11q24.2-25 micro-rearrangements in autism spectrum disorders: Relation to brain structures.

Anna Maruani, Guillaume Huguet, Anita Beggiato, Monique Elmaleh, Roberto Toro, Claire S Leblond, Alexandre Mathieu, Frederique Amsellem, Nathalie Lemière, Alain Verloes, et al.

11q24.2-25 Micro-rearrangements in Autism Spectrum Disorders:

Relation to Brain Structures

Anna Maruani,[1,2,3] Guillaume Huguet,[1,2] Anita Beggiato,[1,2,3] Monique ElMaleh,[4] Roberto Toro,[1,2] Claire S Leblond,[1,2] Alexandre Mathieu,[1,2] Frederique Amsellem,[3] Nathalie Lemière,[1,2] Alain Verloes,[6] Marion Leboyer,[9,10,11] Christopher Gillberg,[5,7] Thomas Bourgeron,[1,2,12] and Richard Delorme[1,2,3,11] *

Anna Maruani,[1,2,3]
Email: maruani.anna@gmail.com

Guillaume Huguet,[1,2]
Email: guillaumeaf.huguet@gmail.com

Anita Beggiato,[1,2,3]
Email: anita.beggiato@gmail.com

Monique ElMaleh,[4]
Email: monique.elmaleh@rdb.aphp.fr

Roberto Toro,[1,2]
Email: roberto.toro@gmail.com

Claire S Leblond,[1,2]
Email: leblond.claire.s@gmail.com

Alexandre Mathieu[1,2]
Email: alexandre.mathieu@pasteur.fr

Frederique Amsellem,[3]
Email: frederiqueamsellem@gmail.com

Nathalie Lemière,[1,2]
Email: nathalie.lemiere@pasteur.fr

Alain Verloes,[6]
Email: alain.verloes@rdb.aphp.fr

Marion Leboyer,[9,10,11]

Email: marion.leboyer@inserm.fr

Christopher Gillberg,[5,7]
Email: christopher.gillberg@pediat.gu.se

Thomas Bourgeron,[1,2,12]
Email: thomasb@pasteur.fr

Richard Delorme[1,2,3,11] *
*Corresponding author
Email: richard.delorme@rdb.aphp.fr

[1] Human Genetics and Cognitive Functions, Institut Pasteur, Paris, France
[2] CNRS URA 2182, Institut Pasteur, Paris, France
[3] Department of Child Psychiatry, Robert Debré Hospital, APHP, Paris, France
[4] Department of Radiology, Robert Debré Hospital, APHP, Paris, France
[5] Gillberg Neuropsychiatry Centre, Gothenburg University, Göteborg, Sweden
[6] Department of Human Genetics, Robert Debré Hospital, APHP, Paris, France
[7] Saint George's Hospital Medical School, London, United Kingdom
[8] Department of Clinical Sciences, Lund University, Lund, Sweden
[9] INSERM U955, Team 15, Faculty of Medicine, Creteil, France
[10] Department of Adult Psychiatry, Henri Mondor-Albert Chenevier Hospitals, AP-HP, Creteil, France
[11] Fondation FondaMental, French National Science Foundation, Creteil, France
[12] University Paris Diderot Paris 7, 75013 Paris, France

Correspondence should be addressed to: Richard Delorme, M.D., Ph.D, Human Genetics and Cognitive Functions, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. Tel: + 33140613311. Fax: +33140613953. E-mail: richard.delorme@rdb.aphp.fr

Running title: NTM in ASD: relation to brain structures

ABSTRACT

Jacobsen syndrome (JS) is characterized by intellectual disability and higher risk for autism spectrum disorders (ASD). All patients with JS are carriers of contiguous *de novo* deletions of 11q24.2-25, but the causative genes remain unknown. Within the critical interval, we hypothesized that haploinsufficiency of the neuronal cell adhesion molecule Neurotrimin (NTM) might increase the risk for ASD and could affect brain structure volumes. We searched for deleterious mutations affecting *NTM* in 1256 ASD patients and 1287 controls, using SNP arrays, and by direct sequencing of 250 ASD patients and 180 controls. We compared our results to those obtained from independent cohorts of ASD patients and controls. We identified two patients with Copy Number Variants (CNV) encompassing *NTM*, one with a large *de novo* deletion, and a clinical phenotype of JS (including macrocephaly), and a second with a paternally inherited duplication, not consistent with JS. Interestingly, no similar CNVs were observed in controls. We did not observe enrichment for deleterious *NTM* mutations in our cohort. We then explored if the macrocephaly in the patient with JS was associated with a homogeneous increase of brain structures volumes using automatic segmentation. Compared to subjects without *NTM* micro-rearrangements (n=188), the patient had an increased volume of the sub-cortical structures but a decrease of the occipital gray matter. Finally our explorations could not incriminate *NTM* as a susceptibility gene for ASD, but provides new information on the impact of the 11q24.2-25 deletion on brain anatomy.

Keywords: autism, brain, gene, Jacobsen syndrome, macrocephaly

Introduction

Autism spectrum disorders (ASD) are characterized by impairments in reciprocal social communication, and repetitive, stereotyped and ritualistic behaviors [Kanner, 1968]. Advances in high throughput array-based genome screening technologies allowed the detection of copy number variants (CNVs) and single nucleotide variants (SNVs) in large cohorts of patients [De Rubeis et al., 2013; Iossifov et al., 2014; O'Roak et al., 2014; Pinto et al., 2014]. *De novo* CNVs or SNVs affecting genes have conferred high risk for ASD [Iossifov et al., 2012; Pinto et al., 2014] but the majority of these de novo events are rare or unique. Several recurrent CNVs have been associated with ASD, reaching genome-wide significance, such as duplications at 15q11-13, or deletions/duplications at 16p11.2 and 22q11.2 [Hadley et al., 2014; Pinto et al., 2014; Sanders et al., 2011]. Whole exome studies also revealed only a slightly elevated rate of *de novo* SNVs in probands compared with their unaffected siblings (1.02 *vs.* 0.79 mutations per offspring), but patients had two- to threefold more disruptive *de novo* mutations in comparison to their siblings, or to a random model of mutation [De Rubeis et al., 2013; Iossifov et al., 2014].

Although the number of causative genes for ASD could be more than 250, [Huguet et al., 2013] they affect a restricted number of biological pathways, including chromatin remodeling, mRNA translation and synaptic functions, [De Rubeis et al., 2013; Iossifov et al., 2014; Toro et al., 2010]. Among the synaptic genes associated with ASD, Ig-like cell adhesion molecules such as *CNTN3* [Girirajan et al., 2011], *CNTN4* [Fernandez et al., 2004] and *CNTNAP*2 [Alarcon et al., 2008; Arking et al., 2008] have important roles in neuronal interactions for synaptic targeting, neuronal migration, and axon guidance. Neurotrimin (NTM), which belongs to the same molecular family, is a

glycosylphosphatidylinositol (GPI)-anchored cell adhesion protein and a member of the IgLON subfamily (containing also LAMP and OBCAM). Predominantly expressed in the brain, NTM promotes neurite outgrowth and adhesion via a homophilic mechanism [Sellar et al., 2003]. Interestingly, two genome wide association studies (GWAS) reported an association between *NTM* and cognitive function performances [Liu et al., 2007; Pan et al., 2011] and two studies have detected CNVs altering *NTM* in ASD and ID [Cooper et al., 2011; Vorstman et al., 2006].

