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The 16p11.2 rearrangements: genetic paradigms for obesity and neurodevelopmental disorders

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The 16p11.2 rearrangements: genetic paradigms for obesity and neurodevelopmental disorders

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Prof. Luc Tappy

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Summary

Recent discoveries of recurrent and reciprocal Copy Number Variants (CNVs) using genome-wide studies have led to a new understanding of the etiology of neuropsychiatric disorders. CNVs represent loss (deletion) or gain (duplication) of genomic material. This thesis work is focused on CNVs at the 16p11.2 BP4-BP5 locus, which are among the most frequent etiologies of neurodevelopmental disorders and have been associated with Autism Spectrum Disorders (ASD), schizophrenia, cognitive impairment, alterations of brain size as well as obesity and underweight. Because deletion and duplication of the 16p11.2 locus occur frequently and recurrently (with the same breakpoints), CNVs at this locus represent a powerful paradigm to understand how a genomic region may modulate cognitive and behavioral traits as well as the relationship and shared mechanisms between distinct psychiatric diagnoses such as ASD and schizophrenia.

The present dissertation includes three studies: 1) The first project aims at identifying structural brain-imaging endophenotypes in 16p11.2 CNVs carriers at risk for ASD and schizophrenia. The results show that gene dosage at the 16p11.2 locus modulates global brain volumes and neural circuitry, including the reward system, language and social cognition circuits. 2) The second investigates the neuropsychological profile in 16p11.2 deletion and duplication carriers. While deletion carriers show specific deficits in language and inhibition, the profile of duplication carriers is devoid of specific weaknesses and presents enhanced performance in a verbal memory task. 3) The third study on food-related behaviors in 16p11.2 deletion and duplication carriers shows that alterations of the reponse to satiety are present in CNV carriers before the onset of obesity, pointing toward a potential mechanism driving the Body Mass Index increase in deletion carriers.

Dysfunctions in the reward system and dopaminergic circuitries could represent a common mechanism playing a role in the phenotype and could be investigated in future studies. Our data strongly suggest that complex cognitive traits correlate to gene dosage in humans. Larger studies including expression data would allow elucidating the contribution of specific genes to these different gene dosage effects. In conclusion, a systematic and careful investigation of cognitive, behavioral and intermediate phenotypes using a gene dosage paradigm has allowed us to advance our understanding of the 16p11.2 BP4-BP5 locus and its effects on neurodevelopment.

Résumé

La récente découverte de variations du nombre de copies (CNVs pour 'copy number variants') dans le génome humain a amélioré nos connaissances sur l'étiologie des troubles neuropsychiatriques. Un CNV représente une perte (délétion) ou un gain (duplication) de matériel génétique sur un segment chromosomique. Ce travail de thèse est focalisé sur les CNVs réciproques (délétion et duplication) dans la région 16p11.2 BP4-BP5. Ces CNVs sont une cause fréquente de troubles neurodéveloppementaux et ont été associés à des phénotypes « en miroir » tels que obésité/sous-poids ou macro/microcéphalie mais aussi aux troubles du spectre autistique (TSA), à la schizophrénie et au retard de développement/déficience intellectuelle. La fréquence et la récurrence de la délétion et de la duplication aux mêmes points de cassure font de ces CNVs un paradigme unique pour étudier la relation entre dosage génique et les traits cognitifs et comportementaux, ainsi que les mécanismes partagés par des troubles psychiatriques apparemment distincts tels que les TSA et la schizophrénie.

Ce travail de thèse comporte trois études distinctes : 1) l'étude en neuroimagerie structurelle identifie les endophénotypes chez les porteurs de la délétion ou de la duplication. Les résultats montrent une influence du dosage génique sur le volume cérébral total et certaines structures dans les systèmes de récompense, du langage et de la cognition sociale. 2) L'étude des profils neuropsychologiques chez les porteurs de la délétion ou de la duplication montre que la délétion est associée à des troubles spécifiques du langage et de l'inhibition alors que les porteurs de la duplication ne montrent pas de faiblesse spécifique mais des performances mnésiques verbales supérieures à leur niveau cognitif global. 3) L'étude sur les comportements alimentaires met en évidence une altération de la réponse à la satiété qui est présente avant l'apparition de l'obésité. Un dysfonctionnement dans le système de récompense et les circuits dopaminergiques pourrait représenter un mécanisme commun aux

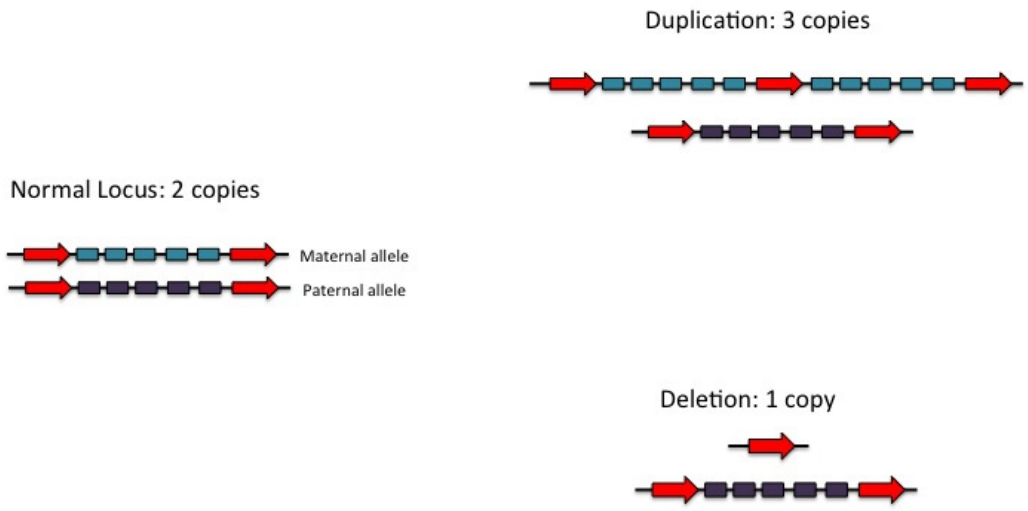
différents phénotypes observés chez ces individus porteurs de CNVs au locus 16p11.2. En conclusion, l'utilisation du dosage génique comme outil d'investigation des phénotypes cliniques et endophénotypes nous a permis de mieux comprendre le rôle de la région 16p11.2 BP4-BP5 dans le neurodéveloppement.

Introduction

1. Copy Number Variants

In recent years, research has moved from a "phenotype first" approach where group of patients were described based on their shared clinical phenotypes to a "genotype first" approach to study patients sharing similar molecular alterations (Watson et al., 2014). Advances in genomic techniques including comparative genome hybridization have made it possible to interrogate the whole genome for single nucleotide polymorphisms (SNPs) and copy number variants (CNVs, Watson et al., 2014). SNPs represent changes in an individual base of Deoxyribonucleic Acid (DNA). They are common (prevalence > 1 %) and contribute to phenotypic variation in the general population (Sachidanandam et al., 2001). The predictive value of one SNP is usually very small and genetic predisposition score associating multiple SNPs are often used to index genetic predisposition to a disease. Although research on rare SNPs associated with large effects in neuropsychiatric disorders is extremely active, they have not been investigated on a very large scale and designing genotype first cohorts remains difficult for most of these newly identified SNPs. CNVs differ from the SNPs in that they represent stretches of DNA with a deletion or a duplication ranging from a few hundred base pairs to several megabases **(Figure 1;** Morrow, 2010). There are on average >1000 CNVs widespread in the human genome, each one including none, a few or multiple genes (Conrad et al., 2010; Watson et al., 2014). CNVs significantly contribute to inter-individual variation and while some are benign, others predispose to diseases (Feuk et al., 2006; Watson et al., 2014). There are two major types of CNVs: recurrent and non-recurrent. Recurrent CNVs arise by non-allelic homologous recombination events during meiosis, with breakpoints in large duplicated blocks of sequence flanking the CNV event, which can confer genomic instability. In contrast, non-recurrent CNVs have breakpoints that generally lie within unique sequences and do not result from a predisposing genomic architecture (Watson et al., 2014).

Figure 1: Recurrent Copy Number Variants: deletion or duplication



Adapted from Morrow et al., 2010

Each chromosome contains two alleles: one copy of the paternal allele and one copy of the maternal allele. A segment of one allele containing few or many genes can be either deleted resulting in the presence of only one copy of the segment or duplicated resulting in three copies of the segment. Rectangles represent genes. Arrows represent flanking segmental duplications which underly the recurrent breakpoints.

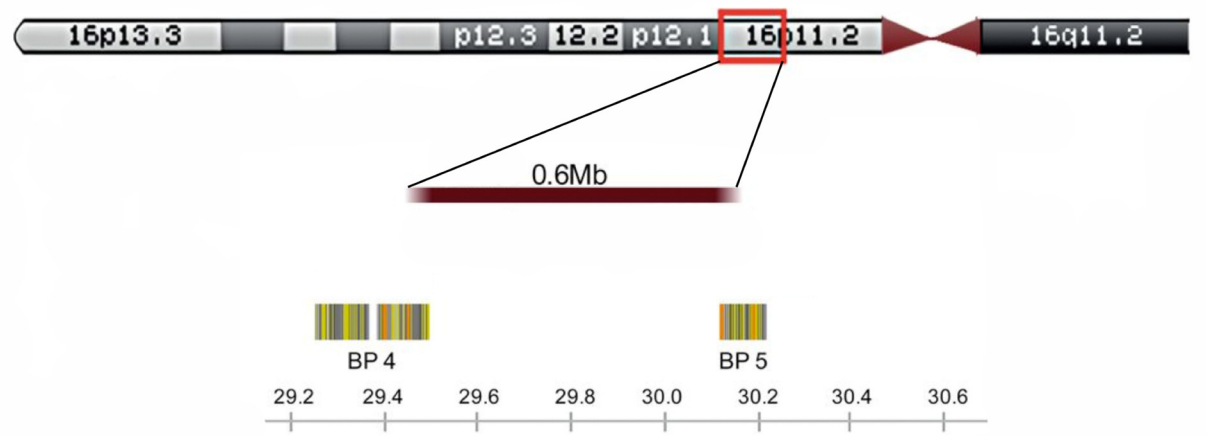
CNVs have been studied in neurodevelopmental and neuropsychiatric diseases and are now recognized as major contributors to mental disorders. Deleterious CNVs are identified in approximately 5-10% of patients presenting with autism spectrum disorders (ASD), schizophrenia, attention deficit/hyperactivity disorder (ADHD), developmental delay/intellectual disability (DD/ID), depressive and anxiety disorders (Doherty and Owen, 2014 ; Malhotra and Sebat, 2012). Interestingly, some of the identified CNVs are shared across disorders (Morrow, 2010). However, mechanisms by which these CNVs lead to clinical manifestations remain unknown. Current research aims at establishing links between genomic variations and their underlying mechanisms predisposing to phenotypic differences or disease. This thesis project focuses on the recurrent CNVs (deletion and duplication) at the 16p11.2 locus [BP4-BP5 or 29.6-30.2 – according to the human genome build GRCh37/hg19].

2. Deletion and duplication at the 16p11.2 locus

2.1 Genomic region

The 16p11.2 BP4-BP5 region (**Figure 2,** Online Mendelian Inheritance in Man [OMIM] #611913) contains 28 genes excluding those within the segmental duplications BP4 and BP5: *SPN, QPRT, C16orf54, ZG16, KIF22, MAZ, PRRT2, PAGR1 (C16orf53), MVP, CDIPT, CDIPT-AS, SEZ6L2, ASPHD1, KCTD13, TMEM219, TAOK2, HIRIP3, INO80E, DOC2A, C16orf92, FAM57B, ALDOA, PPP4C, TBX6, YPEL3, GDPD3, MAPK3 (Erk1), CORO1A* (Walters et al., 2010; Zufferey et al., 2012). The majority of these genes is expressed in the brain and can potentially be important for neurodevelopment. Recent studies in humans and animal models point towards contributions of some genes in this region to specific phenotypes. Genotype-phenotype correlation studies in humans have found an association between *PRRT2* and epilepsy (Dimassi et al., 2014), as well as *TBX6* and vertebral malformation (Fei et al., 2010). Animal studies demonstrated an association between *ALDOA/KIF22* or *KCTD13/MVP* and brain anatomy (Blaker-Lee et al., 2012; Golzio et al., 2012) and *TAOK2* has been found to be important for dendrite morphogenesis (de Anda et al., 2012).

