly allocate CNV present versus absent

status, or regression analyses between the relative copy number and the outcome of interest, in which a CNV was prevalent and hypervariable.

Bioinformatic analysis of detected CNVs. The expression of genes in the mouse and the human brains was established using the Allen Brain Atlas (http://www.brain-map.org/). The CNV regions were mapped to the human genome using the UCSC genome browser (http://genome.ucsc.edu/). The association of these loci with human disease was established with the Sullivan Lab Evidence Project (https://slep.unc.edu/evidence/)[32] and the Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)[33,34] databases. The occurrence of CNVs in the syntenic genomic regions in humans in control cases and cases with neurocognitive dysfunction was explored using the Database of Genomic Variants (http://projects.tcag.ca/variation/project.html),[35,36] the DECIPHER (https://decipher.sanger.ac.uk/) and the National Centre of Biotechnology Information (http://www.ncbi.nlm.nih.gov/) databases.

Results

Identification and prevalence of CNVs. Seven CNVs were identified in the array-based sample. All of these CNVs were successfully validated by Sequenom (Supplementary Material S2). Each CNV contained or overlapped with at least one gene (Table 1, Supplementary Material S3). The prevalence of these CNVs in the combined samples is shown in Figure 1. The prevalence of CNVs within the total cohort of mice tested in this study (including the relationship of animals to each other, the experimental group status and the individual relative copy numbers (rCNs)) can be found in Supplementary Material S4.

Both CNV1 and CNV7 were found to be prevalent and hypervariable in both the parents and the offspring (the wide distribution of rCN for these CNVs in Figure 1 must be noted). Therefore, it was not possible to determine whether these particular CNVs in the offspring were *de novo* or inherited. The detailed counts for the remaining five CNVs are shown in Table 2. On the basis of these CNVs, in the combined samples, we identified a total of six *de novo* CNVs, in the offspring of aged sires — one offspring with a deletion in CNV2, four offspring with deletions in CNV4 and one of CNV6. The latter was a reversion of an X-linked region in a daughter of an aged sire with an expansion in this locus (Supplementary Material S4). No *de novo* CNVs were detected in the offspring of control sires. We estimate that the offspring of aged sires were 16 times more likely to have a *de novo* CNV compared with the offspring of control sires (Haldane correction applied to all counts; odds ratio and 95% confidence intervals = 15.9, 2.2—undefined, mid-*P*-exact = 0.005).

Correlation between CNV load and selected phenotypic measures. We identified significant correlates for two of the CNVs with behavioral and MRI-derived neuroanatomical phenotypes. For CNV1 (a prevalent and hypervariable locus), offspring with higher rCN had significantly more avoidance responses ($R = 0.489$, $F = 8.78$, $df = 28$, $P = 0.006$). For CNV6 (an X-linked locus) both behavioral and

Table 1 Details of CNVs identified in the array-based sample

CNV number	*CNV1*	*CNV2*	*CNV3*		*CNV4*		*CNV5*		*CNV6*	*CNV7*
Array	*B*	*A*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*A*
Total number of probes	15	35	25	32	76	1172	33	428	12	8
Size (bp)	2 025 740	252 355	3 164 549	2 543 203	506 199	428 532	211 341	157 741	85 722	198 616
Genomic coordinates	Chr4qA5 41.6–43.6 Mb	Chr5qG2 132.5–132.7 Mb	Chr7qA3 21.0–24.1 Mb		Chr14qA3 26.7–27.2 Mb		Chr14qD2 69.9–70.1 Mb		ChrXqF5 166.3–166.4 Mb	ChrXqF5 166.4–166.6 Mb
Genes	*Cntfr, Dctn3, Arid3c, Sigmar1, Galt, Il11ra1, Il11ra2, Ccl27a, Ccl27b, Ccl19, Ccl21a, Ccl21b, Ccl21c, Dnajb5, Fancg, Pigo, Stoml2, Unc13b, Atp8b5, Vcp, Rusc2, Fam166b, Tesk1, Cdf72, Sit1, Rmp, Ccdc107, Car9, Tpm2, Tln1, Creb3, Gba2, Rgp1, Msmp,1700022I11Rik, 2810432D09Rik, 4933409K07Rik, B230312A22Rik, BC049635, E130306D19Rik, N28178*	*Auts2*	*EG667283, Nlrp4e, Vmn1r* gene family		*Anxa11, Plac9, D14Ertd449e, Cphx, Duxbl*		*Slc25a37, D930020E02Rik, Entpd4, Loxl2*		*Mid1*	*Mid1*