Deletions of 11q24.2-25 and of the telomeric region of 11q are associated with Jacobsen syndrome (JS), a rare genetic condition sometimes associated with ASD, occurring in 1/100000 birth and considered as a continuous gene disease with highly variable clinical presentations [Cooper et al., 2011; Grossfeld et al., 2004; Penny et al., 1995]. The most common clinical features include mild to severe intellectual disability (ID) associated with autistic symptoms, pre- and postnatal physical growth delay, skull and facial dimorphism (specifically macrocephaly) (for review [Mattina et al., 2009]). The attempts to correlate the clinical findings to the extent of the deletion led to the identification of candidate regions within 11q24.2-25 for several abnormalities such as the Paris-Trousseau thrombocytopenia [Grossfeld et al., 2004]. Difficulties in finding the gene(s) associated with cognitive impairment in patients with JS is due to the relatively high number of compelling candidate genes located at 11q24.2-25 such as *NRGN and KIRREL3* involved in synaptic plasticity [Guerin et al., 2012; Zhong et al., 2009], *ARHGAP32, NTM, OPCML* involved in axon guidance and outgrowth [Akshoomoff et al., 2014; Iossifov et al., 2014; McNamee et al., 2002] or *BSX* involved in brain specific transcriptional activity [McArthur and Ohtoshi, 2007]. Critical regions for ASD and ID were suggested, mainly based on the report of small deletions identified in patients

(Figure 1) [Coldren et al., 2009; Penny et al., 1995]. A recent publication suggested a minimal region containing four genes including *KCJN1*, *KCJN5*, *TP53AIP1* and *ARHGAP32* [Akshoomoff et al., 2014]. However, no gene was formally identified as causing the cognitive phenotype of patients with JS.

Here, we first screened for 11q24.2-25 CNVs in 1256 independent patients with ASD, and detected one *de novo* deletion and one inherited duplication, both encompassing exons of *NTM and SNX19*. Hypothesizing that haploinsufficiency of *NTM* might increase the risk for ASD, we sequenced all coding exons of this gene in 250 patients with ASD and ID, and 180 controls evaluated for the absence of personal and familial history of psychiatric disorders. In addition, to better understand the macrocephaly frequently reported in patients with JS, we quantified the cortical and subcortical brain volumes using magnetic resonance imaging (MRI).

METHODS

Patients and controls

A sample of 1256 patients with ASD from the Paris Autism Research International Sibpair Study (PARIS; N=260) and from the Autism Genome Project (AGP; N=996), meeting stringent quality control (QC) criteria, were included in the study (Table S1 Supplemental material). Information concerning the phenotypic assessment of patients enrolled was described previously [Leblond et al., 2014; Pinto et al., 2010]. To summarize, we used the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) for clinical evaluation and diagnosis. In addition, 1287 control subjects, from the Autism Genome Project were also used to compare our findings [Pinto et al, 2010]. The subjects were from the SAGE cohort

(n=1261) [Bierut et al., 2010] and from HapMap (n= 26) [International HapMap, 2003] (Table S1 Supplemental material).

In parallel to the clinical and genetic exploration, some subjects enrolled in the PARIS cohort were systematically scanned using 3DT1 MRI: 188 subjects including 149 patients with ASD and 39 control subjects were included in the present study (for additional information, see Table S2 Supplemental material).

Ethics Statement

This study was approved by the local Institutional Review Board (IRB) and written inform consents were obtained from all participants of the study. The local IRB are the Hôpital Pitié-Salpêtrière Ethics committee (Paris, France) and the Sahlgrenska Academy Ethics committee (Gothenburg, Sweden). Written informed consent was obtained from all participating subjects. If the proband was under 18 years old, the proband's consent and written parental consent were obtained.

CNV detection and validation for the PARIS cohort

DNA was extracted from blood leukocytes or B lymphoblastoid cell lines. CNV was detected with the Illumina Human 1M and 1M-Duo BeadChip technologies, which interrogate 1 million SNPs distributed over the human genome. Both Illumina technologies are highly similar. Genotyping was performed at the Centre National de Génotypage (CNG), and at the Institut Pasteur. Only samples that met stringent quality control (QC) criteria were included: call rate ≥99%; standard deviation of the log R ratio (LRR) ≤0.35 and of the B allele frequency (BAF) ≤0.13. We used two CNV calling algorithms, QuantiSNP and PennCNV, and one CNV viewer, Snip Peep

(http://snippeep.sourceforge.net/). Similarly to Pinto et al. [2010], CNVs were selected by the number of consecutive probes ≥5, their size ≥1 kb and the confidence score log Bayes factor ≥15. The CNVs were validated by visual inspection of the Log R ratio and B allele frequency values in order to obtain high-confidence calls. To homogenize our CNV detection techniques with those of Pinto et al. [2010], we excluded CNVs if they had a size under 30 kb, if they were within the centromeric region or in extreme GC content regions (>70%). PennCNV was then used to confirm inheritance status of the resulting CNV calls. CNVs were validated by qPCR analysis using the Universal Probe Library (UPL) system from Roche. UPL probes were labeled with 6-FAM™ fluorescein (www.exiqon.com) and the fluorescence was read with the Applied Biosystems 7500 Real-Time PCR System. Each assay was conducted in four replicates for target region probe-set and control region probe-set. Assuming that there were two copies of DNA in the control region, the relative levels of region dosage were determined using the comparative CT method. The relative copy number for each target region was calculated as 2^{-DDCT} with confidence interval as $2^{-(DDCT\pm SD)}$. UPL probes and primers were designed using the Universal Probe Library Assay Design Center from Roche Applied Science.

DNA Sequencing

The genomic structure of *NTM* was obtained from http://genome.ucsc.edu/ (hg18). Among the two main isoforms referenced (NM_001144059, NM_001048209), we sequenced all exons corresponding to the longest isoform (NM_001048209) in 250 subjects with ASD from the PARIS cohort (From European descent) and 180 Caucasian controls (From European descent) evaluated for the absence of personal and familial history of psychiatric disorders by direct assessment using the Diagnostic Interview for

Genetics Study Interview (DIGS) and the Familial Interview for Genetics Study (FIGS) (For additional details about clinical assessment of these patients and controls, see [Leblond et al., 2012]. Primers spanning all seven intron-exon boundaries of *NTM* were designed and used to amplify the regions of interest. Amplicons were produced from genomic DNA, and sequencing was performed using ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA). Sequence comparison to reference sequence was performed using GenalysCarbon 2.8.2b (http://software.cng.fr/).

Protein Function Prediction

Several *in silico* applications were used to predict the functional effect of the amino acid substitutions, including SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and SNAP (http://rostlab.org/services/snap/).

Intracranial and brain volume estimations based on magnetic resonance imaging

For subjects included in this cohort, MRI data were collected using the following parameters: spoiled gradient recalled echo (SPGR), 1mm isotropic, repetition time (TR)=25ms, echo time (TE)=6ms, flip angle=30º. To estimate the intracranial volume, the different datasets were first reoriented to correspond with the orientation of the MNI152 atlas. The brain was removed from the skull using AFNI tools [Cox, 1996], and linearly normalized to the (skull-less) MNI152 atlas using FSL tools [Jenkinson et al., 2002; Smith et al., 2004]. We then used the affine matrix of this transformation to initialize the linear normalization of the reoriented datasets (with skull) to the MNI152 atlas (with skull). We used the inverse of the determinant of the affine matrix produced

by this transformation as an estimation of the intracranial volume, as in Buckner et al [2004][Buckner et al., 2004]. All steps of the process were visually inspected for accuracy using in-house software. In the cases where the skull stripping was inappropriate (the most frequent type of failure of the automatic segmentation procedure), we manually corrected the brain extraction using in-house software, and relaunched the processing pipeline. Also, the estimation of brain volume was obtained by segmenting automatically the grey and white matter. We then labeled the frontal, parietal, occipital, temporal lobes and subcortical structures by non-linearly warping the individual datasets into an atlas, using FSL tools [Smith et al., 2004; Zhang et al., 2001] and our own software [Toro et al., 2009]. Intracranial and brain volume values were converted into Z-scores. We used an open online tool to visually control the accuracy of the segmentations (http://siphonophore.org/qccc). The subjects for whom the segmentation not fulfilled the quality criteria were excluded.

RESULTS

A *de novo* deletion and a rare inherited duplication at 11q24-25 encompassing *NTM*

Among the 1256 independent patients with ASD genotyped using Illumina SNP arrays, we detected one large *de novo* heterozygous deletion within 11q24.2-25 in one boy with autism and moderate intellectual disability (see clinical data section for details / Family I). The CNV size is about 5.4Gb (chr11: 126,633,940 to 132,060,375; hg18). Among the 20 genes within the deletion (Figure 2), three are mainly expressed in the brain: *ARHGAP32, NTM and OPCML*. Haplotype analysis revealed that the deletion originated on the paternal chromosome (data not shown). No additional rare CNVs, inherited or *de novo*, encompassing a gene expressed in the brain was detected in the patient.