Figure 2: The 16p11.2 Locus



Adapted from Zufferey et al. (2012)

This figure shows the proximal 600 kb region, delineated by BP4 and BP5 coordinates 29.6 and 30.2 Mb, respectively. Striated blocks indicate breakpoint regions and common sequence stretches within the segemental duplication blocks.

The prevalence of each CNV in the general population is 1/2000 individuals (Jacquemont et al., 2011; Zufferey et al., 2012). Deletion occurs *de novo* in 64% of the cases and is inherited in the remaining 36% (Zufferey et al., 2012). Maternal transmission is more frequent when it is inherited (Walters et al., 2010). Similar to several neurodevelopmental disorders, a gender bias is present in 16p11.2 CNVs with an overrepresentation of males (2:1) ascertained for DD/ID (Jacquemont et al., 2014).

2.2 Psychiatry

Deletion and duplication of this locus are among the most frequent genetic etiologies of mental disorders including DD/ID, ASD and schizophrenia (Bijlsma et al., 2009; Kumar et al., 2008; McCarthy et al., 2009; Weiss et al., 2008; Zufferey et al., 2012). Deletion was first described and associated with ASD in 2008 (Weiss et al., 2008) followed a year later by the association between duplication at the same locus and schizophrenia (McCarthy et al., 2009). We now know that both deletion and duplication carriers are at risk for ASD but only the

duplication is associated with schizophrenia and bipolar disorder (Malhotra and Sebat, 2012; McCarthy et al., 2009). Both CNVs represent one of the most frequent causes of ASD and explain about 1% of all ASD cases (Fernandez et al., 2010; Kumar et al., 2008; Marshall et al., 2008; Sanders et al., 2011; Weiss et al., 2008). While 15-20% of deletion carriers present with an ASD (Jacquemont et al., 2011; McCarthy et al., 2009; Zufferey et al., 2012), more than 70% of the carriers without ASD carry other mental diagnoses (APA, 2000) including ADHD, disruptive behavior as well as anxiety and/or depression (Zufferey et al., 2012). Although duplication carriers also have a significantly increased risk of developing ASD (McCarthy et al., 2009), the psychiatric phenotype is more complex with a 30-50% increased risk of presenting schizophrenia, bipolar, psychosis and related disorders (McCarthy et al., 2009; Steinberg et al., 2014). More specifically, duplication carriers have a 14.5 fold increased risk of developing schizophrenia on top of the significant association with bipolar disorder and psychosis (McCarthy et al., 2009).

2.3 Energy balance dysregulation

Deletion and duplication have also been associated with obesity and underweight respectively (Jacquemont et al., 2011; Walters et al., 2010; Zufferey et al., 2012). Obesity is defined by a BMI > 30 kg/m^2 in adults and > 2 standard deviation (SD) in children whereas underweight is characterized by a BMI < 18.5 kg/m^2 and < 2 SD in children (WHO, 2000). The 16p11.2 locus represents to date, with *MC4R*, the most frequent "mono-locus" form of obesity. The mechanism by which the deletion at this locus results in dysregulated energy balance remains unknown. While the deletion predisposes to obesity by increasing the risk 43-fold (Bochukova et al., 2010; Walters et al., 2010; Zufferey et al., 2012), duplication shows the reciprocal effect, leading to an 8-fold increased risk of being underweight in adulthood (Jacquemont et al., 2011; Shinawi et al., 2010). The penetrance of obesity in deletion carriers

is age-dependent (Jacquemont et al., 2011; Yu et al., 2011; Zufferey et al., 2012). While birth weight is below average (-0.61 SD), BMI z-score rapidly increases by 3.5 years of age and obesity is present in more than 50% of the carriers by age seven. 75% of adults are obese, of which 45% are morbidly obese (Zufferey et al., 2012). In duplication carriers, we know that birth parameters are normal but the natural history of the growth curve has not been described yet (Jacquemont et al., 2011). Studies using mouse models mimicking 16p11.2 deletion and duplication showed an inversed gene dosage effect on weight, with deletion mice being underweight whereas the duplicated mice are obese (Horev et al., 2011; Portmann et al., 2014).

Prevalence of obesity in the general adult population has doubled in the past two decades and prevalence of overweight has tripled in children and adolescents, making of obesity one of the current greatest healthcare problems (Chen et al., 2010; Kelly et al., 2008). Obesity has a multifactorial etiology including genetic and non-genetic factors. Although environment and life style play a significant role in obesity, heritability of BMI is 40 to 70% (Carnell et al., 2008; Maes et al., 1997). Common SNPs within the *FTO* gene have been associated with obesity (Frayling et al., 2007; Scuteri et al., 2007). More than 30 obesity susceptibility loci have been associated with BMI, many of which are expressed in or influence the central nervous system (Speliotes et al., 2010). Although the predictive value of these SNPs is weak, they point toward genes or pathways that may harbor rare mutation with large effect (Phan-Hug et al., 2012). Monogenic forms of obesity (e.g. *MC4R, POMC*) are thought to cause severe obesity through disruption of the hypothalamic functions (Valette et al., 2012) suggesting the implication of the central nervous system. The current literature suggests that obesity caused by genetic factors is associated with appetitive dysregulation and suggests that diminished satiety is one of the possible underlying mechanisms (Acosta et al., 2014; Ho-Urriola et al., 2014; Llewellyn et al., 2014; Wardle et al., 2008).

2.4 Impaired cognition

Since 2006, more than 20 recurrent CNVs have been associated with DD/ID (Watson et al., 2014). The latter is defined by an impaired cognitive functioning along with decreased adaptive skills in everyday life (APA, 2013). Studies on the 16p11.2 deletion have demonstrated a 27 to 32 points decrease in global cognition (Full Scale Intellectual Quotient - FSIQ) in carriers when compared to intra-familial controls (Hanson et al., 2014; Zufferey et al., 2012). Language is the only cognitive domain that has been more specifically explored and deficits have been reported in several case series on the deletion (Hanson et al., 2010; Shinawi et al., 2010). Recently, Hanson and colleagues investigated a large cohort of deletion carriers and documented below-average performances in comprehension, expression and reading skills along with 71% rate of speech and language disorder as defined in DSM-IV-TR (Hanson et al., 2014). Case series on duplication carriers have reported an important variability in global cognitive functioning and adaptive skills encompassing cases with moderate to severe DD/ID as well as seemingly unaffected transmitting parents (Fernandez et al., 2010; Rosenfeld et al., 2010; Shinawi et al., 2010). Along with the DD/ID, deletion carriers (37.6%) present with a delayed age of walking (mean age = 20.5 months) and later diagnosis of developmental coordination disorders (Hanson et al., 2014). A recent case-control association study showed that epilepsy - known to be associated with DD/ID - occurs at a rate of 18% in both CNVs, with the duplication being specifically associated with typical and atypical rolandic epilepsy (Reinthaler et al., 2014).

2.5 Brain development and structure

Head circumference (HC) constitutes another reciprocal phenotype in 16p11.2 CNVs. While macrocephaly (HC ≥ 2 SD) is present in 17% of the 16p11.2 deletion carriers, microcephaly (HC ≤ 2 SD) is present in 26.7% of the duplication carriers (Jacquemont et al., 2011; McCarthy et al., 2009; Zufferey et al., 2012). Deletion carriers have an HC lowered by 0.57 SD and at birth, which then increases during infancy (Zufferey et al., 2012). This pattern of brain growth is reminiscent of what has been reported in idiopathic ASD (Stanfield et al., 2008). Typical brain growth trajectory in ASD consists of an early overgrowth in overall brain volume followed by a cortical dysmaturation during childhood-adolescence and normal brain volume in adulthood (Baribeau and Anagnostou, 2013 ; Stanfield et al., 2008).

Animal models have given additional insight into the effect of the 16p11.2 locus on brain growth. Over- and under-expression of the *KCTD13* gene in zebrafish induce mirror alterations in brain volume interpreted as correlates of micro- and macrocephaly (Golzio et al., 2012). Under- and over-expression of *KCTD13* were also associated with increased and decreased neurogenesis in mouse (Golzio et al., 2012). Neuroimaging performed in murine models demonstrate reciprocal regional brain volume changes along with subtle changes in the midbrain (Horev et al., 2011).

In conclusion, the literature on the 16p11.2 CNVs provides first descriptions of the disorders, but is still sparse particularly regarding the duplication, which seems to involve a more complex and variable phenotype. The associations between the 16p11.2 CNVs and psychiatric disorders such as ASD and schizophrenia make of the 16p11.2 CNVs an interesting paradigm to better understand shared mechanisms between distinct diagnoses. Because deletion and duplication of the 16p11.2 locus occur frequently and recurrently (with

the same breakpoints), CNVs at this locus represent a powerful paradigm to understand how a genomic region may modulate brain anatomy, cognitive and behavioral traits.

3. Aims of the Thesis

Our main goal was to explore whether gene dosage of the 16p11.2 locus defined as the number of genomic copies (deletion =1, control =2, duplication =3) modulates brain structure as well as cognitive and behavioral traits.

The aims specific to each of the studies are as follows:

Study 1:

1/ Explore whether gene dosage at the 16p11.2 locus modulates brain anatomy.

2/ Identify neuroimaging endophenotypes in 16p11.2 CNV carriers at high risk for ASD and schizophrenia.

Study 2:

1/ Investigate the neuropsychological profile of both deletion and duplication carriers assessing specific cognitive domains (language, memory, attention, executive functions, fine motor skills) beyond global cognition.

2/ Explore whether gene dosage at the 16p11.2 locus can modulate cognitive performances.

Study 3:

1/ Investigate eating behaviors traits in both CNV carriers as well as investigate the specificity of these behaviors by comparing with clinical cohorts presenting with obesity or eating disorders.

2/ Investigate the relationship between BMI z-score and eating behaviors as well as inhibition skills.

4. General Methods

The participants included in these studies are taking part in a larger phenotyping project (16p11.2 European Consortium). The study protocol was reviewed and approved by the local Ethics committee and signed consents were obtained from participants or legal representatives prior to investigation. Participants were assessed at Lausanne University Hospital, Switzerland. The clinical geneticist who had established the diagnosis of CNV at the 16p11.2 locus in the context of a neurodevelopmental or mental disorder referred CNV probands to the study. Cascade testing allowed identification of additional relative carriers in the family. All had whole genome arrays confirming either a recurrent deletion or duplication in the 16p11.2 BP4-BP5 region. Inclusion criteria: Presence of a 16p11.2 deletion or duplication comprising the BP4-BP5 region (29.6-30.2 – according to the human genome build GRC37/hg19). Controls were non-carriers in the same families. Exclusion criteria: Age < 3 years.

Methodology specific to each part of this project is described in each of the three studies.

Study 1: The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity

The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity

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The supplemental material is presented in **Appendix 1**.

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Running head title: 16p11.2 gene dosage and brain structure

Key words: 16p11.2 CNV, voxel-based morphometry, surface-based morphometry, diffusion-weighted imaging, autism, schizophrenia, obesity.

Abstract

Anatomical structures and mechanisms linking genes to neuropsychiatric disorders are not deciphered. Reciprocal copy number variants at the 16p11.2 BP4-BP5 locus offer a unique opportunity to study intermediate phenotypes in carriers at high risk for autism spectrum disorder (ASD) or schizophrenia (SZ). We investigated variation in brain anatomy in 16p11.2 deletion and duplication carriers. Beyond gene dosage effects on global brain metrics, we show that the number of genomic copies is negatively correlated to grey matter volume and white matter tissue properties in cortico-subcortical regions implicated in reward, language and social cognition. Despite the near absence of ASD or SZ diagnoses in our 16p11.2 cohort, the pattern of brain anatomy changes in carriers spatially overlaps with the well-established structural abnormalities in ASD and SZ.

Using measures of peripheral mRNA levels, we confirm our genomic copy number findings. This combined molecular, neuroimaging and clinical approach, applied to larger datasets, will help interpret the relative contributions of genes to neuropsychiatric conditions by measuring their effect on local brain anatomy.