Abbreviation: CNV, copy number variant.
CNVs were detected using a (A) commercial, genome-wide aCGH array and/or (B) a targeted, custom CNV array, pending on suitable probes being present. The total number of probes supporting each CNV, its estimated size and genomic location are listed together with the genes that were either contained or affected by the CN.

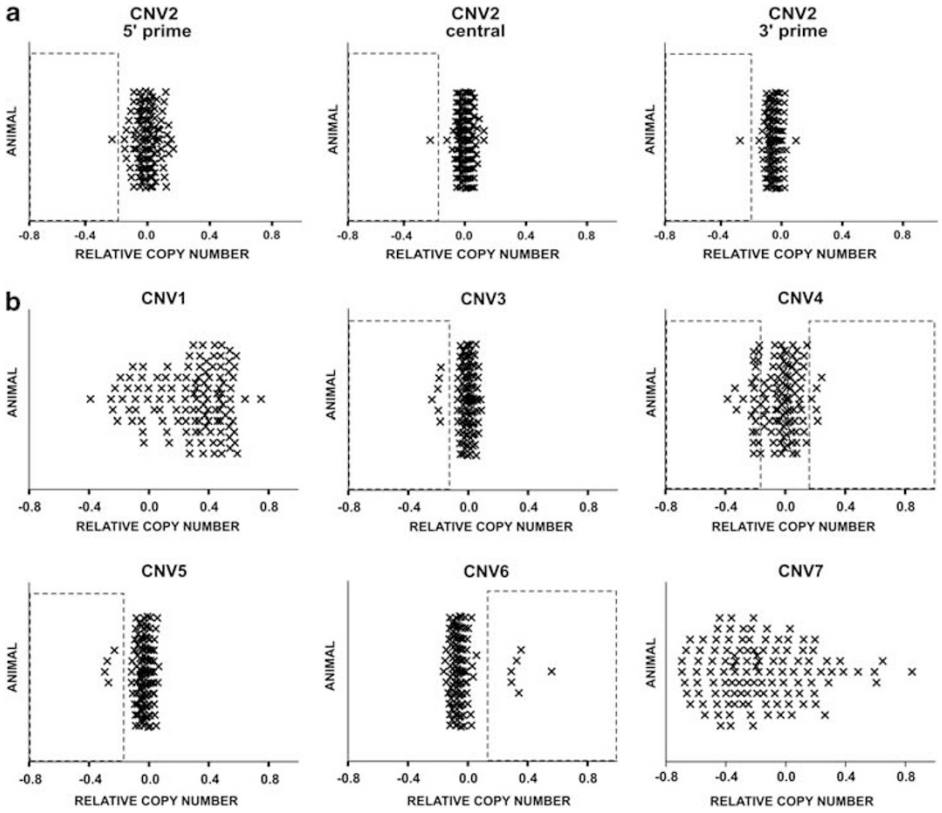

Figure 1 Relative copy number (rCN) in the combined samples established by Sequenom. rCN in the combined samples was examined by Sequenom competitive quantitative PCR. The data from both sets of animals are shown with all values scaled to set a relative CN of 1 at 0. (**a**) Variation in CNV2 was established using three separate assays targeting the 5′ prime, central or 3′ prime region of this CNV, respectively. (**b**) The remaining CNVs were detected by one assay, each. CNV2–CNV6: Boxes denote positive calls for amplifications or deletions. No formal assignment of calls was performed for the highly variable loci CNV1 and CNV7. CNV, copy number variant.