We also detected a 938 kb duplication located within the same region (11q24.3-q25; chr11: 130,144,889 to 131,083,311; hg18) in an independent patient with ASD. The micro-rearrangement affects two genes: *SNX19* and the first exon of *NTM,* a gene that is mainly expressed in the brain. The duplication was inherited from the father and shared by the affected sister (Figure 2). Both CNVs were validated by quantitative PCR (qPCR). We then compared our results to the CNVs detected in the 1287 independent controls from the AGP [Pinto et al., 2010]. We found only one CNV (a duplication) affecting NTM (B209941_1007852571; chr11: 130,064,162 to 131,099,383; hg 18), which was very similar to the one observed in family II (Figure 2).

We then compared our results to those obtained in different databases [Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/), Decipher (http://decipher.sanger.ac.uk)] (Figure 2). No similar heterozygous CNVs (sharing at least 80% overlap with these CNVs) were previously referenced in DGV. In Decipher, several subjects presenting with a development delay (no additional clinical information was provided) carried a large deletion or duplication within 11q24.2-q25, which encompassed *NTM* (Figure 2). Three distinct CNVs affecting only *NTM* were reported: a duplication of the first exon (chr11: 130209116 -13077274) in one subject (Id: 250240, clinical information not known), a deletion of the third exon (chr11: 131406957-131574563) in one subject (Id: 250733 showing global development delay), and a deletion of the third and fourth exons (chr11: 131506987-131598665) in four individuals (A in Figure 2; Id: 1617, 2049, 2050, 2054). For these subjects, clinical information was as follow: 2049 and 2050 showed ID; 1617 had microcephaly, prominent ears, delayed language development and clinodactyly.

Deleterious mutations in *NTM* coding sequence

All *NTM* exons were directly sequenced in a subset of patients with ASD and ID (n=250) from the PARIS study and in controls (n=180). Two heterozygous non-synonymous mutations, both in exon 8, were identified: p.V327I, observed in 11/250 patients (4.4%) and 5/180 controls (2.8%) (Fisher's exact test, Odd ratio=1.6, p=0.4), and p.S335L, observed in 1/250 patients (0.4%) and 1/180 controls (0.6%) (Fisher's exact test, Odd ratio=0.7, p=1). No additional mutations were found on the remaining allele of *NTM* in the affected probands of families I and II. *In silico*, the mutations p.V327I and p.S335L were predicted to be benign whatever the model of prediction used (Polyphen-2/Prediction: benign; PSIC score difference: 0.056 for p.V327I and 0.002 for p.S335L - SNAP/Prediction: neutral; reliability index: 6 for p.V327I and 0 for p.S335L; expected accuracy 92% for p.V327I and 53% for p.S335L - SIFT/prediction: tolerated; SIFT median sequence conservation score: 3.11 for p.V327I and 3.05 for p.S335L). Clinically, the 11 patients carrying the p.V327I mutation were not different from the whole cohort of patients in terms of severity of ID or autistic symptoms, based on ADI-R sub-scores and cognitive assessment measure (data not shown). Concerning the patient carrying p.S335L, he displayed a non-verbal autism with a severe ID. Clinical examination was in the normal range, but with minor signs of dimorphism including hypertelorism and divergent strabismus. The mutation was inherited from the mother, which displayed no personal or familial history of psychiatric illness. We then compared our results to those reported previously in two independent cohorts, which were whole-exome sequenced. We collected the data from 767 independent probants with ASD and 573 unaffected siblings from the Simons Simplex Collection (SSC) [Iossifov et al., 2012; O'Roak et al., 2012; Sanders et al., 2012] and 4477 controls from the Exome Variant Server

(evs.gs.washington.edu). Based on the NM_001144058 isoform, we observed 9 non-synonymous variants (7 within the patients, 2 in the siblings) in the SSC (Table1), but no stop-gain, in-del or *de novo* mutations (no variation has been found only in patients). Variants were predicted as benign (Table 1). The variants identified in our cohort of patients, p.V327I and p.S335L, were also observed in SSC cohorts of patients and controls and were not significantly associated with ASD (respectively : p= 0,43 et p=1; odds ratio=0,82, [0.5295; 1.2528] et 0,70 [0.0769; 3.1108]). These results were also confirmed in the recent studies from the SSC (over 3800 ASD and 9000 controls) that did not find any stop-gain, in-del or *de novo* mutations within ASD patients. Latest studies from the SSC did not find transmitted missense variants, this can been explained by new pipeline and filtered MAF (minor allele frequency ≤ 0.001) used [Iossifov et al., 2014; O'Roak et al., 2014]

Clinical characteristics of the patients with CNVs at 11q24-25

Family I. The patient carrying the large 11q24 deletion was a 21-year-old male, second child of non-consanguineous healthy parents. The father, of North African origin, had no personal medical or psychiatric history, but reported a paternal cousin with symptoms of ID and ASD, and a paternal uncle with brief recurrent delirium episodes. The maternal personal and family history revealed no prior instances of mental or neurological disorders. The clinical profile of the proband's older sister was normal with no specific medical history.

The patient was born following an unremarkable pregnancy. The delivery was preterm (35 weeks of gestation) for unknown reason. A cesarean section was performed in emergency after painful and inefficient labor due to a foetomaternal

disproportion. All birth measurements were over the 97th percentile (the head circumference of the newborn was 37 cm, the birth weight was 3.440 kg and the height was 52 cm). Fetal resuscitation was performed initially (with APGAR scores at 2 and 9 at 1 and 10 minutes respectively), but the patient was not transferred to an intensive care unit. Although early neonatal development was considered as normal by pediatricians (motor skills were in the normal range at 6 months and walking was acquired at 14 months), the mother was concerned with sleep difficulties and the appearance of body rocking at the age of 8 months. Verbal language was delayed with first words emerging at 3 years of age and sentence constructions only at 5 years. Initially echolalic, the language was considered functional at 7 years. At that time, the cognitive assessment, using the Wechsler Intelligence Scale for Children, objectified the intellectual disability, with an intellectual quotient estimated fewer than 50. Clinically, the patient had clear abnormalities of social interactions. He also had various stereotypies and self-injurious behaviors associated with major impulsivity. The clinical diagnosis of autism (in accordance with DSM-IVTR criteria) was confirmed by standard assessment using the Autism Diagnostic Interview-Revised and the Autism Diagnosis Observation Scale. Clinical examination showed various signs of dimorphism similar to those reported in JS, *i.e.,* low-set ears, large nasal bridge, slight nose protrusion, high forehead, short neck, macrocephaly (+4 SD) and short stature (-0.5 SD). Neurological examination was in the normal range. Also, results from routine biological screens (including a complete blood count), electrocardiogram and ultrasound explorations were all in the normal ranges.

Family II. The patient carrying the duplication was a 15-year-old male, born at term after an uneventful twin pregnancy and delivery (38th week) from non-consanguineous

Caucasian parents. The maternal personal and family history revealed no prior instances of mental or neurological disorders, except for a putative post-partum depression after her twin pregnancy for which she did not receive any treatment. Her clinical exam produced normal results. The father reported a general anxiety disorder that emerged during childhood alongside a diagnosis of attention deficit / hyperactive disorder (ADHD). At the time of assessment, only the anxiety disorder persisted. His clinical exam was normal, except the presence of a unilateral left thumb macrodactyly. The older sister, aged 17 years at the time of the assessment, did not report any psychiatric disorder, but had experienced an idiopathic pubertal delay during childhood. Her clinical exam was normal except for a mild strabismus. Birth weight of the patient was 3170 g (25th percentile), length 50 cm (49th percentile), and occipito-frontal head circumference (OFC) 36 cm (53rd percentile). APGAR scores were 10 at 1 and 5 min after birth. Despite normal development during infancy, including walking at 15 months, the parents reported a pronounced intolerance to frustrations and noises at age 2. They also noted a paucity of interactions accompanied by stereotyped body movements. The patient exhibited severely delayed speech, with first words at 4 years of age. Referred to a psychiatric unit at the age of 5 years, he was diagnosed clinically with autism, based on DSM-IV and ICD-10 criteria. He also met criteria for autism according to the ADI-R and the Autism Diagnostic Observation Scale (ADOS). Assessment of his cognitive performance with the Raven's Colored Progressive Matrices test indicated that his intelligence quotient (IQ) was below the first percentile for his age. When examined at the age of 15, the patient's height, weight and OFC were all in the normal range, but he presented with minor dimorphic features (not typical of JS) including a long and narrow face, arachnoid hand fingers and a supernumerary nipple. The neurological exam was

normal. His expressive language was limited to restricted and stereotyped sentences. His dizygotic twin sister who shared the *NTM* duplication also exhibited a severe intellectual disability with autistic features, but also displayed a severe psychomotor delay and microcephaly. These symptoms were quite different from those reported in her twin brother, but concordant with Rett syndrome, which led to a screening of the *MECP2* gene when she was 4. A *de novo* deletion (c.1126del50) in exon 3 resulting in the truncation of the MECP2-protein was found. None of the other members of the family carried this *MECP2* deletion.