Introduction

Copy number variants (CNVs) are major contributors to common neuropsychiatric disorders and cognitive deficits[1]. Investigations of cohorts with specific CNVs allow the characterization of endophenotypes[2] associated with neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (SZ). The 16p11.2 CNV (breakpoint 4 to 5, BP4-BP5, 29.6-30.2Mb- Hg19) phenotypes are characterized by both reciprocal and overlapping deficits that include energy imbalance, language impairment, ASD and SZ [3-8]. Notably, both 16p11.2 deletion and duplication have been associated with ASD while only the duplication is enriched in SZ cohorts [9,10]. Deletion carriers present with increased head circumference[6,8] and body mass index (BMI)[3,7], while duplication carriers show microcephaly and are underweight [5]. Manipulations of zebrafish embryos and mouse models suggest a close relationship between gene dosage at this locus and brain anatomy. Over- and under-expression of the *KCTD13* gene in zebrafish induces mirror alterations interpreted as correlates of micro- and macrocephaly[11]. Murine models mimicking 16p11.2 deletion and duplication demonstrate reciprocal regional brain volume changes [12].

The aim of this study is to identify imaging endophenotypes in a group of 16p11.2 CNV carriers at high risk for ASD and SZ. We investigate the effects of gene dosage, defined as the number of genomic copies at the 16p11.2 locus on brain structure using state-of-the-art structural magnetic resonance imaging (sMRI). We find a correlation between gene dosage and alterations in brain structure with diametrically opposite changes in both global and local brain volumes that parallel specific changes in tissue microstructure. The anatomical areas affected by gene dosage are also key areas involved in ASD, SZ and obesity, supporting the notion that common molecular mechanisms may be involved in these conditions.

Methods

Participants: The study was reviewed and approved by the local Ethics committee and signed consents were obtained from participants or legal representatives prior to investigation. Participants (**Table 1**) were taking part in a larger phenotyping project on the deletion/duplication of the 16p11.2 region. Carriers were referred to the study by the clinical geneticist who had initially established the genetic diagnosis in the context of a neuro-developmental disorder. Inclusion criteria: Participants were selected based on the presence of a 16p11.2 deletion or duplication comprising the BP4-BP5 region. Controls were non-carriers in the same families. Exclusion criteria: None beside an age < 6 years. Seventeen participants were unable to complete the scan due to incompliance related to moderate or severe intellectual disability, anxiety, significant behavioral issues or extreme BMIs with waist circumference beyond the limit of scanning safety standards.

All had whole genome arrays confirming either a recurrent deletion or duplication of the BP4-BP5 region. The larger project aims at phenotyping a European cohort of 16p11.2 rearrangement carriers. It includes neuropsychological and behavioral assessments, medical, psychiatric and neurological examinations.

Anthropometric measures, psychiatric and cognitive assessment: We collected anthropometric data such as height and weight to calculate the Body Mass Index (BMI). Obesity is defined as BMI > 30 kg/m^2 in adults and > 2 standard deviations in children[13]. Underweight is considered significant < 18.5 kg/m^2 and < 2 standard deviations in children[13]. Z-scores were computed for all data using gender, age, and geographically matched reference population as previously described in Zufferey et al.[8]. Overall cognitive functioning was measured using the Wechsler Intelligence scales for children (WISC-IV)[14] as well as the Wechsler Intelligence scale for adults (WAIS-III)[15]. All assessments were performed by a board certified neuropsychologist. DSM-IV-TR[16] diagnoses were made by licensed

psychologist and psychiatrist using history, parent report as well as the Diagnostic Interview for Genetic Studies (DIGS)[17]. An additional assessment was performed to investigate prodroms of schizophrenia using the Schizophrenia Proneness Instrument Adult version (SPI-A)[18]. The diagnosis of ASD was established by a certified clinician using the Autism Diagnostic Interview-Revised (ADI-R)[19] and the Autism Diagnostic Observation Schedule (ADOS)[20]. Of note, only two duplication carriers were on medication: lithium, aripiprazol and valproate.

Quantitative RT-PCR: For QPCR, 100 ng of high-quality total RNA isolated from Epstein-Barr virus transformed lymphoblastoid cell lines was converted to cDNA using Superscript VILO (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Primers were designed using PrimerExpress 2.0 software (Applied Biosystems, Foster City, CA, USA), with default parameters except for the primer- and minimal amplicon lengths, which were set at 17-26 bp and 60 bp respectively. The amplification factor of each primer pair was tested using a cDNA dilution series and only assays with amplification factors between 1.75 and 2.00 were retained. A representative set of samples was tested for genomic contamination. QPCR experiments were performed in triplicates using SYBR-Green (Roche, Basel, Switzerland) as reporter. The reaction mixtures were prepared in 384-wells plates using a Freedom Evo robot (Tecan, Männedorf, Switzerland) and run in an ABI 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA) using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and then 60°C for 1 minute, after which dissociation curves were established. Applicable normalization genes were included in each experiment to enable compensation for fluctuations in expression levels between experiments. Using SDS v2.4 software (Applied Biosystems, Foster City, CA, USA) the threshold and baseline values were adjusted when necessary to obtain raw cycle threshold (Ct) values. The Ct values were further analysed

using qBase plus software (Biogazelle, Zwijnaarde, Belgium), which calculates relative expression values per sample per tested gene upon designation of the normalization genes and corrects for the amplification efficiency of the performed assay.

MRI Data Acquisition and processing: All participants were examined on a 3T whole body scanner (Magnetom TIM Trio, Siemens Healthcare, Erlangen, Germany) using a 12-channel RF receive head coil and RF body transmit coil. Participants presenting with moderate to severe clinical phenotype were unable to undergo the scanning procedure. A total of 57 participants underwent the MRI protocol, which included T1-weighted (T1w) and diffusion-weighted (DWI) data acquisition. Anatomical images were acquired using a multi-echo magnetization prepared rapid gradient echo sequence (ME-MPRAGE: 176 slices; 256x256 matrix; echo time (TE): TE1: 1.64 ms, TE2: 3.5 ms, TE3: 5.36ms, TE4: 7.22ms; repetition time (TR): 2530ms; flip angle 7°). The DWI protocol consisted of 2 mm contiguous slices covering the whole brain (TE = 83ms, TR = 9020ms) along 60 spherically-distributed gradient directions with b-value = 700s/mm2 with 10 reference images with no diffusion weighting (b-value = 0 s/mm2). Due to movement artifacts, 3 out of 57 T1w images and 5 DWI out of 45 who were able to complete the DWI protocol were excluded.

Multi-echo T1w images were averaged then classified into probability of belonging to gray matter (GM), white matter (WM) or cerebrospinal fluid (CSF) using Gaussian mixture model within the "unified segmentation" framework[21]. Images were transformed non-linearly to standard Montreal Neurological Institute (MNI) space using the diffeomorphic spatial registration algorithm (DARTEL) implemented in SPM8 [22]. GM probability maps were subsequently "modulated" by the Jacobian determinants of the deformations to account for local compression and expansion due to linear and non-linear transformation [23]. Finally, GM probability maps were smoothed with an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM).

Cortical surface extraction was performed on the averaged multi-echo T1-w images using the default settings of the Freesurfer software [24] (http://surfer.nmr.mgh.harvard.edu/). Individual images were examined for potential defects of surface reconstruction. Mean cortical thickness and cortex surface area were then computed for each subject.

Diffusion-weighted images were corrected for Eddy current and motion artifacts with the Artefact correction in diffusion MRI (ACID toolbox) [25]. Diffusion tensor based indices - fractional anisotropy (FA) and mean diffusivity (MD), were computed with the Camino Diffusion MRI toolkit [26]. FA and MD maps were aligned to the T1w images using affine transformation. In order to enhance the specificity for a particular tissue class and to avoid diffusion indices value changes due to Gaussian smoothing kernel during the spatial registration to MNI space, we applied a previously described combined weighting/smoothing procedure [27].

For voxel-based statistical analysis of gene dosage-dependent regional effects we used a linear regression model in the General Linear Model framework of SPM8. Age effects were analyzed separately for GM and WM sub-space by creating two corresponding design matrices. Explicit masking using binary masks of GM and WM ensured inclusion of the same number of voxels in all analyses. All GM/WM data were included in the same model with gender, total intracranial volume (the sum of GM, WM and CSF volume) and 1st order polynomial expansion of age as regressors. We tested for overall cognitive functioning (FSIQ), but as results did not show significant effect on whole brain or regional structures, we did not add it as a covariate in the SPM analyses. Statistical thresholds were applied at $p<0.05$ after family-wise error (FWE) correction for multiple comparisons over the whole volume of the GM/WM mask. Trends were assessed by using an auxiliary uncorrected voxel threshold of $p<0.001$[28].

Multivariate linear models analysis: Multivariate Linear model (MLM) was used to discover high-order correlation mapping between the two datasets: gene expression and brain volume. The multivariate approach takes into account the full dimensionality of both data sets (all voxels and all mRNA levels for all individuals). The method simultaneously determines the best representations in each datasets in order to explain maximum covariance between gene expression and volume. The representation in the brain space is called eigenimage and show the level contribution (either positive or negative) for each voxel to the correlation mapping. Similarly, the gene loadings (positive or negative) show the contribution of each gene to the correlation mapping. The number of mappings and the significance of the mapping is assessed with Wilks' Lambda statistic [28,29].

Statistical Analyses: Statistical analyses were performed using Matlab and R 3.0.2. Plots and heat maps have been generated using R libraries ggplot2 and gplots, respectively.

Results

Study participants and MRI measures: We acquired data on fourteen 16p11.2 BP4-BP5 deletion and seventeen duplication carriers as well as twenty-three intra-familial controls. The median age of participants was 34 years (range 7-58). The three groups were not significantly different in terms of age, gender or handedness (**Table 1**). Overall cognitive functioning (full scale intellectual quotient- FSIQ) in deletion and duplication carriers was 2 standard deviations (≈ 27 points) below that in the control group, which is consistent with previous studies [5,8]. Two duplication carriers met diagnostic criteria for ASD and none of the participants had clinical signs of SZ (all clinical data and neuro-radiological findings are presented in **Supplementary Tables 1 and 2**).

Table 1: Population characteristics

	Deletion **n = 14**	Controls **n = 23**	Duplication **n = 17**
Age in years (mean± SD)	24.6 ± 13.3	33 ±12.3	35.7 ±12.5
Gender (M/F)	8 / 6	11/ 12	10 / 7
Handedness (L/R)	3 / 11	0 / 23	3 / 14
BMI z-score (mean ±SD)	2.37 ± 2.14 ¶	0.37 ± 1.07	-0.56 ± 1.61
FSIQ (mean± SD)	74 ± 14§	98 ± 13	72 ± 16§
NVIQ (mean±SD)	79 ± 11§	102 ± 13	73 ± 15§

M, male; F, female; L, left; R, right; BMI, body mass index; FSIQ, full-scale intellectual quotient; NVIQ, non-verbal intellectual quotient.
¶ significantly different from the two other groups, ANCOVA, post-hoc group comparisons, p <.05 Bonferroni corrected
§ significantly different from the control group, ANCOVA, post-hoc group comparisons, p < .05 Bonferroni corrected

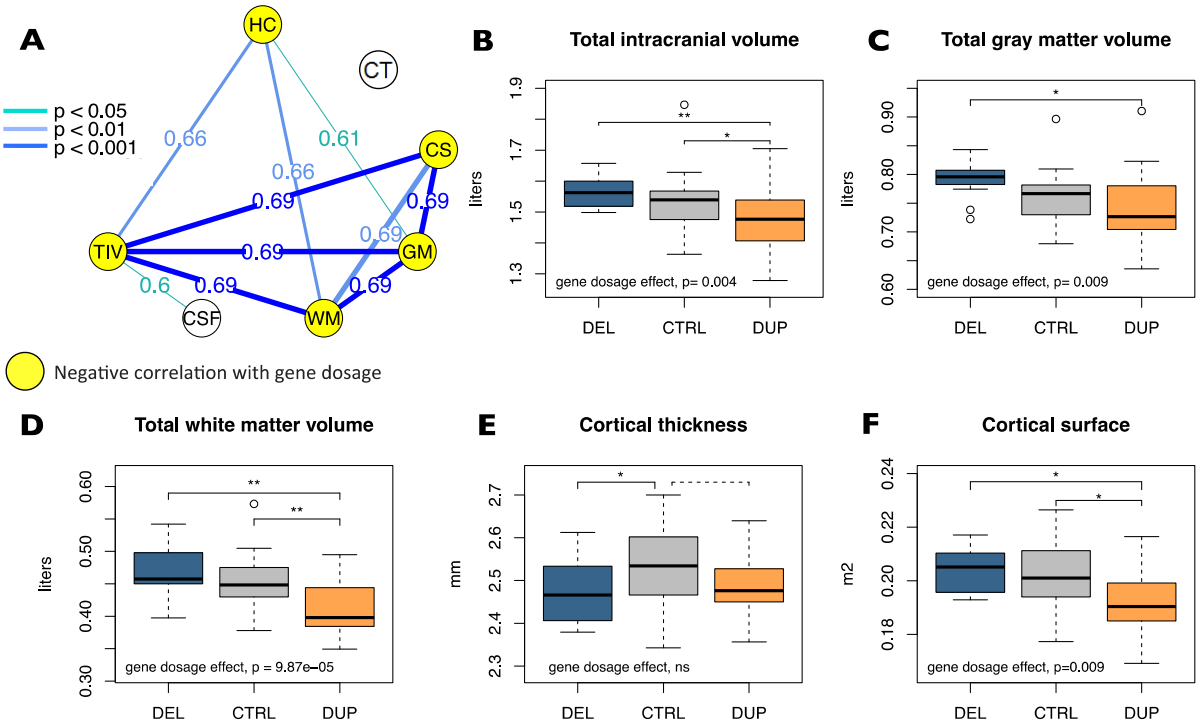
In an unbiased whole-brain approach we analyzed regionally derived sMRI estimates of gray (GM) and white matter (WM) volume, cortical thickness and surface area, and also total intracranial volume (TIV)[24,30]. We also investigated brain microstructure using an independent data set of diffusion-weighted images from the same subjects, from which we computed fractional anisotropy (FA) and mean diffusivity (MD) as indices of local tissue integrity [31]. Gene dosage effects on brain anatomy were tested explicitly over the cohorts of deletion carriers, controls and duplication carriers using differential contrasts and conjunction analyses.