Table 2 Occurrence of CNV2, 3, 4, 5 and 6 in the combined samples

Parameter	n	*CNV2*	*CNV3*	*CNV4*	*CNV5*	*CNV6*
Advanced paternal age group						
Sire	9	0	1	2	0	1
Dam	11	0	0	0	2	0
Female offspring	21	1	6	4	0	6
Male offspring	19	0	0	2	2	0
Sum	60	1	7	8	4	7
Control group						
Sire	10	0	0	3	0	0
Dam	10	0	0	1	0	0
Female offspring	27	0	0	13	0	0
Male offspring	22	0	0	3	0	0
Sum	69	0	0	20	0	0
Total number of animals (%)	129	1 (1)	14 (5)	28 (22)	8 (3)	14 (5)
De novo calls						
Advanced paternal age group	6	1	0	4	0	1
Control group	0	0	0	0	0	0

Abbreviation: CNV, copy number variant.
The number of sires, dams, male and female offspring tested in each experimental group by either array and/or Sequenom is shown (*n*) together with the number of those animals affected by an aberration. In addition, the total number and proportion of all animals (%) affected and the number of *de novo* calls for each CNV and each experimental group is detailed.

neuroanatomical features were significantly correlated (see Figure 2). As expected, the X-linked CNV6 expansion was only seen in female offspring. When compared with CNV-negative APA females, those with CNV6 expansion had a higher score for tail flick (a measure related to pain threshold; $t(14) = 4.0$, $P < 0.001$) and higher scores on head dip on the hole board test (a measure related to exploratory behavior; $t(14) = 2.7$, $P = 0.02$). With respect to the MRI measures, those with CNV6 expansion had smaller striatal ($t\ (14) = 3.89$, $P < 0.01$) and hippocampal volumes ($t\ (14) = 4.84$, $P < 0.01$).

Bioinformatic analysis of syntenic regions in humans. Copy number variant 1 spanned 33 characterized genes on mouse chromosome 4 and their orthologs in the syntenic region of this CNV in humans on chromosome 9. Within this region, amplifications and deletions have been observed. A gene encompassed by CNV1 (Sigmar1) has been associated with alcoholism,[37] schizophrenia[38] and dementia.[39]

Copy number variant 2 spanned exons 3 and 4 in the mouse and the syntenic human *Auts2* gene (see Figure 3). This locus has been associated with autism,[40] schizophrenia,[41] bipolar disorder,[42] attention deficit hyperactivity disorder[43] and alcoholism.[44] Moreover, this gene was reported as differentially methylated in a study on patients with schizophrenia and major psychosis in schizophrenics.[45] Although several small

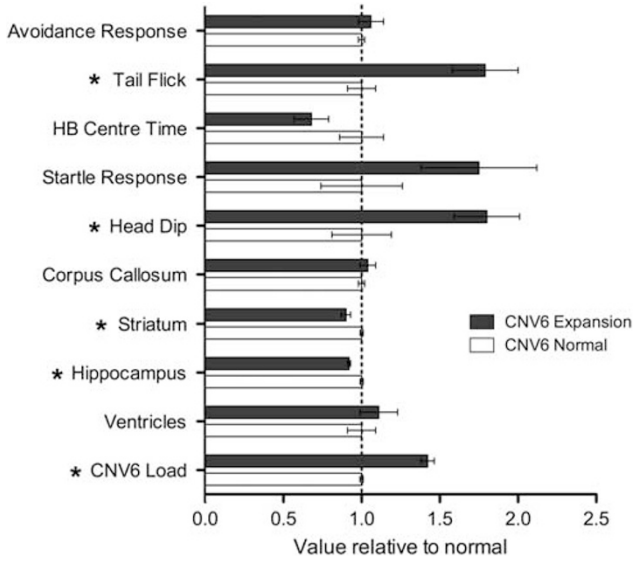

Figure 2 Normalized values on CNV6 load—MRI and behavioral measures. HB center time = proportion of time spent in center zone of the hole board arena. Avoidance response and head dip are count variables. Tail flick and HB center time are timed outcomes (in seconds). Startle response is average amplitude of response to 120 decibels. MRI outcomes include volume of the striatum, hippocampus and ventricles, and width of the corpus callosum. Statistically significant group differences by CNV6 status are shown with an asterisk. CNV, copy number variant; HB, hole board; MRI, magnetic resonance imaging.