Macrocephaly and exploration of brain structures volume in a patient with JS

Results from brain MRI obtained in the proband from Family I were compared to similar data collected in 188 individuals including 149 subjects with ASD and 39 controls from our cohort Surprisingly, the macrocephaly observed in our patient (>2SD) was not associated with an increase in brain volume. Indeed, patient's total brain volume was less than 1SD from the mean (Figure 3). Thus, the macrocephaly was explained by the increased volume of the skull and not by the brain itself. In parallel, automatic segmentation of brain structures suggested that grey and white matter volumes of the frontal, parietal and temporal lobes were in the normal range when compared to subjects from the cohort (Figure 3). However, right and left occipital grey matter was significantly decreased (<2SD and approximately 2SD from the mean, respectively). In addition, when exploring the volume of the subcortical structures, we observed an increased volume of left thalamus (approximately 2SD), left and right putamen (both >2SD), left and right pallidum approximately 2SD and >2SD, respectively), and a slight increase of the left hippocampus (>1SD).

DISCUSSION

In the literature, several minimal regions and genes were proposed to delineate the location of the gene(s) that could increase the risk for ASD and ID in patients with JS: *BSX* and *NRGN* have been described at 11q24.2 [Coldren et al., 2009]; *KIRREL3* at 11q24.2 [Guerin et al., 2012], *ARHGAP32* at 11q24.3 [Akshoomoff et al., 2014] and few others [Bernaciak et al., 2008; Ji et al., 2010; Penny et al., 1995]. A recent morbid map of the genome based on the investigation of 29,085 patients with developmental disorders and 19,584 controls, showed that all genes within the JS region are strongly associated with developmental disorders ($10^{-5} < P < 10^{-10}$) [Coe et al., 2014].

In the present study, we completed our initial finding of a 11q24.2-25 *de novo* deletion in a patient with ASD by a screening for new CNVs in that region. The identification of a duplication of the first exon of NTM that could disrupt the gene prompted us to search for mutations in this gene. *NTM* seemed to be a compelling candidate gene with a strong association with neurodevelopmental disorders ($P=1.13.10^{-9}$) based on the morbid map of Coe et al. [2014]. In addition, NTM belongs to the IgG-like cell adhesion molecules and several members of this family of genes such as Contactins were previously shown to be associated with ASD [State and Levitt, 2011]. Finally, *NTM* is predominantly expressed in the brain and promotes neurite outgrowth [Gil et al., 1998].

Direct sequencing of *NTM* did not provide evidence for its involvement in ASD and ID. Two heterozygous non-synonymous mutations (p.V327I and p.S335L), both considered as benign by predictive models, were detected at the same frequency in patients and controls in our study and in larger independent cohorts [Iossifov et al., 2012;

Sanders et al., 2012]. Based on the whole exome sequence data from patients with ASD, no association was observed between *NTM* and ASD. Our study indicates that *NTM* mutations do not play a major role in the susceptibility to ASD, but cannot formally exclude that haploinsufficiency of *NTM* could be a risk factor for ASD in patients with JS. In addition to the structural similarities with other genes related to ASD such as *CNTN3* [Girirajan et al., 2011] and *CNTN4* [Fernandez et al., 2004]. NTM participates in the control of the axonal pathfinding and synaptogenesis, but also in the proliferation of glial cells [Hashimoto et al., 2009; Sugimoto et al., 2012]. Overexpression of NTM in rat cortical astrocytes, results in an inhibition of cell proliferation and an increase of the cell size [Sugimoto et al., 2012]. Thus, haploinsufficiency of *NTM* reported in Family I, and in patients with JS, might impact the development of cortical astrocytes. Increased in astrocyte proliferation and decreased in the astrocyte size could participate in the white matters lesions reported on patients with JS [Mattina et al., 2009]. These lesions are mainly reported in the cerebellum and in the neocortex, both regions in which *NTM* is strongly expressed all along the life (http://hbatlas.org/). Interestingly, mutations in *HEPACAM*, a member of IgG-like cell adhesion molecules, affect the astrocyte-astrocyte junctions and cause macrocephaly and ID (with or without ASD), or benign familial macrocephaly [Mattina et al., 2009].

The exploration of brain structures using MRI in the proband from Family I has revealed that the macrocephaly was not the consequence of increased brain volume, but of increase circumference of the skull. On average, 20% of patients with JS [Tyson et al., 2008] display macrocephaly, but no case report has mentioned structural changes in the skull of the patients. Previous studies have rather highlighted the white matter lesions, thought to be due to delayed myelinisation [Tyson et al., 2008]. In caudal regions

(reported with a high level of *NTM* mRNA in Allen Humain Brain Atlas data), we observed a bilateral decrease in grey matter volume, with a maximum in the occipital region (approximately -2SD). Such an inverse correlation was also observed for subcortical structures, with an increased volume (approximately +2SD) of the left thalamus, left and right putamen, left and right pallidum, regions for which the level of *NTM* mRNA was reported low. Although these results were obtained in a single patient, they provide an interesting hypothesis on the mechanism of macrocephaly in patients with JS. Region where NTM needs to be at a high level would be more sensitive to haploinsufficiency. The major decrease in volume observed in the occipital region and the increased volume of left thalamus, the putamen and the pallidum could be explained by aberrant growth rates in regions implicated in repetitive and stereotyped behaviors and in contrast, by growth rates deceleration in cortical regions reported in young adults with ASD [Zielinski et al., 2014].

The clinical impact of the duplication reported in Family II is difficult to ascertain. The paternally transmitted rearrangement was shared by the proband and his affected sister but not by the healthy sister. The father had a lifetime diagnosis of general anxiety disorder and ADHD. He was also evaluated for autistic symptoms but he scored in the normal range on the Social Responsiveness Scale [Constantino and Todd, 2005]. A paternal cousin was also diagnosed with ID, but unfortunately, we were unable to directly assess him and his DNA was not available. Interestingly, *SNX19* is located in the duplication and might be involved in the thumb macrodactyly observed in the father. *SNX19* codes for a chrondrogenic factor, and a gain of function resulting from the duplication might affect the differentiation of chondrogenic cells and chondrocyte hypertrophy [Kan et al., 2009]. Indeed, mutations of family members of SNX were

associated with skeletal dysplasia or growth retardation in humans and mice [Carlton et al., 2005; Vervoort et al., 2002].

The identification of the gene(s) involved in the cognitive phenotype of patients with JS warrants more genetic and functional studies. *OPCML* is a tail-to-head tandem duplication of *NTM* and is very similar at the protein level (>75% identity). *OPCML* codes for the Opioid binding Protein/Cell adhesion Molecule-Like and is highly specifically expressed in the brain. It might also have an accessory role in opioid receptor function. In the rat, the orthologous protein binds opioid alkaloids in the presence of acidic lipids and exhibits selectivity for mu ligands. Another gene located in the deleted region, *ARHGAP32* is highly expressed during neuronal development and plays an important role in postsynaptic NMDA signaling and neurite outgrowth [Hayashi et al., 2007]. In mice, *ARHGAP32* is expressed in the brain, involved in early brain development, including extension of axons and dendrites, and postnatal remodeling and fine-tuning of neural circuits [Nasu-Nishimura et al., 2006]. *ARHGAP32* has been recently reported to be deleted in a few patients and proposed as a good candidate gene for ASD traits in JS [Akshoomoff et al., 2014]. The *KIRREL3* gene has also been proposed as a candidate gene for autism [Guerin et al., 2012], but this gene was not included in the CNVs reported here.