Global brain differences: We first tested the sensitivity of our whole-brain imaging approach to detect global effects of gene dosage since early brain overgrowth is a feature common to both ASD and the 16p11.2 deletion[8,32]. We confirm the strong correlation between head circumference and gene dosage in the studied group (p=0.0001) [5,7] and demonstrate the

negative correlation between TIV and number of 16p11.2 copies (p=0.004, **Fig. 1B**). Both GM (p=0.009) and WM volume (p=9.8 e-05) contribute to the observed effect on TIV (**Fig. 1C, D**). There is no significant correlation between gene dosage and cerebrospinal fluid volume (CSF) suggesting that there are no compensatory CSF changes secondary to brain atrophy. The analysis of cortical anatomy shows correlation between gene dosage and global cortical surface area (p=0.009, **Fig. 1F**). As opposed to global measures of volume and surface, which are all inter-correlated (**Fig. 1A**) and modulated by gene dosage, only cortical thickness is decreased in both deletions and duplication carriers (**Fig. 1A, E**). This may be related to previously reported relationship between intellectual disability and cortical thickness [33,34]. Of note, adjusting for IQ does not affect our findings (**Supplementary Fig. 1).** Analyses on global metrics are detailed in **Supplementary Table 3**.

There is a strong correlation between BMI and head circumference in 16p11.2 CNV carriers [8]. We also find a negative correlation between BMI and gene dosage ($p < 0.001$, **Table 1**). Total GM volume (p=0.008) and TIV (p= 0.019) are correlated to BMI (**Supplementary Table 4**) and this relationship becomes borderline significant (p=0.06) after adjusting for the copy number effect on BMI.

Figure 1. Effects of gene dosage on global brain metrics



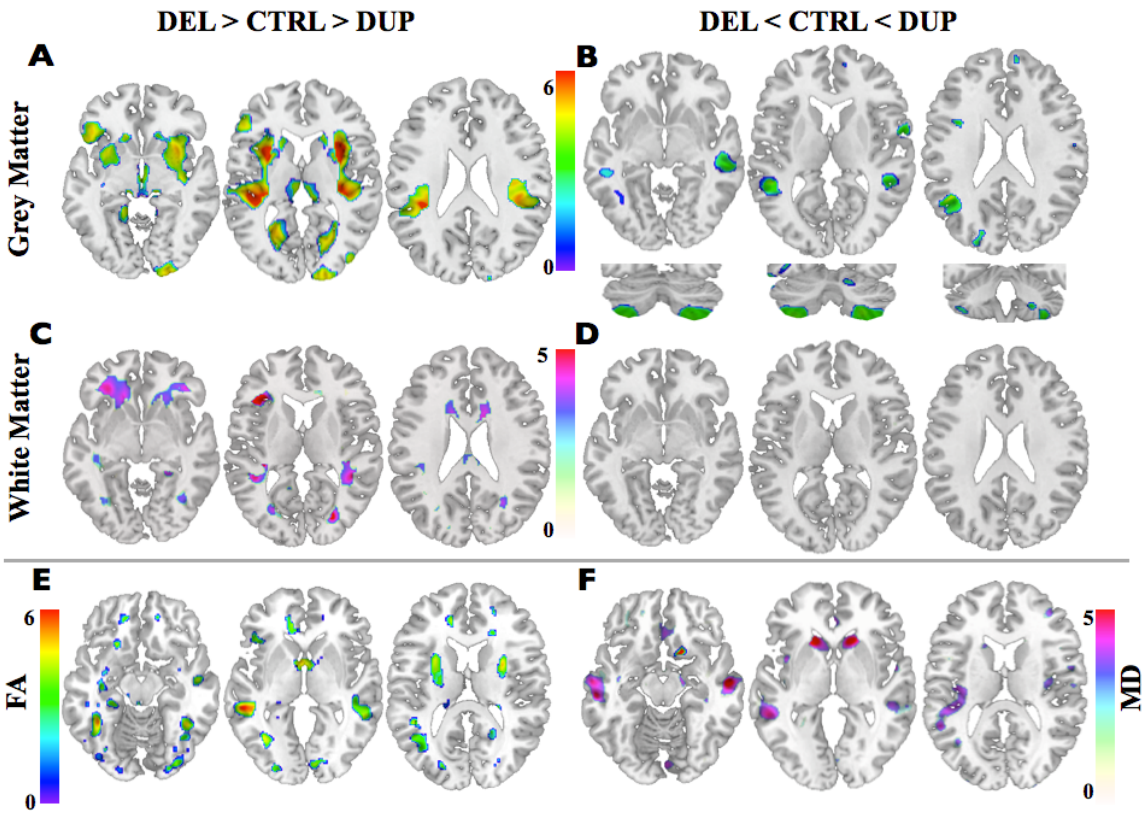
*(A): Correlation between global brain metrics in 16p11.2 CNV carriers and controls. The intensity of blue lines represents positive r Pearson's coefficient correlations at different statistical thresholds. All global measures are inter-correlated except for CT and CSF. (B - F): Boxplots representing TIV, GM, WM volume, cortical surface area and cortical thickness adjusted for age and gender in deletion, duplication carriers and intra-familial controls. ** p ≤ 0.001; * p ≤ 0.05 uncorrected. Gene dosage effect is estimated in a linear regression analysis using the number of copies (1, 2 or 3), and including age, gender as covariates. Significant differences between groups after Bonferroni correction (threshold at p<0.01) are represented by solid black lines, trends – by dashed lines.*

HC - head circumference; TIV - total intracranial volume; GM - gray matter; WM - white matter; CSF - cerebrospinal fluid; CT - cortical thickness; CS - cortical surface area; DEL-deletion carriers; CTRL - intra-familial controls; DUP - duplication carriers

Regional volume brain differences: Using the same statistical design and voxel-based morphometry, we analyzed local GM and WM changes beyond global volume effects. The spatial pattern of gene dosage associated changes overlaps with key areas of the reward system including the medio-dorsal thalamus, insula, ventral striatum, orbito-frontal cortex and white matter corresponding to fronto-striatal projections (**Fig. 2A, C**). The language circuitry, comprising the left middle temporal gyrus, bilateral supramarginal and superior temporal gyri

and adjacent white matter connections, also shows strong gene dosage dependence (**Fig. 2A, B, C** and **Supplementary Table 5**). We further observe gene dosage effects on cerebellar anatomy, including lobules VIIb, VIII and crus II bilaterally (**Fig. 2B**). Subsequent gender-by-gene dosage interaction analysis demonstrates that this effect is mainly driven by decreased volume in male deletion carriers (**Supplementary Fig. 2**). The analysis of an independent dataset of diffusion-weighted images sensitive to water diffusion properties of brain tissue in a subset of forty-five participants confirms a reciprocal gene dosage effect on reward and language circuit associated regions – striatum, middle and superior temporal gyrus (**Fig. 2 E, F**). The changes of diffusion-tensor derived indices (FA and MD) suggest a dosage dependent effect on brain microstructure beyond the volume changes already described [35].

Figure 2. Effects of gene dosage on local brain volume and tissue properties



Results of voxel-based whole brain general linear analyses showing: (A) negative gene dosage effect (DEL > CTRL > DUP) on GM volume in ventral striatum, thalamus, superior temporal region, fusiform, precuneus, insula and calcarine sulci bilaterally as well as in right occipital region. (B) positive gene dosage effect (DEL

< CTRL < DUP) on GM volume in the middle temporal gyrus and in cerebellar lobule VIII, VIIb and crus II. (C) negative gene dosage effect on WM volume within fronto-striatal projections, anterior thalamic and superior longitudinal fasciculus. (D) absence of significant positive gene dosage effect on WM volume. (E-F) FA and MD changes overlapping with GM changes in superior temporal gyrus and caudate bilaterally.
For representation purposes, results significant at a voxel level at threshold of p < 0.05 family-wise error corrected for multiple comparisons are displayed at significance threshold of p < 0.001 uncorrected at voxel level in standard Montreal Neurological Institute space. Color bars represent T scores.
DEL – deletion carriers; CTRL – intra-familial controls; DUP – duplication carriers; GM - gray matter; WM - white matter; FA – fractional anisotropy; MD – mean diffusivity

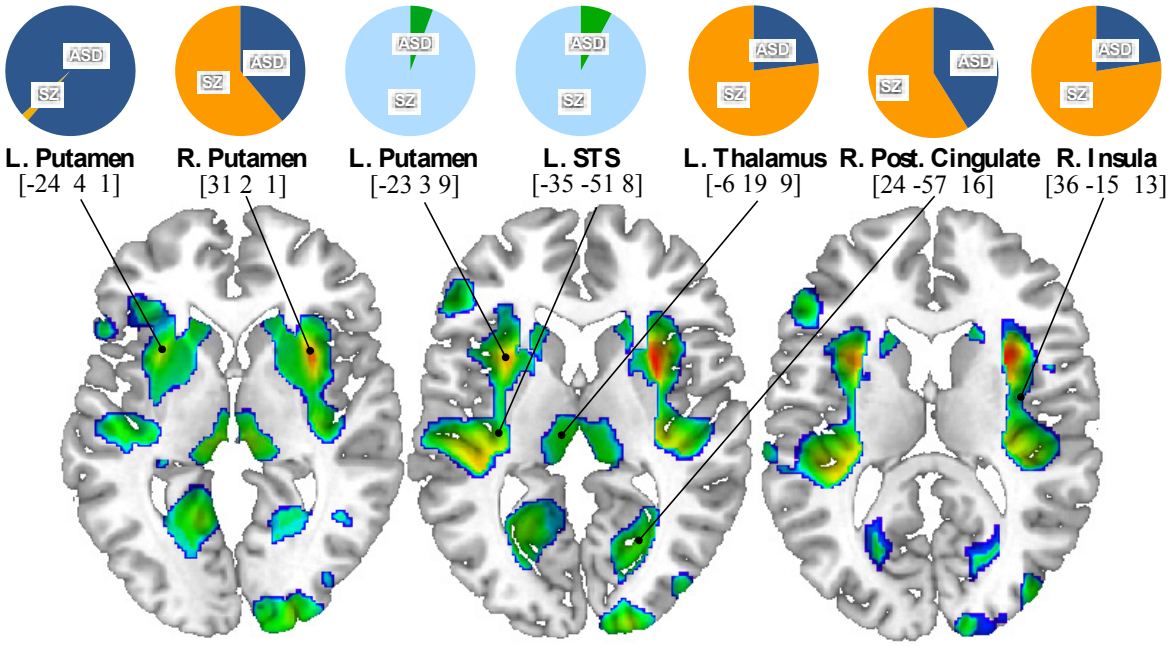
We further investigated the contribution of the deletion and duplication to the gene dosage analysis (deletion > control > duplication) results on brain structure. We performed a conjunction analysis testing the intersection of the two differential contrasts: (deletion > control) ∩ (control > duplication) (**Supplementary Fig. 3)**. This stringent analysis shows that both the deletion and duplication contribute to the negative correlation between gene dosage and putamen volume. The two differential contrasts suggest that changes in the reward system are driven by the duplication, while deletion carriers contribute to modifications in language and social cognition networks (**Supplementary Fig. 3** and **Supplementary Table 5**). Due to the stringency of the conjunction analysis, we cannot exclude rejecting modest brain changes following the gene dosage dependent pattern. A larger sample size would be required to identify brain anatomy changes specific to either deletion or duplication carriers.