CNVs have been observed in the general population,[46–51] separate aberrations within this gene and overlapping with CNV2 have been reported in cases with neurodevelopmental disorders.[19,52–54] Balanced translocations disrupting *Auts2* have been observed in additional cases with autism spectrum disorder and mental retardation.[55] In addition, larger aberrations encompassing *Auts2* have been reported in cases with mental retardation,[54,56–58] Zellweger's syndrome[59] and social cognitive delay.[60]

For CNV4, three (*Anxa11*, *Plac9* and *D14Ertd449e*) of the five genes affected by this CNV mapped to human chromosome 10. One patient with schizophrenia[61] and two with autism spectrum disorder[54] carried aberrations <600 kb in size that also contained these genes. CNV5 mapped to a locus in human chromosome 8 that has been associated with schizophrenia in a meta-analysis of genome-wide linkage studies.[62] An aberration containing this region was present in a patient with mental retardation.[54]

Both CNV6 and CNV7 affected the *Mid1* gene on chromosome X. In the mouse, a pseudoautosomal boundary is situated between exons 3 and 5 of *Mid1*.[63] The region upstream of exon 3 is specific to the X chromosome, whereas the region downstream of this exon is located on both sex chromosomes.[63] The pseudoautosomal portion is highly variable within C57BL/6J.[28,64,65] In our current study, CNV6 spanned exons 2 and 3 and is, therefore, X-linked. The two breakpoints for CNV7 were between exons 3 and 4 and $\sim$11 kb downstream of this gene in the pseudoautosomal region. CNV6 and CNV7 map to the same location in *Mid1* in humans, where the entire gene is X linked. Genetic loss of function mutations in *Mid1*, has been found causative for the Opitz G/BBB syndrome, which is characterized by a varied phenotype that might include a range of midline birth defects and mental retardation.[66] A patient with Opitz G/BBB syndrome and autism carried a discrete deletion of *Mid1* exon 2 (contained in CNV6).[67] A separate case with autism but without Opitz G/BBB syndrome carried a duplication that included the *Mid1* gene from exon 2 onwards to a region $\sim$500 kb downstream of his gene.[68]

Discussion

We report, for the first time, experimental evidence indicating that the offspring of older males have an increased risk of *de novo* CNVs. Although the field has long appreciated that male germ cells would be at risk of more copy error mutations compared with female germ cells,[69,70] the empirical evidence has rested on the observation that particular types of paternally derived translocations seem to be more common in offspring of older men,[71,72] and studies that compared the counts of candidate point mutations in the sperm of men of different ages.[73,74] Here, we used a well-controlled mouse model to investigate the impact of paternal age on CNVs in the offspring. Although our sample size was small, we identified six *de novo* CNVs in this study, and all *de novo* CNVs were in the offspring of aged sires.

On the basis of detailed comparisons between C57BL/6J and closely related strains, Egan *et al*.[65] estimated that *de novo* CNVs occurred once in every 46–139 offspring. The lack of *de novo* CNVs in the offspring of control animals is consistent with these estimates. In contrast, for the offspring of aged sires, we estimate that the incidence of *de novo* CNVs is once in every six or seven offspring. Although we cannot be certain that the *de novo* CNVs originated in the paternal germline, because the age of the dams was the same in the two groups, we would have expected an even distribution of maternally derived mutations in the two groups. Similarly, somatic mutations after fertilization should be equally represented in both groups. On the balance of probability, it is likely that these CNVs were derived from the male germline. Future studies could use dams and sires from different mouse strains to allocate *de novo* CNVs to maternal or paternal chromosomes.