CONCLUSION

In conclusion, our study provides the screening of *NTM* mutations in ASD and the first report that the macrocephaly observed in patients with JS is associated to increased skull

size and abnormal volumes of subcortical structures. More studies linking rare and common genetic variants to brain anatomy will be helpful to better define the heterogeneity of ASD and to understand the complex mechanisms leading to neurodevelopmental disorders in humans.

Abbreviations

ADHD, Attention Deficit / Hyperactive Disorder; ADI-R, Autism Diagnostic Interview-Revised; Acc, Nucleus accumbens; ADOS, Autism Diagnostic Observation Schedule; ADOS-G, Autism Diagnostic Observation Schedule-Generic; AGP, Autism Genome Project Consortium; Amy, Amygdala; ARHGAP32, RHO GTPase activating protein 32; ASD, Autism Spectrum Disorder; BSX, Brain-Specific Homebox ; Ca, Caudate nucleus; CGH, comparative genomic hybridization ; CNTN, Contactin; CNV, copy number variation; DIGS, Diagnostic Interview for Genetics Study Interview; DISCO, Diagnostic Interview for Social and Communication Disorders; FIGS, Familial Interview for Genetics Study; EEG, electroencephalography; GPI, glycosylphosphatidylinositol ; GWAS, genome-wide association study; Hip, Hippocampus; ID, intellectual disability; IgLON, subgroup of the immunoglobulin superfamily cell adhesion molecules ; IQ, intelligence quotient; JS, Jacobsen syndrome ; KIRREL3, Kin Of Irre-Like 3 ; LAMP, lysosomal-associated membrane protein ; MAF, minor allele frequency ; MRI, magnetic resonance imaging; mRNA, messenger ribonucleic acid ; NRGN, Neurogranin; NTM, Neurotrimin; OPCML, Opioid-Binding Cell Adhesion Molecule ; Pa, Pallidum; Pu, Putamen; OFC, occipito-frontal head circumference; QC, quality control; SNP, single-nucleotide polymorphism; SNV, single nucleotide variation; SNX19, Sorting Nexin 19; SPGR, spoiled gradient recalled echo; SRS, Social Responsiveness Scale; SSC, Simons

Simplex Collection; Th, Thalamus; TP53AIP1, Tumor Protein P53 Regulated Apoptosis Inducing Protein 1.

Competing interest

The authors declare that they have no competing interests.

Author's contributions

AM: conception and design; data collection; psychiatric evaluation, analysis, manuscript writing; AB, CSL, GH, ME, NL and RT: extraction data, analysis; FA, ME & AV: data collection, analysis. ML, CG and TB are the main investigators with RD the corresponding authors that drafted the manuscript. All authors red and approved the final manuscript.

Acknowledgements

We thank the platform for Ressources Biologiques, Groupe Hospitalier Albert-Chenevier-Henri Mondor, APHP, Crident and the Centre d'Investigation Clinique of the Robert Debré Hospital for collecting and processing the biological samples used in this study. This work was supported by the Institut Pasteur, University Paris Diderot, CNRS, Fondation Orange, ANR (ANR-08-MNPS-037-01 - SynGen), Neuron-ERANET (EUHF-AUTISM), Fondation FondaMental, Fondation Recherche Médicale, and Fondation Bettencourt-Schueller. Written informed consent was obtained from the participant for publication of their individual details and accompanying images in this manuscript. The consent form is held by the authors' institution and is available for review by the editor-in-Chef.

References

Akshoomoff N, Mattson SN, Grossfeld PD. 2014. Evidence for autism spectrum disorder in Jacobsen syndrome: identification of a candidate gene in distal 11q. Genet Med.

Alarcon M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH. 2008. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet 82:150-159.

Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH, Chakravarti A. 2008. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. Am J Hum Genet 82:160-164.

Bernaciak J, Szczaluba K, Derwinska K, Wisniowiecka-Kowalnik B, Bocian E, Sasiadek MM, Makowska I, Stankiewicz P, Smigiel R. 2008. Clinical and molecular-cytogenetic evaluation of a family with partial Jacobsen syndrome without thrombocytopenia caused by an approximately 5 Mb deletion del(11)(q24.3). Am J Med Genet A 146A:2449-2454.

Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Grucza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman

RJ, Nothen MM, Nurnberger JI, Jr., Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP, Gene EASC. 2010. A genome-wide association study of alcohol dependence. Proc Natl Acad Sci U S A 107:5082-5087.

Buckner RL, Head D, Parker J, Fotenos AF, Marcus D, Morris JC, Snyder AZ. 2004. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage 23:724-738.

Carlton JG, Bujny MV, Peter BJ, Oorschot VM, Rutherford A, Arkell RS, Klumperman J, McMahon HT, Cullen PJ. 2005. Sorting nexin-2 is associated with tubular elements of the early endosome, but is not essential for retromer-mediated endosome-to-TGN transport. J Cell Sci 118:4527-4539.

Coe BP, Witherspoon K, Rosenfeld JA, van Bon BW, Vulto-van Silfhout AT, Bosco P, Friend KL, Baker C, Buono S, Vissers LE, Schuurs-Hoeijmakers JH, Hoischen A, Pfundt R, Krumm N, Carvill GL, Li D, Amaral D, Brown N, Lockhart PJ, Scheffer IE, Alberti A, Shaw M, Pettinato R, Tervo R, de Leeuw N, Reijnders MR, Torchia BS, Peeters H, O'Roak BJ, Fichera M, Hehir-Kwa JY, Shendure J, Mefford HC, Haan E, Gecz J, de Vries BB, Romano C, Eichler EE. 2014. Refining analyses of copy number variation identifies specific genes associated with developmental delay. Nat Genet 46:1063-1071.

Coldren CD, Lai Z, Shragg P, Rossi E, Glidewell SC, Zuffardi O, Mattina T, Ivy DD, Curfs LM, Mattson SN, Riley EP, Treier M, Grossfeld PD. 2009. Chromosomal microarray mapping suggests a role for BSX and Neurogranin in neurocognitive

and behavioral defects in the 11q terminal deletion disorder (Jacobsen syndrome). Neurogenetics 10:89-95.

Constantino JN, Todd RD. 2005. Intergenerational transmission of subthreshold autistic traits in the general population. Biol Psychiatry 57:655-660.

Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. 2011. A copy number variation morbidity map of developmental delay. Nat Genet 43:838-846.

Cox RW. 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29:162-173.

De Rubeis S, Pasciuto E, Li KW, Fernandez E, Di Marino D, Buzzi A, Ostroff LE, Klann E, Zwartkruis FJ, Komiyama NH, Grant SG, Poujol C, Choquet D, Achsel T, Posthuma D, Smit AB, Bagni C. 2013. CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron 79:1169-1182.

Fernandez T, Morgan T, Davis N, Klin A, Morris A, Farhi A, Lifton RP, State MW. 2004. Disruption of contactin 4 (CNTN4) results in developmental delay and other features of 3p deletion syndrome. Am J Hum Genet 74:1286-1293.

Gil OD, Zanazzi G, Struyk AF, Salzer JL. 1998. Neurotrimin mediates bifunctional effects on neurite outgrowth via homophilic and heterophilic interactions. J Neurosci 18:9312-9325.

Girirajan S, Brkanac Z, Coe BP, Baker C, Vives L, Vu TH, Shafer N, Bernier R, Ferrero GB, Silengo M, Warren ST, Moreno CS, Fichera M, Romano C, Raskind WH,

Eichler EE. 2011. Relative Burden of Large CNVs on a Range of Neurodevelopmental Phenotypes. PLoS Genet 7:e1002334.

Grossfeld PD, Mattina T, Lai Z, Favier R, Jones KL, Cotter F, Jones C. 2004. The 11q terminal deletion disorder: a prospective study of 110 cases. Am J Med Genet A 129A:51-61.

Guerin A, Stavropoulos DJ, Diab Y, Chenier S, Christensen H, Kahr WH, Babul-Hirji R, Chitayat D. 2012. Interstitial deletion of 11q-implicating the KIRREL3 gene in the neurocognitive delay associated with Jacobsen syndrome. Am J Med Genet A 158A:2551-2556.