Regional cortical thickness and surface area differences: We observed cortical thickness changes mainly driven by deletion carriers in the fronto-temporal regions, particularly in insula, supramarginal and superior temporal gyrus (**Supplementary Table 6 and Supplementary Fig. 4**). These regions overlap spatially with the cortical volume changes derived in the whole-brain voxel-based morphometry analysis. For measures of cortical surface area, the overlap is restricted to the frontal pole.

16p11.2 dosage-related brain alterations common to idiopathic ASD and SZ. The 16p11.2 CNVs that confer high risk for ASD and SZ exemplify the concept of shared genetic factors in psychiatric disorders. We formally tested the spatial overlap between the described brain

patterns and the results of a recent meta-analysis in ASD and SZ brain morphometry [36] (**Fig. 3**). Areas subject to strong 16p11.2 gene dosage effects overlap with six out of the eight brain structures most commonly affected in ASD and SZ - left and right putamen, insula, posterior cingulate, thalamus and superior temporal gyrus, but with none of the SZ-specific regions [36] (**Fig. 3** and **Supplementary Table 7**).

Figure 3. Spatial mapping of meta-analysis data in autism spectrum disorders and schizophrenia on to 16p11.2 gene dosage brain pattern



Projection of meta-analysis data in ASD and SZ [36] on the statistical map of 16p11.2 gene dosage effects on gray matter volume in deletion and duplication carriers (DEL > CTRL > DUP). Pie charts represent previously published data on the relative contribution of ASD and SZ to brain volume change at particular location [36]. Orange-blue pie charts indicate gray matter decreases; cyan-green pie charts show gray matter increases in ASD and SZ. Coordinates correspond to Montreal Neurological Institute standard space.

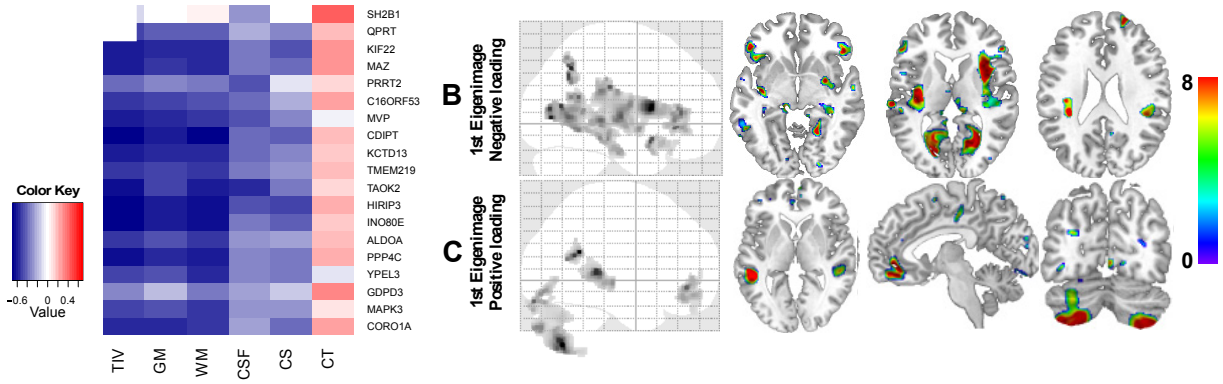
ASD - Autism spectrum disorder; SZ – schizophrenia; STS – superior temporal sulcus

mRNA expression levels and brain anatomy: 16p11.2 BP4-BP5 CNVs either delete or duplicate 28 genes. We therefore tested whether the effects of gene dosage on brain anatomy

are mediated by changes in gene expression measured in lymphoblastic cell lines of twenty-seven of our participants. We investigated in a linear regression analysis mRNA levels of eighteen genes mapping within and one gene *(SH2B1)* outside the BP4-BP5 interval. While *SH2B1* expression is not affected by changes in copies of the BP4-BP5 CNVs, mRNA levels for all eighteen genes within the interval are correlated to the number of genomic copies and twelve of these genes show strong correlation (Pearson r >0.75) with each other (**Supplementary Fig. 5**).

mRNA levels of all assessed genes within the BP4-BP5 interval except *GDPD3* and *PRRT2* show negative correlation with global metrics of brain volume (**Fig. 4 A** and **Supplementary Table 8**). To characterize the differential contributions of highly correlated gene expression levels on brain structure, we performed whole-brain multivariate analyses based on singular value decomposition. This method identifies the linear combination of brain voxels – eigenimages - that are best predicted by a linear mixture of gene expression levels. Despite the reduced subject sample size, this analysis replicated the anatomical pattern described by categorical analyses of gene dosage effects (**Figs. 2, 4**). The first eigenimage is characterized by negative loadings on the striatum, fusiform gyrus and thalamus bilaterally (**Fig. 4B**), as well as positive loadings on both cerebellar hemispheres (**Fig. 4C**). Put simply, voxels with negative loadings indicate that low mRNA levels are associated with increased GM volumes while those with positive loadings with GM volume reduction. In our cohorts the first three eigenimages explain more than thirty percent of variance in brain anatomy, and are mainly driven by the number of genomic copies (p=0.003, p=0.01, p=0.07 for the first three eigenvariates respectively). The correlation between gene expression and the number of genomic copies (**Supplementary Fig. 5)** as well as the high level of shared variance between genes at this locus preempts identification of the contributions of individual gene to specific anatomical patterns (**Supplementary Table 9 and Fig. 6**).

Figure 4. Effects of mRNA levels on global and local metrics of brain anatomy



Brain anatomy changes explained by mRNA levels of 18 genes within the 16p11.2 BP4-BP5 interval in a subset of 27 participants. (A) Matrix correlation between mRNA levels and global measures of brain volume. The color key represents the Pearson correlation coefficient. Most mRNA levels, except for SH2B1, PRRT2 and GDPD3 are correlated to TIV, GM and WM. There are no correlations with cortical thickness and CSF. Statistical p values for all correlations are detailed in Supplementary table 7. (B-C) Eigenimages represent the statistical parametric maps resulting from singular value decomposition analysis. This method simultaneously determines the best combination in each dataset (gene expression and voxels) in order to explain maximum covariance between gene expression and local brain volume. Negative loading indicates that low mRNA levels are associated with increased GM volumes while positive loading represents the inverse effect.

Discussion

Using an unbiased whole-brain approach, we demonstrate that genomic copy number at the 16p11.2 BP4-BP5 locus is associated with brain anatomy changes in a dosage dependent manner, and that these structural changes are present in the absence of either an ASD or SZ diagnosis. Our findings including areas implicated in reward, language and social cognition allow generating new hypotheses on how gene dosage results in reciprocal and overlapping phenotypes observed in 16p11.2 deletion and duplication carriers. This provides a general framework to study the effects of CNV on cognition and behavior in common neuropsychiatric disorders such as ASD and SZ. This approach circumvents a number of confounders such as the effects of ascertainment, medication and clinical symptoms [2,37] and

has recently been used to investigate predefined anatomical regions related to early psychosis in 15q11.2 CNVs carriers [38].

There is a strong correspondence between the changes in brain anatomy patterns and the phenotypic traits characterizing 16p11.2 deletion or duplication carriers. In particular, opposing volume changes in key nodes of the reward circuitry - striatum, medio-dorsal thalamus, orbito-frontal cortex and insula - which are associated with eating behavior [39-41], may explain the mirror BMI phenotype in 16p11.2 CNVs carriers. Similarly, the reciprocal changes in the language areas - middle, superior temporal gyrus and caudate [42] may underlie the language deficits reported in deletion but not in duplication carriers. Our results showing involvement of the striatum have recently been corroborated by the findings of Portmann et al. [43] in a mouse model of 16p11.2 deletion syndrome.

Whole genome studies have identified genetic factors, including 16p11.2 CNVs, shared between ASD and SZ. This led to the assumption that the two disorders may represent opposite manifestations of the same underlying mechanism or trait [44]. There is a large overlap in the reported patterns of gene dosage-dependent brain anatomy changes with well-established structural signatures of ASD and SZ. Notably, alterations in reward system structure, influenced by 16p11.2 gene dosage, is also the main structural change shared by ASD and SZ [36,45,46]. This finding supports the notion of a common abnormal mechanism underlying these two conditions. None of the studied participants met diagnostic criteria for SZ and only two did so for ASD (**Supplementary Table 1**), which suggests that the observed brain modifications are not the consequence of a long standing ASD or SZ diagnosis, but that they may be considered as intermediate phenotypes. This is also in keeping with previous findings of brain modifications predating the onset of psychosis [47].

Obesity is a well-known comorbidity of ASD or SZ [48]. It has been hypothesized that the reward system, and the striatum in particular, may underlie the frequent co-occurrence of

metabolic and psychiatric manifestations [37,39,41,49]. This idea is in line with our findings and suggests that modulation of the reward system by genes at the 16p11.2 locus is driving a group of disorders as opposed to the notion that different genes lead to different symptoms in CNV carriers.

Analyses of cortex anatomy showed cortical thickness reduction in 16p11.2 deletion carriers and a similar trend in duplication carriers. This shared feature may be explained by decreased IQ and risk for ASD in both deletion and duplication carriers. However, both cortical thickness and surface area are correlated to IQ and this relationship changes with age [50]. Of note, SZ is also associated with widespread cortical thining[51]. The dissociation between cortical surface and thickness with regards to 16p11.2 gene dosage supports the notion of distinct genetic mechanisms regulating these cortical anatomy features[52,53]. As shown in the general population and ASD, it is most likely that the 16p11.2 gene dosage effects on brain volume are related to changes in total cortical surface[52,54,55]. Therefore, we refrain from drawing conclusions on topology overlap and causal links between regional thickness/surface changes and brain volume alterations[54].

In agreement with the known effect of gender in ASD and in carriers of 16p11.2 CNVs [5,56], we observe a volume reduction in both cerebellar hemispheres restricted to male deletion carriers (**Supplementary Fig. 2**). This finding supports the notion of differential neurodevelopmental effects of genetic variants in males and females as well as mounting evidence for cerebellar involvement in intellectual disability, language impairment and ASD [57 58].

The reciprocal effects of gene dosage on global metrics of brain volume and cortical surface area corroborate previous reports on head size measurements in humans and zebrafish [5,11]. In the absence of gene dosage-dependent cortical thickness changes, we interpret the effects of 16p11.2 CNVs on brain volume and cortical surface area as evidence of abnormal

neurogenesis [59]. *TAOK2*, *MAPK3, MVP, KIF22, ALDOA* and *KCTD13,* are 16p11.2 genes previously linked to neurogenesis and/or apoptosis[60,61], and could represent candidate genes implicated in the control of brain growth.

Whole brain analysis using continuous measures of mRNA levels further validates results using the number of genomic copies (1, 2 or 3), and both approaches identify that the same anatomical structures are involved. Given the significant amount of shared variance between mRNA levels of the studied genes at the 16p11.2 BP4-BP5 locus we refrain from drawing conclusions about potential differential contribution of single genes on brain anatomy.

One of the anticipated limitations of the study is the inability to acquire imaging data in participants with significant behavioral deficits and BMI/waist circumference beyond limits of MRI scanning safety standards. Results might not generalize to other 16p11.2 CNV carriers with extreme obesity or full-blown clinical symptoms of ASD or SZ. Nevertheless, with respect to global cognition, our cohort is highly representative of 16p11.2 with IQ measures identical to what has been previously published [4,8].

Conclusion

In this study, we demonstrate that gene dosage at the 16p11.2 locus modulates specific neural circuitry including foremost the reward system. The patterns of brain anatomy changes in fronto-subcortical networks could be interpreted as endophenotypes of ASD, SZ and obesity associated with 16p11.2 rearrangements in the absence of diagnostic criteria for ASD and SZ. The complementary analysis using peripheral measures of gene expression levels brings further evidence for the correlation between gene dosage and brain structure. This combined approach applied to larger datasets should allow dissection of the relative contributions of genes to human behavior and cognition through a fine-grained analysis of human brain anatomy.