With respect to the clinical relevance of our findings in humans, our mouse model detected CNVs that impact on genes previously linked to autism, schizophrenia and mental retardation. Three of the CNVs occurred in regions of prominent segmental duplication, which suggests that non-allelic homologous recombination may underlie at least some of the age-related CNVs. The two prevalent and hypervariable CNVs (CNV1 and CNV7) are of interest and potentially important for interpreting within-colony variation in this strain of mouse. However, these may be less relevant to human health outcomes as disease-related CNVs tend to be rare and 'privileged' within pedigrees.[23] It should also be noted that mechanisms other than CNVs may contribute to the association between APA and increased risk of disorders, such as schizophrenia and autism. For example, it is feasible that epigenetic changes identified in sperm from older males may also contribute to adverse health outcomes in the offspring.[75]

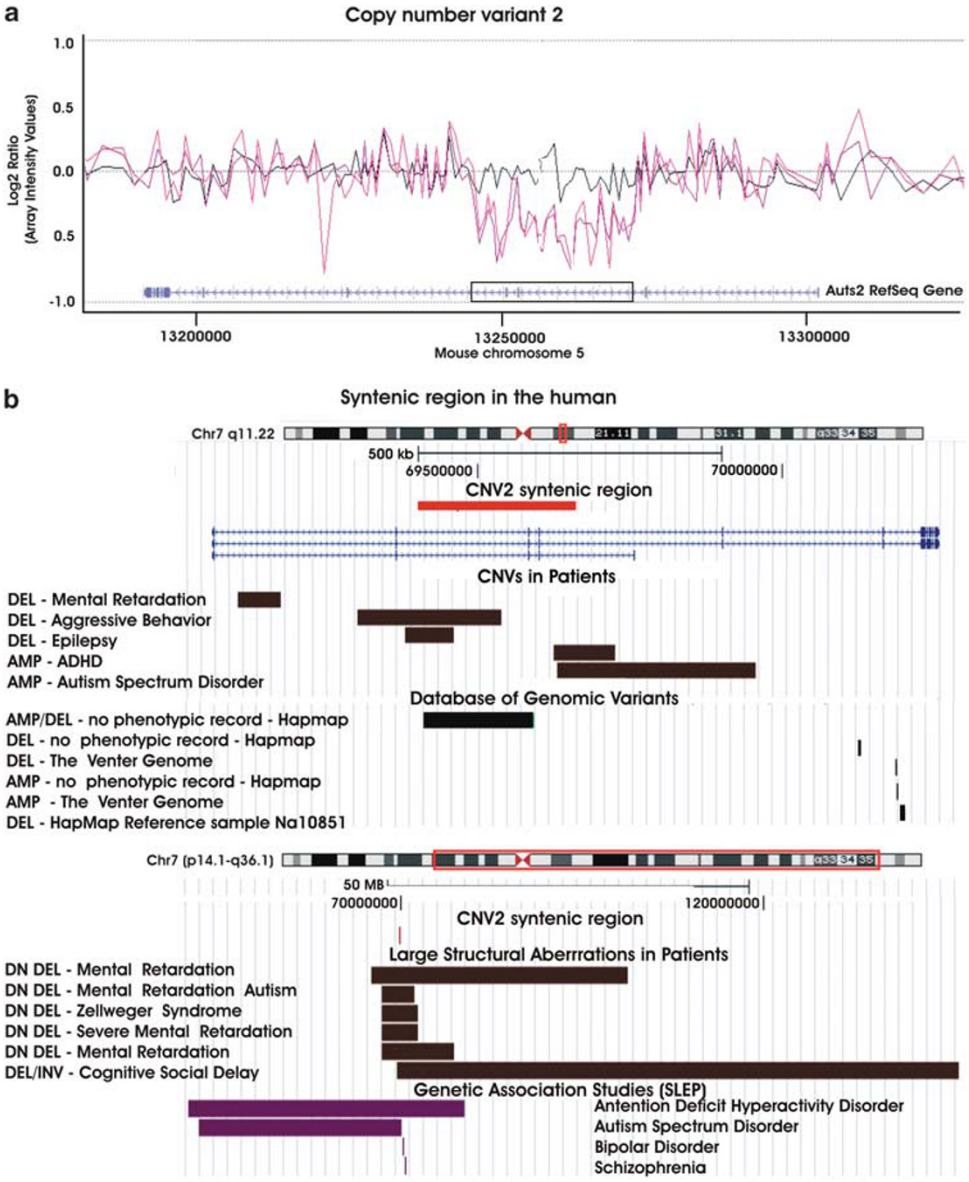

Figure 3 CNV2 details. (**a**) Array log(2) signal intensity ratios of the individual probes are plotted along mouse chromosome 5 and connected with a trend line based on triangular smoothening. A *de novo* deletion (CNV2) affecting the *Auts2* gene was detected in one female offspring of an old sire. The two purple lines depict the result of a technical replicate with the animal containing the deletion. The black line visualizes the result obtained from a self-hybridization array, which was used to control for noise. The location of the CNV on the mouse genome is boxed. (**b**) CNV2 was aligned to the human genome using the UCSC genome browser (http://genome.ucsc.edu/) together with copy variants observed in the human and genomic regions associated with neurocognitive disease. HapMap: Case from the International Hapmap Project. SLEP, Sullivan Laboratory Evidence Project; CNV, copy number variant; DEL, deletion; AMP, amplification; DN, *de novo*.

The study has several important limitations. As our study was exploratory in nature, the sample size was small, and we used readily accessible technology to assess CNVs. The use of (1) aCGH arrays with greater probe coverage, and/or deep sequencing technology and (2) larger sample sizes, would optimize more complete CNV discovery in our APA model. Unfortunately, our experimental protocols required killing animals for *ex vivo* MRI before the results of the CNV analysis were completed, thus we were not able to establish breeding lines from the offspring carrying APA-related *de novo* CNVs. Adequate numbers of CNV-bearing offspring would be required for optimal behavioral and neuroanatomical phenotyping. Furthermore, the broad range of phenotypic measures used in our standard APA screening battery involve many comparisons, inflating type I error (our results were not adjusted for multiple comparisons). As such, the phenotypic findings reported in this study should be considered exploratory in nature and require replication in well-powered samples.