Hadley D, Wu ZL, Kao C, Kini A, Mohamed-Hadley A, Thomas K, Vazquez L, Qiu H, Mentch F, Pellegrino R, Kim C, Connolly J, Consortium AGP, Glessner J, Hakonarson H. 2014. The impact of the metabotropic glutamate receptor and other gene family interaction networks on autism. Nat Commun 5:4074.

Hashimoto T, Maekawa S, Miyata S. 2009. IgLON cell adhesion molecules regulate synaptogenesis in hippocampal neurons. Cell Biochem Funct 27:496-498.

Hayashi T, Okabe T, Nasu-Nishimura Y, Sakaue F, Ohwada S, Matsuura K, Akiyama T, Nakamura T. 2007. PX-RICS, a novel splicing variant of RICS, is a main isoform expressed during neural development. Genes Cells 12:929-939.

Huguet G, Ey E, Bourgeron T. 2013. The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 14:191-213.

International HapMap C. 2003. The International HapMap Project. Nature 426:789-796.

Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA, Witherspoon KT, Vives L, Patterson KE, Smith JD, Paeper B, Nickerson DA, Dea J, Dong S, Gonzalez LE, Mandell JD, Mane SM, Murtha MT, Sullivan CA,

Walker MF, Waqar Z, Wei L, Willsey AJ, Yamrom B, Lee YH, Grabowska E, Dalkic E, Wang Z, Marks S, Andrews P, Leotta A, Kendall J, Hakker I, Rosenbaum J, Ma B, Rodgers L, Troge J, Narzisi G, Yoon S, Schatz MC, Ye K, McCombie WR, Shendure J, Eichler EE, State MW, Wigler M. 2014. The contribution of de novo coding mutations to autism spectrum disorder. Nature 515:216-221.

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepansky A, Troge J, Andrews P, Bekritsky M, Pradhan K, Ghiban E, Kramer M, Parla J, Demeter R, Fulton LL, Fulton RS, Magrini VJ, Ye K, Darnell JC, Darnell RB, Mardis ER, Wilson RK, Schatz MC, McCombie WR, Wigler M. 2012. De novo gene disruptions in children on the autistic spectrum. Neuron 74:285-299.

Jenkinson M, Bannister P, Brady M, Smith S. 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17:825-841.

Ji T, Wu Y, Wang H, Wang J, Jiang Y. 2010. Diagnosis and fine mapping of a deletion in distal 11q in two Chinese patients with developmental delay. J Hum Genet 55:486-489.

Kan A, Ikeda T, Saito T, Yano F, Fukai A, Hojo H, Ogasawara T, Ogata N, Nakamura K, Chung UI, Kawaguchi H. 2009. Screening of chondrogenic factors with a real-time fluorescence-monitoring cell line ATDC5-C2ER: identification of sorting nexin 19 as a novel factor. Arthritis Rheum 60:3314-3323.

Kanner L. 1968. Autistic disturbances of affective contact. Acta Paedopsychiatr 35:100-136.

Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, Huguet G, Konyukh M, Chaste P, Ey E, Rastam M, Anckarsater H, Nygren G, Gillberg IC, Melke J, Toro R, Regnault B, Fauchereau F, Mercati O, Lemiere N, Skuse D, Poot M, Holt R, Monaco AP, Jarvela I, Kantojarvi K, Vanhala R, Curran S, Collier DA, Bolton P, Chiocchetti A, Klauck SM, Poustka F, Freitag CM, Waltes R, Kopp M, Duketis E, Bacchelli E, Minopoli F, Ruta L, Battaglia A, Mazzone L, Maestrini E, Sequeira AF, Oliveira B, Vicente A, Oliveira G, Pinto D, Scherer SW, Zelenika D, Delepine M, Lathrop M, Bonneau D, Guinchat V, Devillard F, Assouline B, Mouren MC, Leboyer M, Gillberg C, Boeckers TM, Bourgeron T. 2012. Genetic and Functional Analyses of SHANK2 Mutations Suggest a Multiple Hit Model of Autism Spectrum Disorders. PLoS Genet 8:e1002521.

Leblond CS, Nava C, Polge A, Gauthier J, Huguet G, Lumbroso S, Giuliano F, Stordeur C, Depienne C, Mouzat K, Pinto D, Howe J, Lemiere N, Durand CM, Guibert J, Ey E, Toro R, Peyre H, Mathieu A, Amsellem F, Rastam M, Gillberg IC, Rappold GA, Holt R, Monaco AP, Maestrini E, Galan P, Heron D, Jacquette A, Afenjar A, Rastetter A, Brice A, Devillard F, Assouline B, Laffargue F, Lespinasse J, Chiesa J, Rivier F, Bonneau D, Regnault B, Zelenika D, Delepine M, Lathrop M, Sanlaville D, Schluth-Bolard C, Edery P, Perrin L, Tabet AC, Schmeisser MJ, Boeckers TM, Coleman M, Sato D, Szatmari P, Scherer SW, Rouleau GA, Betancur C, Leboyer M, Gillberg C, Delorme R, Bourgeron T. 2014. Meta-analysis of SHANK Mutations in Autism Spectrum Disorders: a gradient of severity in cognitive impairments. PLoS Genet 10:e1004580.

Liu F, Arias-Vasquez A, Sleegers K, Aulchenko YS, Kayser M, Sanchez-Juan P, Feng BJ, Bertoli-Avella AM, van Swieten J, Axenovich TI, Heutink P, van Broeckhoven C, Oostra BA, van Duijn CM. 2007. A genomewide screen for late-onset Alzheimer disease in a genetically isolated Dutch population. Am J Hum Genet 81:17-31.

Mattina T, Perrotta CS, Grossfeld P. 2009. Jacobsen syndrome. Orphanet J Rare Dis 4:9.

McArthur T, Ohtoshi A. 2007. A brain-specific homeobox gene, Bsx, is essential for proper postnatal growth and nursing. Mol Cell Biol 27:5120-5127.

McNamee CJ, Reed JE, Howard MR, Lodge AP, Moss DJ. 2002. Promotion of neuronal cell adhesion by members of the IgLON family occurs in the absence of either support or modification of neurite outgrowth. J Neurochem 80:941-948.

Nasu-Nishimura Y, Hayashi T, Ohishi T, Okabe T, Ohwada S, Hasegawa Y, Senda T, Toyoshima C, Nakamura T, Akiyama T. 2006. Role of the Rho GTPase-activating protein RICS in neurite outgrowth. Genes Cells 11:607-614.

O'Roak BJ, Stessman HA, Boyle EA, Witherspoon KT, Martin B, Lee C, Vives L, Baker C, Hiatt JB, Nickerson DA, Bernier R, Shendure J, Eichler EE. 2014. Recurrent de novo mutations implicate novel genes underlying simplex autism risk. Nat Commun 5:5595.

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE. 2012. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485:246-250.

Pan Y, Wang KS, Aragam N. 2011. NTM and NR3C2 polymorphisms influencing intelligence: family-based association studies. Prog Neuropsychopharmacol Biol Psychiatry 35:154-160.

Penny LA, Dell'Aquila M, Jones MC, Bergoffen J, Cunniff C, Fryns JP, Grace E, Graham JM, Jr., Kousseff B, Mattina T, Syme J, Voullaire L, Zelante L, Zenger-Hain J, Jones OW, Evans GA. 1995. Clinical and molecular characterization of patients with distal 11q deletions. Am J Hum Genet 56:676-683.

Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z, Vorstman JA, Thompson A, Regan R, Pilorge M, Pellecchia G, Pagnamenta AT, Oliveira B, Marshall CR, Magalhaes TR, Lowe JK, Howe JL, Griswold AJ, Gilbert J, Duketis E, Dombroski BA, De Jonge MV, Cuccaro M, Crawford EL, Correia CT, Conroy J, Conceicao IC, Chiocchetti AG, Casey JP, Cai G, Cabrol C, Bolshakova N, Bacchelli E, Anney R, Gallinger S, Cotterchio M, Casey G, Zwaigenbaum L, Wittemeyer K, Wing K, Wallace S, van Engeland H, Tryfon A, Thomson S, Soorya L, Roge B, Roberts W, Poustka F, Mouga S, Minshew N, McInnes LA, McGrew SG, Lord C, Leboyer M, Le Couteur AS, Kolevzon A, Jimenez Gonzalez P, Jacob S, Holt R, Guter S, Green J, Green A, Gillberg C, Fernandez BA, Duque F, Delorme R, Dawson G, Chaste P, Cafe C, Brennan S, Bourgeron T, Bolton PF, Bolte S, Bernier R, Baird G, Bailey AJ, Anagnostou E, Almeida J, Wijsman EM, Vieland VJ, Vicente AM, Schellenberg GD, Pericak-Vance M, Paterson AD, Parr JR, Oliveira G, Nurnberger JI, Monaco AP, Maestrini E, Klauck SM, Hakonarson H, Haines JL, Geschwind DH, Freitag CM, Folstein SE, Ennis S, Coon H, Battaglia A, Szatmari P, Sutcliffe JS, Hallmayer J, Gill M, Cook EH, Buxbaum JD, Devlin B, Gallagher

L, Betancur C, Scherer SW. 2014. Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 94:677-694.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bolte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J,

Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI, Jr., Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C. 2010. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466:368-372.

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, Mason CE, Bilguvar K, Celestino-Soper PB, Choi M, Crawford EL, Davis L, Wright NR, Dhodapkar RM, DiCola M, DiLullo NM, Fernandez TV, Fielding-Singh V, Fishman DO, Frahm S, Garagaloyan R, Goh GS, Kammela S, Klei L, Lowe JK, Lund SC, McGrew AD, Meyer KA, Moffat WJ, Murdoch JD, O'Roak BJ, Ober GT, Pottenger RS, Raubeson MJ, Song Y, Wang Q, Yaspan BL, Yu TW, Yurkiewicz IR, Beaudet AL, Cantor RM, Curland M, Grice DE, Gunel M, Lifton RP, Mane SM, Martin DM, Shaw CA, Sheldon M, Tischfield JA, Walsh CA, Morrow EM, Ledbetter DH, Fombonne E, Lord C, Martin CL, Brooks AI, Sutcliffe JS, Cook EH, Jr., Geschwind D, Roeder K, Devlin B, State MW. 2011. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863-885.

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, Walker MF, Ober GT, Teran NA, Song Y, El-Fishawy P, Murtha RC, Choi M, Overton JD, Bjornson RD, Carriero NJ, Meyer KA, Bilguvar K, Mane SM, Sestan N, Lifton RP, Gunel M,

Roeder K, Geschwind DH, Devlin B, State MW. 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485:237-241.

Sellar GC, Watt KP, Rabiasz GJ, Stronach EA, Li L, Miller EP, Massie CE, Miller J, Contreras-Moreira B, Scott D, Brown I, Williams AR, Bates PA, Smyth JF, Gabra H. 2003. OPCML at 11q25 is epigenetically inactivated and has tumor-suppressor function in epithelial ovarian cancer. Nat Genet 34:337-343.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM. 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 Suppl 1:S208-219.

State MW, Levitt P. 2011. The conundrums of understanding genetic risks for autism spectrum disorders. Nat Neurosci.

Sugimoto C, Morita S, Miyata S. 2012. Overexpression of IgLON cell adhesion molecules changes proliferation and cell size of cortical astrocytes. Cell Biochem Funct.

Toro R, Chupin M, Garnero L, Leonard G, Perron M, Pike B, Pitiot A, Richer L, Veillette S, Pausova Z, Paus T. 2009. Brain volumes and Val66Met polymorphism of the BDNF gene: local or global effects? Brain Struct Funct 213:501-509.

Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, Coleman M, Leboyer M, Gillberg C, Bourgeron T. 2010. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends Genet 26:363-372.

Tyson C, Qiao Y, Harvard C, Liu X, Bernier FP, McGillivray B, Farrell SA, Arbour L, Chudley AE, Clarke L, Gibson W, Dyack S, McLeod R, Costa T, Vanallen MI, Yong SL, Graham GE, Macleod P, Patel MS, Hurlburt J, Holden JJ, Lewis SM, Rajcan-Separovic E. 2008. Submicroscopic deletions of 11q24-25 in individuals without Jacobsen syndrome: re-examination of the critical region by high-resolution array-CGH. Mol Cytogenet 1:25.

Vervoort VS, Viljoen D, Smart R, Suthers G, DuPont BR, Abbott A, Schwartz CE. 2002. Sorting nexin 3 (SNX3) is disrupted in a patient with a translocation t(6;13)(q21;q12) and microcephaly, microphthalmia, ectrodactyly, prognathism (MMEP) phenotype. J Med Genet 39:893-899.

Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. 2006. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. Mol Psychiatry 11:1, 18-28.

Zhang Y, Brady M, Smith S. 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 20:45-57.

Zhong L, Cherry T, Bies CE, Florence MA, Gerges NZ. 2009. Neurogranin enhances synaptic strength through its interaction with calmodulin. EMBO J 28:3027-3039.

Zielinski BA, Prigge MB, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, Fletcher PT, Zygmunt KM, Travers BG, Lange N, Alexander AL, Bigler ED, Lainhart JE. 2014. Longitudinal changes in cortical thickness in autism and typical development. Brain 137:1799-1812.

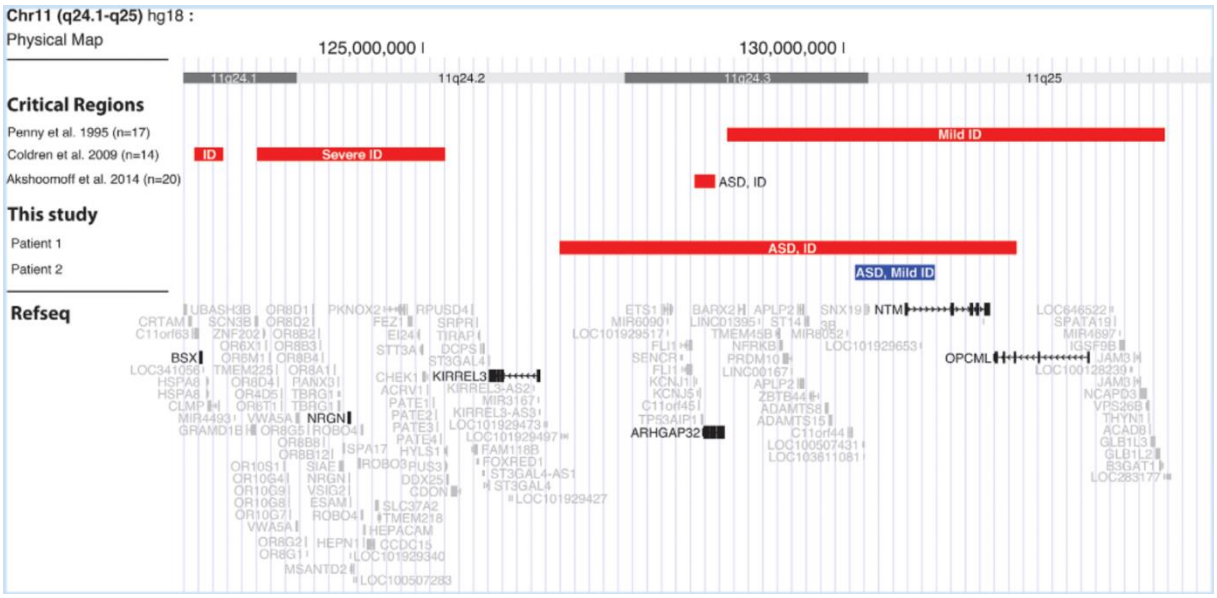

Figure 1. Location of the critical regions related to cognitive impairment in the Jacobsen syndrome region. Several critical regions could be causative for cognitive impairments in the Jacobsen syndrome region. These regions were delineated by the minimum overlap of 11q24.2-q25 micro rearrangements reported in studies with more than 10 patients with ASD and/or ID. Locations of both CNVs reported in this study were also mentioned. Deletions are in red and duplications in blue. In parallel, genes located in the region were added, based on Refseq information (https://genome.ucsc.edu/). Genes expressed in the brain are in black and the others in grey.