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Competing Financial Interests

The authors declare no competing financial interests

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Study 2: Gene dosage at the 16p11.2 locus modulates language, verbal memory and inhibition

Gene dosage at the 16p11.2 locus modulates language, verbal memory and inhibition

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The supplemental material is presented in **Appendix 2**.

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Abstract

Background: Deletions and duplications of the 16p11.2 BP4-BP5 locus are prevalent Copy Number Variants (CNVs), highly associated with autism spectrum disorders (ASD) and schizophrenia. Beyond language and global cognition, neuropsychological assessments of these two CNVs have not yet been reported. Methods: Our study investigates the relationship between gene dosage at the 16p11.2 locus and cognitive domains assessed in CNV carriers as well as in intrafamilial controls using neuropsychological tools. Results: Beyond the decrease of global cognition common to both CNV carriers, we demonstrate contrasting cognitive profiles in 16p11.2 deletions and duplications. Taking global cognition into account, deletion carriers present with particularly acute deficits in language and executive domains while duplication carriers are devoid of specific deficits, showing significantly enhanced performance in verbal memory. This is reminiscent of special isolated skills characterized in ASD and observed for the first time in individuals with an ASD-associated genetic variant. Neuroimaging analyses reveal that measures of inhibition co-vary with neuroanatomical structures previously identified as sensitive to 16p11.2 gene dosage. Conclusions: The simultaneous study of reciprocal CNVs suggests that the 16p11.2 genomic locus modulates specific cognitive traits in a dosage sensitive manner. Further research is warranted to replicate these findings and elucidate the molecular mechanisms modulating these cognitive performances.

Introduction

Deletions and duplications of the 16p11.2 ~600 kb breakpoints 4-5 (BP4-BP5) region are amongst the most frequent causes of neurodevelopmental and neuropsychiatric disorders (1-5). While both Copy Number Variants (CNVs) are equally and highly enriched in autism spectrum disorders (ASD) cohorts, only duplications are associated with schizophrenia (4). Cognitive studies in 16p11.2 deletion carriers demonstrate a decrease of ~30 points in global cognition (full scale IQ or FSIQ) compared to intrafamilial controls (1, 6). Language impairment has been reported in several case series (7, 8) and specific assessments have shown below-average performance in comprehension, expression and reading skills with a 71% rate of speech and language disorder as defined in DSM-IV-TR (6 , 9). Little is known about the cognitive deficits in carriers of the reciprocal duplication: important variability in global cognition was occasionally reported and in several instances, with seemingly unaffected transmitting parents (8, 10, 11). The duplication has been estimated to decrease global cognition by 25 FSIQ points in probands and 16 points in their siblings who carry the same CNV (d'Angelo et al., submitted).

While most studies of CNVs investigated deletions and duplications separately using a case control design, the simultaneous assessment of reciprocal CNVs of the same genomic region provides a unique opportunity to study how the number (1, 2 or 3) of genomic copies (defined hereafter as gene dosage) may modulate clinical phenotypes and endophenotypes. We and others have previously shown that body mass index (BMI) inversely correlates to the number of genomic copies at 16p11.2 locus (1, 8, 12-14). Gene dosage is also negatively correlated with global brain volume (15) and patterns of neuroanatomical structures involved in reward, language and social cognition circuits have been reported (16).

We therefore hypothesized that specific cognitive traits may also correlate with gene dosage of the 16p11.2 region, and that this relationship may be mediated by changes in brain structure. Our findings suggest that gene dosage of this locus modulates specific cognitive domains resulting in impaired as well as enhanced skills after adjusting for IQ in carriers of reciprocal CNVs. Analysis of structural neuroimaging data suggests that brain regions previously identified as correlating with gene dosage mediate cognitive alterations in 16p11.2 CNV carriers.

Methods

2.1 Participants

Participants were taking part in a larger research project on CNVs at the 16p11.2 locus which aims at phenotyping a European cohort of 16p11.2 rearrangement carriers. The final dataset comprised 56 deletion carriers (31 probands / 25 relatives), 38 duplication carriers (18 probands / 20 relatives) and 45 intrafamilial controls. Participants' main characteristics are presented in **Table 1**.

Inclusion criteria: Presence of a recurrent 16p11.2 deletion or duplication comprising the BP4-BP5 region (29.6-30.2 Mb according to the human genome build GRCh37/hg19). Controls were non-carriers in the same families. *Exclusion criteria:* (i) Age < 3 years. Eighteen participants who were not able to perform complex cognitive tasks due to low cognitive functioning were excluded from the analyses (**Table S1 in Supplement).**

Additional deleterious CNVs were identified in one deletion and 5 duplication carriers (**Table S2 in Supplement**). They were not removed from the analyses but their potentially confounding effects were taken into account in a sub-analysis. Deleterious CNVs were defined as: i) known recurrent genomic disorder or ii) CNV encompassing published critical

genomic region or disrupting a gene which is a known etiology of neurodevelopmental disorders or iii) rare (<1/1000) and large (>500kb).

Table 1: Population characteristics

	Deletion (n = 56)	**Controls** (n = 45)	**Duplication** (n = 38)
Age in years (mean ± SD) [range]	22.4 ± 15.8♦ [4.8-59]	32.4 ±15.2 [3.3-61]	29.3 ±17.5 [3.3-65]
Gender (M/F)	29 / 27	22 / 23	22 / 16
Handedness (R/L/U)	39 / 10† / 7	41 / 2 / 2	28 / 8† / 2
Inheritance (De novo/In /U)	11 / 26 / 19	-	6 / 14 / 18
Kinship (proband / relative)	31 / 25	-	18 / 20
ASD (n)	1	0	3
Schizophrenia (n)	0	0	0
FSIQ (mean ± SD)	72±13.4§	96 ±14.1	75 ±18.7§
NVIQ (mean ± SD)	78 ±11.7§	98 ±14.3	76 ±15.9§

***SD**, standard deviation; **M**, male; **F**, female; **R**, right; **L**, left; **U**, unknown / undefined; **In**, inherited; **ASD**, Autism Spectrum Disorder; **FSIQ**, full scale intellectual quotient (standard score); **NVIQ**, non verbal intellectual quotient (standard score).*
♦ significantly different from the control group, ANCOVA, post-hoc group comparisons, p = 0.007, Bonferroni corrected.
§ significantly different from the control group, Linear mixed model, ps < 0.0001.
† significantly increased compared to the control group, Logistic mixed model, (del: p = 0.041; dup: p = 0.03)

The study was reviewed and approved by the local Ethics committee and signed consent forms were obtained from participants or legal representatives prior to investigation. Participants were assessed at Lausanne University Hospital, Switzerland. More than 87% of the CNV probands carriers were referred to the study by the clinical geneticist who had initially established the genetic diagnosis in the context of a neurodevelopmental disorder. Five probands (3 duplications) were identified in the general population and one duplication carrier was referred for psychiatric problems.

2.2 Cognitive assessment

Trained neuropsychologists performed all cognitive assessments. Participants underwent age and developmentally appropriate neuropsychological tests assessing overall cognitive functioning, fine motor skills, language, memory and executive functions. All z-scores were derived from population norms, except where mentioned otherwise. We used the following psychometric tests:

Overall cognitive functioning: The Wechsler Intelligence Scales or Abbreviated Scale of Intelligence (17-20) were used to obtain Full-scale intellectual quotient (FSIQ), non-verbal IQ (NVIQ) and verbal IQ (VIQ) as outcome measures when available.

Fine motor skills: The Purdue Pegboard test (21) (≥ age 5) assessed 4 conditions: dominant hand, non-dominant hand, bimanual and assembly. Z-scores were used as outcome measures.

Language: *Phonological skills* were assessed with non-word repetition (≥ age 5), oromotor sequences (≥ age 3) and phonological processing (≥ age 3) from the NEPSY battery (22). Z-scores were used as outcome measures (for participants > age 14, based on an adult control group [n=35]). Participants ≥ age 16 performed a sentence repetition task including low frequency words. The outcome score was the number of sentences correctly repeated. *Lexical skills* were assessed with the Wechsler vocabulary subtest (≥ age 4), the Peabody Picture Vocabulary Test Revised (PPVT-R, (23), word comprehension, ≥ age 3), semantic (animal, ≥ age 3) and phonemic (letter M, ≥ age 5) fluencies. Total z-scores were used as outcome measures for word comprehension and word definition tasks, raw scores for verbal fluencies (number of words). *Comprehension and verbal skills* were assessed with a selection of 24 items of the test for reception of grammar 2 (TROG-2 (24), syntax comprehension, ≥ age 15) and the Wechsler similarity subtest (≥ age 7). Total z-score (similarity subtest) and number of success (syntax comprehension) were used as outcome measures. *Written language* was assessed through a text reading (PC robbery (25), ≥ age 12) and a spelling task (ROC (26), ≥

age 12). Reading fluency and reading comprehension z-scores were used as outcomes measures; the total raw score was used for the spelling task.

Memory: *Verbal short-term memory* was assessed using the forward digit span task (18, 19) (≥ age 6). The total raw score was used as outcome measure. *Verbal long-term memory* was assessed using the California verbal learning task (CVLT (27), ≥ age 17). Two z-scores were used as outcome measure: the total number of words correctly recalled across all 5 learning trials (encoding) and the number of words recalled after a 20 minutes delay (delayed recall). *Visuo-spatial short-term memory* was assessed using the forward spatial span task (28, 29) (≥ age 6). The total raw score was used as outcome measure. *Visuo-spatial long-term memory* was assessed with the Rey-Osterrieth complex figure test (ROFC (30), ≥ age 5). Z-scores for immediate and delayed recall (20 minutes) were used as outcome measures.

Executive Functions: *Working memory* was assessed using the backward digit span (18, 19) (≥ age 6) and the backward spatial span tasks (28, 29) (≥ age 6). The total raw scores were used as outcome measures. *Planning skills* were assessed with the tower of London test (31), (TOL, ≥ age 7). Z-scores for the total correct score and the total move score were used as outcome measures. *Inhibition skills:* Stroop task (32) (≥ age 8) was used to assess verbal inhibition. A computerized version of the GoNogo task (33) (≥ age 7) was used to assess motor inhibition. Z-scores for response time and raw score for errors number were used as outcome measures in both tasks.

2.3 Psychiatric Assessments

Experienced, licensed psychologists and psychiatrists performed the autism diagnostic interview-Revised (ADI-R) (34) and autism diagnostic observation schedule (ADOS) (35) to establish a categorical diagnosis in participants presenting symptoms of ASD. All adult carriers underwent the diagnostic interview for genetic studies (DIGS) (36).

2.4 Magnetic resonance imaging (MRI): data acquisition and processing

We use structural MRI data acquired for a previously published study including 14 deletion carriers (age range 8-53 years, mean age 24 ± 13), 17 duplications carriers (age range 14-58 years, mean age 36 ± 11) and 23 intrafamilial controls (age range 11-52 years, mean age 34 ± 11) (16). The three groups did not differ in terms of age and gender (**Table S3 in Supplement)**. MRI data was acquired on a 3-T Siemens Trio scanner (Siemens AG, Erlangen, Germany) using a standard 12-channel head coil. The protocol consisted of a multi-echo magnetization prepared rapid gradient echo sequence (ME-MPRAGE: 176 slices; 256×256 matrix; echo time (TE): TE1: 1.64ms, TE2: 3.5ms, TE3: 5.36 ms, TE4: 7.22 ms; repetition time (TR): 2530 ms; flip angle 7°). For structure-function analysis we use the default settings processed multi-echo T1-weighted images from the aforementioned study. The algorithm consisted of automated tissue classification into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the 'unified segmentation' framework (37), within SPM12 (www.fil.ion.ucl.ac.uk/spm; Wellcome Trust Centre for Neuroimaging, London). Aiming at optimal anatomical precision, data was additionally spatially registered to the Montreal Neurological Institute (MNI) space using the diffeomorphic registration algorithm (DARTEL) (38). An isotropic Gaussian smoothing kernel (8 mm full-width-at-half-maximum (FWHM)) was applied by convolution to the GM volume maps (39, 40).

2.5 Statistical analyses

Neuropsychological data analyses: Variables derived from normative data were converted into z-scores [mean (standard deviation, SD) = 0 (1)]. Raw scores of variables without available normative data were systematically detrended for age. We performed either linear regression or generalized regression analyses depending on the distribution of the data. Cognitive outcome measures were used as dependent variable.