As sequencing technology improves, it may also be feasible to examine the mechanism underpinning APA directly in the sperm of young versus older males. Studies based on point mutations in a candidate gene have already demonstrated the utility of this strategy in pooled sperm samples.[73,74] Furthermore, the mouse may not be the ideal species with respect

to exploring human paternal age-related mutagenesis. For example, the human genome contains slightly less segmental duplication compared with the mouse (2.3 and 4.3%, respectively)[76] and is enriched for different types of retrotransposal elements. As more human genomes are sequenced (especially mother-father-offspring trios), and CNVs are more accurately detected, we predict that the offspring of older fathers will carry more *de novo* CNVs. It will be of interest to explore the taxonomy of paternal age-related CNVs and explore whether these mutations differentially impact on neuropsychiatric health outcomes. It is feasible that these mutations could 'decanalize' brain development and increase the risk of neurodevelopmental disorders, such as schizophrenia and autism.[77]

The age of parenthood is increasing in many societies,[78] and thus it is feasible that the incidence of paternal-age related *de novo* CNVs will increase over time. Worryingly, these CNVs can be inherited and may accumulate over several generations, with some clinical phenotypes 'breaking through' only after a critical threshold of inherited and *de novo* mutations have accumulated.[79] In light of the clues from epidemiology linking APA to increased risk of schizophrenia and autism, and given the convergent evidence linking rare CNVs to these two disorders, the results of our study provide a parsimonious biological mechanism that may contribute to these disabling and poorly understood disorders.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. This study was support by the National Health and Medical Research Council (APP569528). This study makes use of data generated by the DECIPHER Consortium. A full list of centers that contributed to the generation of the data is available from http://decipher.sanger.ac.uk and through e-mail from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust. Those who carried out the original analysis and collection of the data bear no responsibility for the further analysis or interpretation of these data. Dr Naomi Wray from the Queensland Institute for Medical Research assisted with the interpretation of the aCGH data. We acknowledge support from the University of Queensland Centre for Advanced Imaging for access to the animal MRI.

Author contributions. The overall design was conceived by JJM, DWE, TF-B and EW. The animal breeding, behavioral and imaging components were undertaken by CJF. SC designed the custom array. RJM conducted the Sequenom analyses and TF-B performed the microarray assays and all the assessment of CNVs. TF-B and JJM wrote the manuscript with input from all authors.

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