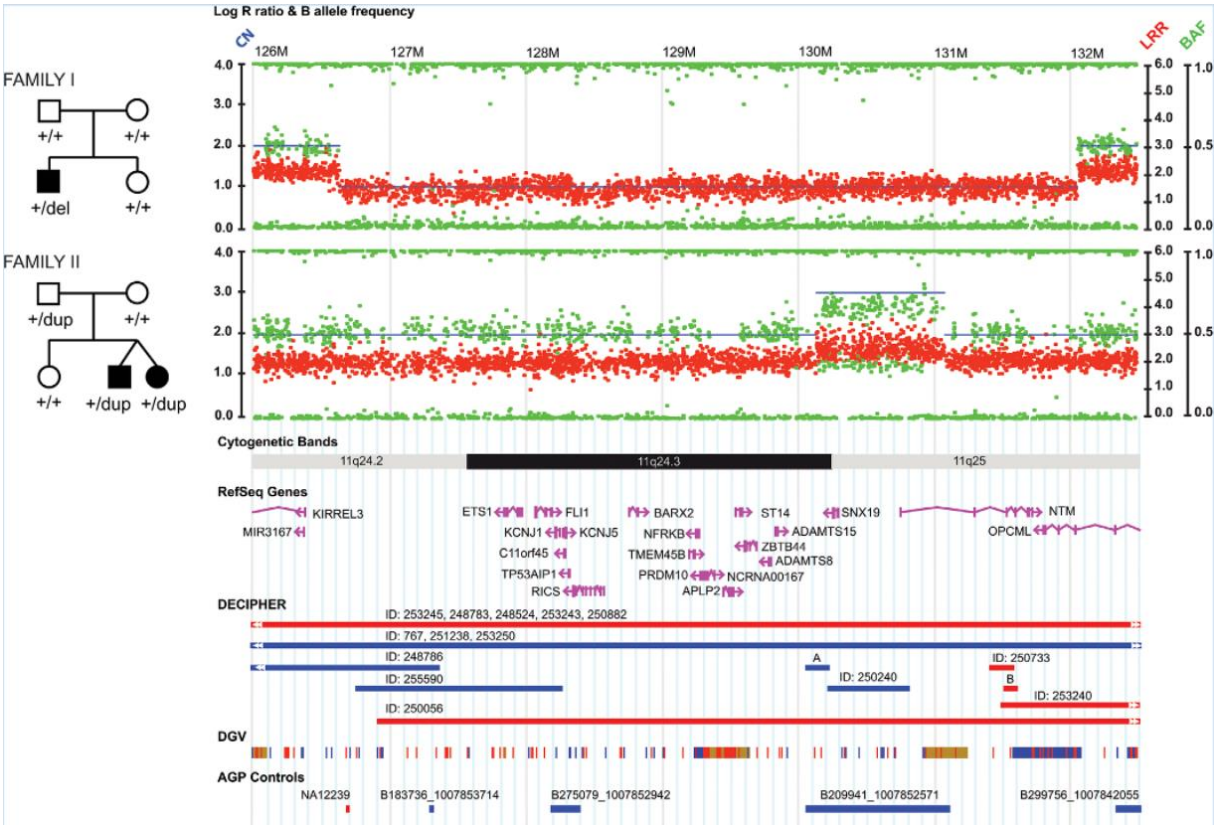

Figure 2. Rearrangements of 11q24.2-q25 were identified with the Human 1M-Duo SNP array from Illumina in both probands of family I and II. In Family I, the *de novo* deletion spans 5.4 Mb, covers 20 genes among which only two expressed in the brain, i.e., *NTM* and *RCIS*. In Familly II, the duplication spans 938 kb involves two genes (*SNX19* and the first exon of *NTM)*, is shared with the affected siblings and transmitted by the father. This latter had a lifetime diagnosis of attention deficit/ hyperactive disorder and general anxiety disorder, and also one of his paternal cousins had an ID. The upper plot shows Log R Ratio (LRR) (in red) and B allele frequency (BAF) (in green). QuantiSNP score is represented with a blue line and indicates the copy number (CN). No similar heterozygous copy number variations (sharing at least 80% overlap with one of these rearrangements) were previously referenced in the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/). At the opposite, in Decipher (http://decipher.sanger.ac.uk), several subjects with a development delay, carrying a large deletion or duplication within 11q24.2-q25, which encompassed *NTM*, are referenced. Also, three distinct CNVs affecting only *NTM* are reported: a duplication of the first exon, a deletion of the third exon, and a deletion of the third and the fourth exons. Red bars are deletions, and blue bars are duplications. **A**: Four patients (1617,2050, 2054,2049) with deletion (affecting NTM) and duplication of the region, **B**: Four patients (1969,1967,1970,2056) with duplications.

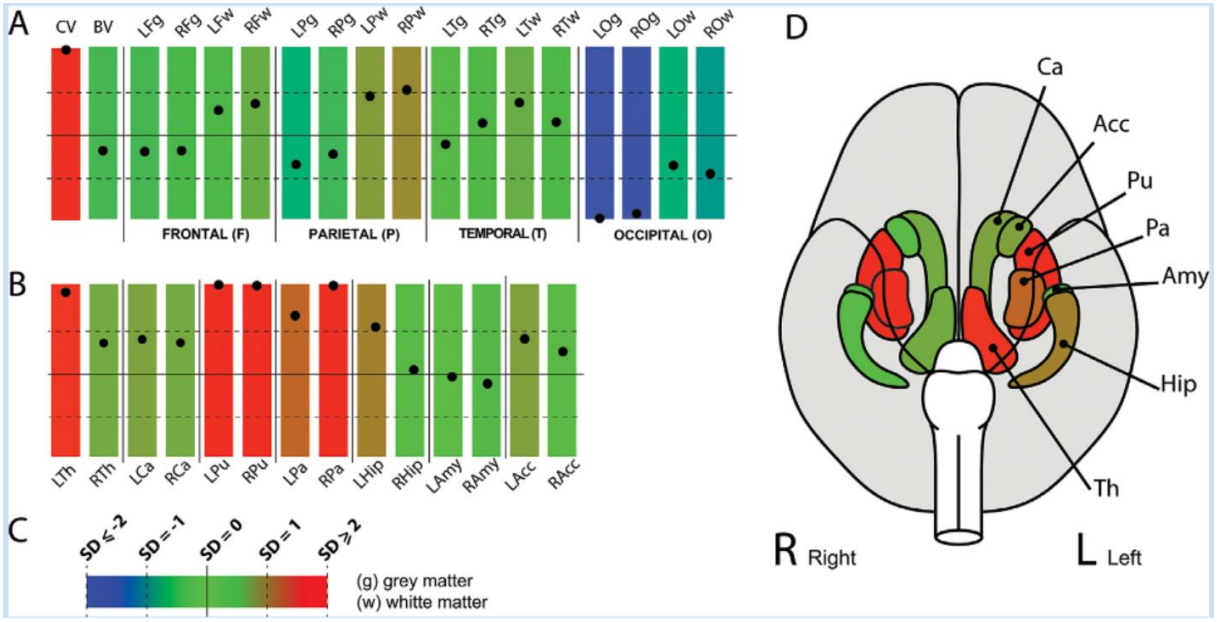

Figure 3. Schematic representation of cortical and subcortical structure volumes of a patient with a 11q24-25 de novo deletion compared to 254 subjects with ASD. **A**: Estimation of brain cortical volumes: whole brain volume (BV) and cortical volumes were obtained by automatic segmentation. Reported brain volumes are the sum of the grey (g) and white matter (w) volumes for each of the four Lobes [frontal (F), parietal (P), occipital (O), temporal (T)]. We converted intracranial and brain volume values into Z-scores. Black points represent the volumes observed in the subject compared to the cohort of 254 independent patients with ASD. **B**: Estimation of subcortical structures: Caudate nucleus (Ca), Nucleus accumbens (Acc), Putamen (Pu), Pallidum (Pa), Amygdala (Amy), Hippocampus (Hip), and Thalamus (Th). **C**: Legend used in this figure [−2 SD (blue), near the average of the cohorts (green), +2 SD (red)]. **D**: Cross sectional picture of brain.