To investigate the effect of gene dosage on cognition we used linear models with the number of genomic copies as a continuous variable (deletion = 1, control = 2, duplication = 3). For contrasts between groups, we used post-hoc t-test subsequently corrected for multiple comparisons. Uncorrected p-values ≤ 0.005 were considered significant and p-values >0.005 up to ≤ 0.01 were considered as trends. Appropriate linear mixed models (LMM) or generalized mixed models (GLMM) were performed taking the variable "family" as random factor to account for correlated measures within family. We also included in the statistical design IQ, gender and their interactions with gene dosage or group in order to control for the effects of these variables. These additional covariates were kept in the final models only when the effect was significant. Non-carrier participants' results coming for deletion or duplication families were pooled, as they did not differ in term of age, gender and cognitive performances. Selected models, estimates and uncorrected p-values are reported in **Table 2 and Tables S4 - S7 in Supplement**. Statistical analyses were conducted using IBM SPSS (Version 21.0, released 2012; IBM Corp., SPSS Statistics for Windows, Armonk, New York) and R 3.0.2 (The R Project for Statistical Computing; http://www.R-project.org/).

Brain structure and behavior correlation analyses: We tested whether the effects of gene dosage on cognitive measures are mediated by changes observed in brain anatomy. Therefore, only cognitive measures (z-scores and raw scores detrended for age and IQ**)** with significant differences between CNV carriers and intrafamilial controls were subsequently used for regression analysis with brain anatomy. The statistical design included also age, gender and total intracranial volume as regressors. Voxel-based statistical analysis of the GM regional changes was assessed by creating voxel-wise statistical parametric maps (SPMs) for the whole extent of the search volume using the General Linear Model (GLM) and Random Field Theory (41). Given that gene dosage negatively correlates with GM volume in language and reward-related areas (16), the a priori hypothesis was to test whether the behavioral deficits

were associated with an increase of local brain volume. Consequently, one-tailed t-tests were used to identify the regions whose volume showed negative correlation with the cognitive score.

We subsequently estimated the degree of overlap between brain areas correlating with behavioral scores and the previously identified regions sensitive to gene dosage (16). Clusters sharing both effects were obtained with a subtraction of the two statistical maps. We further examined how the number of genomic copies interacted with the brain-behavior correlation in these regions, using a multiple linear regression analysis of the summed voxel values for each group. For all whole-brain analyses, we applied a voxel-level threshold of $p < 0.05$ after family-wise error (FWE) correction for multiple comparisons. Trends were assessed by using an auxiliary uncorrected voxel threshold of $p < 0.001$ (41).

Results

Global cognitive impairment in deletion carriers is consistent with previous reports (1, 6) with a 23 points decrease of mean FSIQ compared to controls. Duplication carriers presented similar levels of impairment (deletion FSIQ = 72, duplication FSIQ = 75, **Table S4 in Supplement**). Thus, global cognition (Wechsler Intelligence Scale) is similarly decreased in both deletion and duplication carriers notably across non-verbal subscales (i.e. Block Design). Fine motor skills were decreased in both CNVs after adjustment for NVIQ and age. We performed all subsequent analyses adjusting for NVIQ in verbal tasks and FSIQ otherwise (**Table 2** and **Table S5 in Supplement;** see **Table S6 in Supplement** for results not adjusted for IQ).

Table 2: *Group contrasts and gene dosage effect for cognitive measures adjusting for IQ*

	Deletion versus Duplication		**Deletion versus Control**		**Duplication versus Control**		**Gene dosage**
Cognitive variables	Estimate$^{\sim}$ (Std. err)	*P*-value	Estimate* (Std. err)	*P*-value	Estimate* (Std. err)	*P*-value	Estimate (*P*-value)
Language							
Phonological skills [1]							
Non-word repetition (z-score)	1.97 (0.56)	**0.0007**	-1.74 (0.54)	**0.0017**	0.23 (0.64)	0.73	1.06 **(0.0002)**
Oromotor sequences (z-score)	1.85 (0.63)	**0.002**	-2.52 (0.61)	**1.9e-05**	-0.67 (0.7)	0.3	1.07 **(0.0005)**
Sentence Repetition (age adjusted raw score)	0.79 (0.25)	**0.0015**	-0.75 (0.24)	**0.0017**	0.05 (0.29)	0.86	0.44 **(0.0003)**
Lexical skills [2]							
Word definition (z-score)	0.49 (0.2)	0.02	-0.69 (0.22)	**0.002**	-0.19 (0.24)	0.41	0.27 (0.009)
Comprehension and verbal skills [3]							
Syntax comprehension (age adjusted raw score)	-0.09 (0.18)	0.62	-0.76 (0.25)	**0.003**	-0.85 (0.27)	**0.002**	-0.005 (0.96)
Written language [4]							
Reading fluency (z-score)	1.35 (0.55)	0.016	-1.75 (0.55)	**0.002**	-0.4 (0.6)	0.51	0.73 (0.012)
Memory							
Verbal short-term memory							
Forward digit span (age adjusted raw score)	1.58 (0.42)	**0.0003**	-0.8 (0.48)	0.09	0.76 (0.52)	0.13	0.79 **(0.0002)**
Verbal long-term memory							
CVLT encoding (gender adjusted z-score)	1.41 (0.31)	**3e-05**	0.07 (0.33)	0.82	1.49 (0.37)	**0.0002**	0.65 **(0.0001)**
CVLT delayed recall (gender adjusted z-score)	1.39 (0.34)	**0.0001**	0.29 (0.36)	0.42	1.69 (0.41)	**0.0001**	0.62 **(0.0009)**
Visuo-spatial short-term and long-term memory [5]							
Executive functions							
Working memory and planning [6]							
Inhibition [7]							
Stroop number of success (age adjusted raw score)	0.52 (0.25)	0.037	-0.82 (0.26)	**0.001**	-0.3 (0.3)	0.32	**0.31 (0.012)**
GoNogo response time (z-score) [§]	-0.94 (0.3)	**0.003**	0.31 (0.29)	0.28	-0.63 (0.3)	0.042	-0.47 **(0.003)**
GoNogo number of success (age adjusted raw score) [§]	0.99 (0.37)	0.007	-0.69 (0.01)	0.012	0.29 (0.4)	0.45	0.55 (**0.002**)
Fine motor skills [8]							
Purdue non dominant hand (z-score)	0.47 (0.25)	0.067	-1.1 (0.29)	**0.004**	-0.63 (0.3)	0.038	0.25 (0.067)

Std. Err *- Standard Error;* ***FSIQ*** *- full scale IQ;* ***NVIQ*** *- non verbal IQ;* ***VIQ*** *- verbal IQ;* ***CVLT*** *- California Verbal Learning Test.*
Significant p-values (corrected threshold, p = 0.005) are highlighted in bold.
Linear mixed models were used to account for the correlations of measures within families. When only one family member was included in the analysis, linear models were performed. P-values represent post-hoc unpaired t-tests after assessing the group effect with the ANOVA analysis.

[§] In the absence of a main effect of IQ, the model is not adjusted for IQ.
[~] positive estimate: Deletion carriers' score < duplication.
[*] negative estimate: Deletion or Duplication carriers' score < controls.

*[1-7] For space constraints, cognitive variables showing no significant gene dosage effect and/or no significant group differences (p-values corrected) are presented in **Supplementary Table 5.***

[1]Phonological processing; [2] Word comprehension, Semantic and phonemic fluencies; [3] Verbal reasoning; [4] Reading comprehension and Spelling; [5] Forward spatial span and Rey-Osterrieth Complex Figure (immediate and delayed recall); [6] Backward digit span, Backward spatial span and Tower of London (total correct and total move scores); [7] Stroop response time; [8] Purdue: dominant hand, bimanual and assembly conditions.

Phonology, written language and vocabulary are modulated by gene dosage

Gene dosage positively correlates with phonology measures such as non-word repetition, oromotor sequences and sentence repetition. This effect is mainly driven by the deletion carriers who perform worse than controls and duplication carriers while these two later groups do not significantly differ (**Figure 1A-C**). There are also trends for a positive gene dosage effect on word definition ($p = 0.009$) and reading fluency ($p = 0.01$), mainly driven again by deletion carriers performing worse than the two other groups (**Table 2)**. None of the measures assessing spelling, verbal fluencies, verbal comprehension and reasoning are impacted by the 16p11.2 locus except for the syntax comprehension task in which controls outperform both deletion and duplication carriers **(Table 2 and Table S5 in Supplement)**.

Verbal short term and long term memory processes are modulated by gene dosage

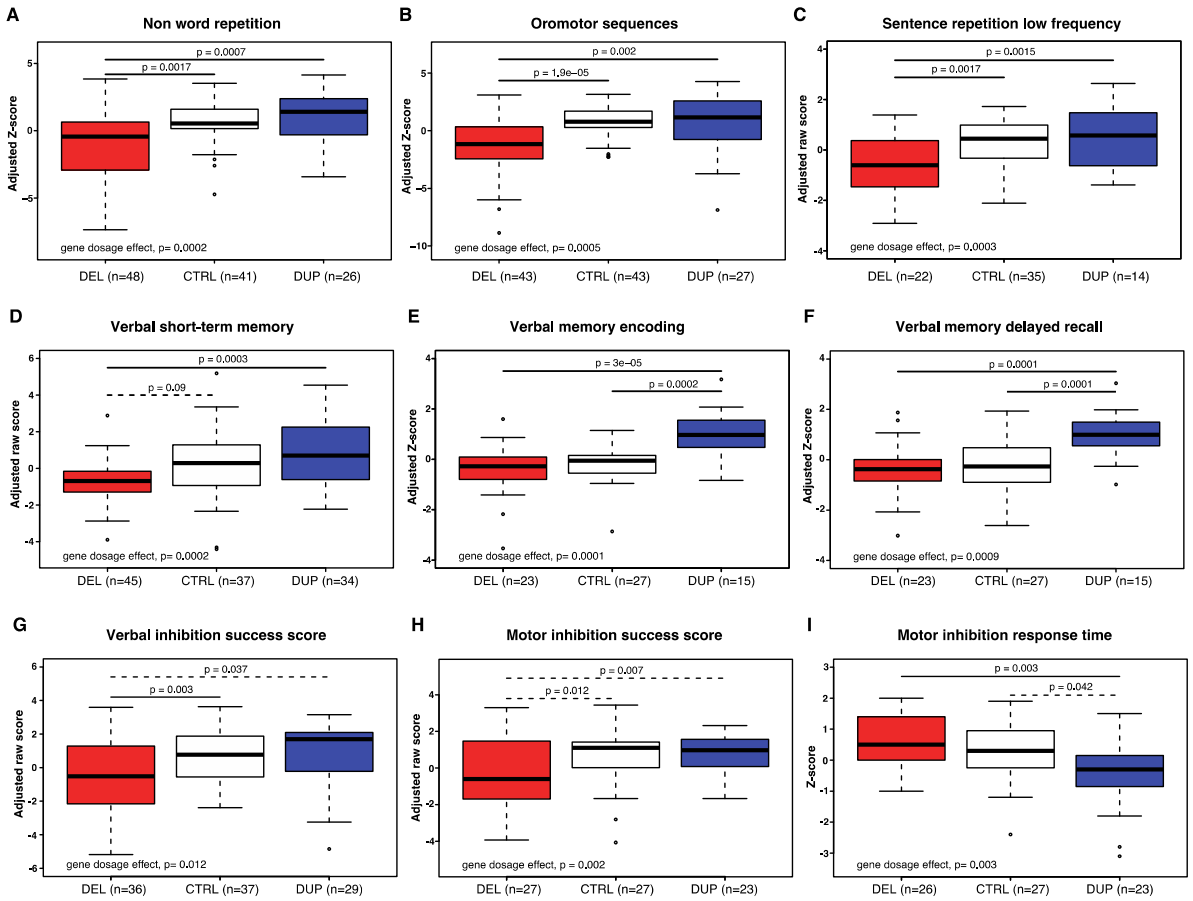
All verbal memory measures significantly and positively correlate to genomic copy number. The preferential contribution of the deletion or the duplication to this gene dosage effect is unclear and varies according to the sub-domains. Remarkably, the duplication carriers outperform both deletion carriers and controls in measures of verbal long-term memory (**Figure 1D-F**) while the deletion carriers score worse than duplication carriers on short-term memory.

The 16p11.2 locus does not significantly affect either short- or long-term visuo-spatial memory. Of note, we corrected for the main effect of gender present in all groups for verbal long-term memory tasks (females > males, **Table S7 in Supplement**).

