Supplementary results

Human genetic data implicates the *CNTNAP4* gene in neuropsychiatric disorders

We observed copy number variations (CNVs) within the *CNTNAP4* locus in human subjects afflicted by a range of neuropsychiatric conditions. Deletions affecting this gene were identified in multiple unrelated individuals with ASD, SCZ, and ADHD, but not in healthy controls (Extended Data Fig. 6a and 8a). Specifically, studies on 2,078 ASD cases versus 2,512 controls and 1,241 ADHD cases versus 4,109 controls (genotyped on Illumina 550K arrays), as well as 965 schizophrenia cases versus 1,466 controls (genotyped on Affymetrix 6.0 arrays) were examined. Among these, two cases from each disease cohort were identified carrying a *CNTNAP4* intronic deletion, whereas none of the controls were found to harbor CNVs affecting the gene, yielding a combined *P*-value of <0.002 (Supplementary Table 1). All 6 CNV calls were positively validated by TaqMan® Copy Number Assay (Extended Data Fig. 8b). We also identified 2 SNPs with gene-wide significant association to SCZ (Extended Data Fig. 6b and Supplementary Table 1). In an effort to provide more direct evidence for a link between *CNTNAP4* loss of function and neuropsychiatric disease we surveyed an independent cohort of 784 patients with ASD. Through this analysis we identified two unrelated patients carrying maternally inherited *CNTNAP4* deletions. The first patient, diagnosed with Asperger syndrome, carried a deletion (191.6 kb) removing the last three exons of *CNTNAP4* (Extended Data Fig. 7 and Supplementary Table 1). The second patient, diagnosed with autism and mild intellectual disability, carried a deletion (916.2 kb) of all *CNTNAP4* exons (Extended Data Fig. 8c and Supplementary Table 1). Furthermore, whole exome sequencing of the Simons simplex family collection has identified one ASD patient bearing a large *de novo* deletion in the *CNTNAP4* gene[1] (Extended Data Fig 6a and Supplementary Table 1). Additional intronic and exonic CNVs affecting *CNTNAP4* have previously been described in the following studies: Mefford et al. reported 1 such CNV out of 527 cases with seizure disorder[2], Pinto et al. described a *CNTNAP4* CNV in 1 patient with ASD out of 996 ASD cases from 876 trios and 1,287 controls[3], and Hanemaaijer et al. reported a *CNTNAP4* CNV in 1 patient with ADHD, mild developmental delay and mental retardation (MR) out of 600 patients and 1,000 control subjects[4]. Taken together, these independent findings, in coding as well as intronic regions, support for inferring that *CNTNAP4* represents a potential susceptibility gene target for neuropsychiatric disease.

Over-grooming behavior in *Cntnap4* mutants appears to be mouse strain dependent

As stated in the methods, two targeted *Cntnap4* (*Cntnap4*) mouse lines were created at the Weizmann Institute in Israel in exactly the same way using two different clones from the same targeting vector. Both lines (#13 and #149) were targeted correctly to the endogenous *Cntnap4* locus as shown by RT-PCR. Western blot analysis showed that both lines lacked the Cntnap4 protein when they were genotypically homozygous null for the mutant allele. Line 13 was backcrossed 5 times to an outbred strain (ICR) and then intercrossed, whereas line 145 was backcrossed once to ICR and then intercrossed and sent over to NYU Medical Center.

While in mice of the 149 knock-in line we have observed the over-grooming phenotype in two separate animal facilities at NYU (at both the Smilow Research Center as well as the Skirball Biomedical Research Center) - it was absent in mice of the 13 knock-in line when housed at another facility in Israel (Weizmann Institute of Science, Israel). To test if environmental factors could account for this difference, we transferred 8 severely over-groomed adult mice to the Weizmann facility to directly address whether the same mice would show an attenuated over-grooming behavior. Although there was some attenuation of the over-grooming in that facility, the mice still displayed this aberrant behavior strongly suggesting that the differences could not be explained by environmental factors.

The reverse was also true. When we transferred line 13 mice from Israel to New York these mice retained normal whisker growth. We also noticed that even WT offspring derived from line 149 heterozygotes parents when housed with mutant mice exhibited severe whisker and hair loss due to allo-grooming by the mutant mice. In our colony, we observed a penetrance of 82% of over-grooming in 164 mice over various genotypes when they were housed together (87% in HET, 88% in KO, 79% in WT mice; Figure 4B). The allo-grooming was not confined only to adult mice, but was apparent from early ages prior to weaning and was initiated by their mutant parents. Overall, in 7 litters, 79 out of 79 pups were groomed by their Cntnap4 heterozygous parents.

We next wanted to test if the presence and the high penetrance of overt self- and allo-over-grooming we saw in our line 149 mouse colony was the result of a learned behavior that was independent of the presence of the mutant allele. To explore this issue, we performed a series of cross-fostering experiments (Supplementary Fig. 17). We cross-fostered mutant pups onto wild type (WT) parents and observed no over-grooming at the time of weaning (4 litters, 35 out of 35 *Cntnap*4 pups). Conversely, when WT pups were raised by *Cntnap*4 mutant parents they were over-groomed (2 litters of WT mice fostered by *Cntnap*4 heterozygous parents, 15 out of 15 were over-groomed). Nevertheless, when we tracked the mice subsequent to weaning we found that all WT mice regained their whiskers and facial hair after a one to two month period subsequent to separation from mutant animals. Conversely in mutant mice cross-fostered onto WT parents, the over-grooming phenotype re-emerged in 5 out of 11 mice after two and a half months. Notably when WT littermates of mutant animals were cross-fostered separately from the mutant mice they retained their normal whisker and fur growth (4 out of 4).

Mutant animals whose over-grooming re-emerged were mated together, and we observed that all their pups were over-groomed (5 out of 5). WT littermates when similarly bred together did not over-groom their pups (8 out of 8). Finally, as expected, no over-grooming was observed in the pups of the WT fostered mice that had re-gained their whiskers (2 litters, 22 out of 22 pups). We therefore conclude that the over-grooming phenotype, including the allo-grooming component, tracks exclusively with the mutant *Cntnap*4 allele in the inbred 149 line, but not the outbred 13 line. All the experiments presented in this manuscript are from examining line 149 mice at NYU Medical Center.

Nevertheless, we were able to reproduce some of the cellular phenotypes in line 13, such as the increased dopamine in the nucleus accumbens (NAc) through voltammetry and some differences in inhibitory innervation of pyramidal cells through recording spontaneous IPSCs. It is interesting to note that we were not able to reproduce the increase in extracellular dopamine in the dorsal striatum, which may provide an explanation for the lack of over-grooming in line 13.

1 O'Roak, B. J. *et al.* Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* **485**, 246-250, doi:10.1038/nature10989 (2012).

2 Mefford, H. C. *et al.* Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* **6**, e1000962, doi:10.1371/journal.pgen.1000962 (2010).

3 Pinto, D. *et al.* Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* **466**, 368-372, doi:10.1038/nature09146 (2010).

4 Hanemaaijer, N. M. *et al.* Practical guidelines for interpreting copy number gains detected by high-resolution array in routine diagnostics. *Eur J Hum Genet* **20**, 161-165, doi:10.1038/ejhg.2011.174 (2012).