The 16p11.2 locus modulates inhibition skills but not working memory and planning

Motor and verbal inhibition measures show a positive gene dosage effect (**Figure 1G-I**). Deletion carriers who perform or tend to perform worse than controls and duplication carriers mainly drive this effect (**Table 2**). We do not observe any group differences in working memory and planning skills (**Table S5 in Supplement**).

Figure 1: Gene dosage and group comparisons on language, memory and executive measures



*Boxplots represent language measures of phonology (**A-C**), memory **(D-F)** and executive functions **(G-I)** in deletion, duplication carriers and intrafamilial controls. Higher scores translate into better performance except for panel **I** where better is represented a shorter response time. The bold line shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whisker ends at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR). Circles are outliers. Gene dosage effect (1, 2 or 3 copies) and group contrasts are estimated using linear mixed model to account for correlated measures within families (**A, B, D, G, H, I**) and linear model when only one family*

*member is included in the analysis (**E** and **F**). Non linear model was required for **C**. Scores are adjusted for NVIQ, age and gender when required (see methods). Significant post-hoc group comparisons (p-corrected threshold = 0.005) are represented by solid lines with exact p-values above, trends by dashed lines with exact p-values above.*

***NVIQ** – non verbal IQ; **DEL**-deletion carriers; **CTRL** - intrafamilial controls; **DUP** - duplication carriers*

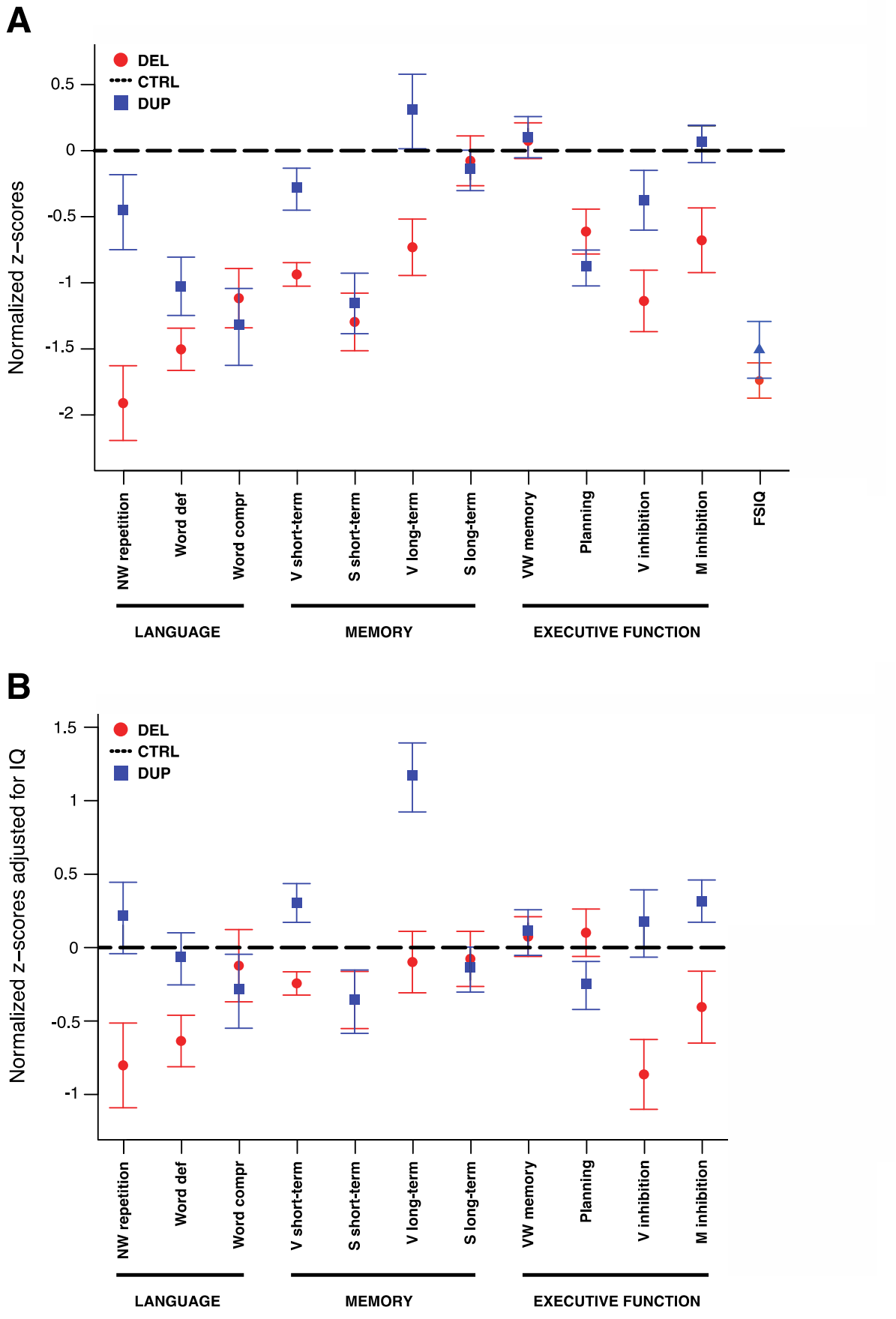
Overall neuropsychological profile

In order to illustrate the neuropsychological profiles of deletion and duplication carriers, we summarize a sample of the cognitive tasks presented above corrected and uncorrected for IQ (**Figure 2A-B**). Carriers' data were converted into z-scores relative to the intrafamilial controls to highlight preserved skills (performance similar to controls after adjusting for IQ), specific deficits and enhanced performances (respectively lower and higher performances than expected for IQ level). Taken together, the cognitive findings in this cohort of carriers (excluding moderate to severe intellectual disability) show that deletion carriers present specific deficits in the language and executive domains, while the profile of duplication carriers is devoid of particular impairments. Remarkably, duplication carriers show enhanced performance in a verbal long-term memory task compared to intrafamilial controls (**Figure S1 in Supplement**).

ASD diagnosis and additional CNVs

We considered possible confounders including a diagnosis of ASD (1 deletion and 3 duplication carriers), additional deleterious CNVs (1 deletion and 5 duplication carriers) and inheritance status in deletion. The sub-analyses performed after exclusion of participants with additional CNVs did not change the results (**Table S8 in Supplement**). Analyses excluding participants with ASD also led to the same results. In the deletion group, *de novo* carriers' performance did not differ from those of the inherited carriers (**Table S9 in Supplement**).

Figure 2: Neuropsychological profile in 16p11.2 deletion and duplication carriers

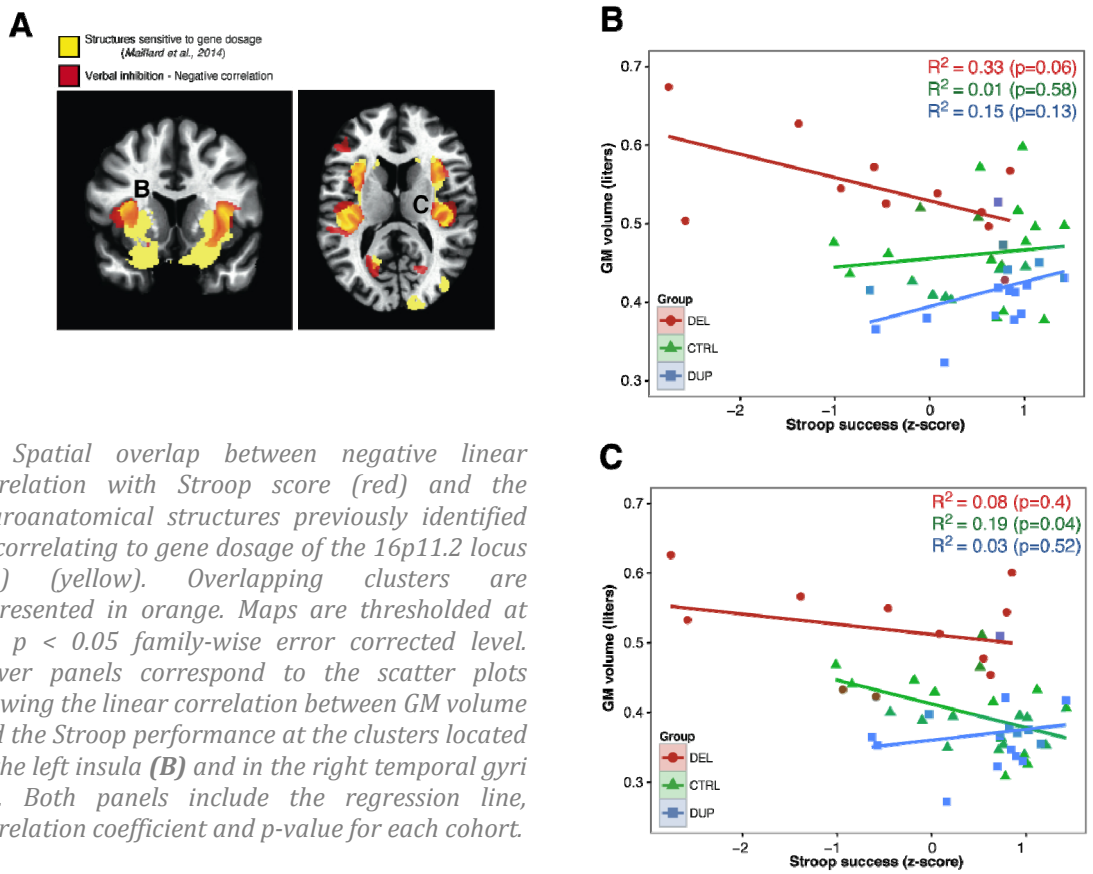


*(**A**) The Y axis shows mean cognitive residual scores for deletion (red circles) and duplication carriers (blue square) converted into z-scores relative to the intrafamilial control group represented by the black dashed line. Error bars represent standard error of the mean. When appropriate, scores are adjusted for age and gender. The X axis lists one task per sub-domain with the most complete dataset: NW repetition, non-word repetition; Word def, word definition; Word compr, word comprehension; V short-term, verbal short-term memory; S short-term, spatial short-term memory; V long-term, verbal long-term memory; S long-term, spatial long-term memory; VW memory, verbal working memory; Planning, Tower of London success score; V inhibition, verbal inhibition number of success; M inhibition, motor inhibition number of success; FSIQ, full scale intellectual quotient.*
***B**) This graph represents the neuropsychological profile of deletion and duplication carriers once the cognitive residual scores (Y axis) are adjusted for IQ.*

Correlation with brain anatomy

Whole-brain analysis showed a positive correlation between GM volume and the verbal inhibition error rate in bilateral insula and transverse temporal gyri (**Figure 3 and Table S10 in Supplement**). The subsequent regression analyses (left cluster: $r^2 = 0.14$, $p = 0.007$; right cluster: $r^2 = 0.24$, $p = 0.0003$) revealed that the effect was mainly driven by deletion carriers, who showed the greatest variance (**Figure 3B-C**). We report a trend (uncorrected p value < 0.001 for all the clusters) for increased GM volume in the left inferior frontal gyrus, bilateral superior temporal gyri and bilateral caudate associated with deficits in two measures related to phonology: non-word repetition and reading fluency. Measures of memory did not co-vary with any brain structure.

Figure 3: Brain structure-behavior correlation analysis between verbal inhibition score and grey matter map



A) *Spatial overlap between negative linear correlation with Stroop score (red) and the neuroanatomical structures previously identified as correlating to gene dosage of the 16p11.2 locus (16) (yellow). Overlapping clusters are represented in orange. Maps are thresholded at the p < 0.05 family-wise error corrected level. Lower panels correspond to the scatter plots showing the linear correlation between GM volume and the Stroop performance at the clusters located in the left insula* ***(B)*** *and in the right temporal gyri* ***(C)****. Both panels include the regression line, correlation coefficient and p-value for each cohort.*

Discussion

By assessing carriers of deletion and duplication at the 16p11.2 locus as well as intrafamilial controls, our study characterizes gene dosage effects of this genomic region on several cognitive domains. After adjusting for global cognition, which is decreased in both CNVs, specific cognitive functions including verbal memory, executive and phonological skills show a positive correlation with gene dosage. Most surprisingly, duplication carriers show either preserved or enhanced cognitive performance, particularly in verbal memory, when compared with intrafamilial controls.

The same gene dosage approach to study cognitive traits was recently investigated in carriers of recurrent CNVs using batteries of neuropsychological tests to evaluate spatial working

memory, verbal fluency, inhibition and mental flexibility (42). Nevertheless, either the small sample size (n=7 for 16p11.2 deletion and duplication carriers respectively) or the small effect size (for 15q11.2 BP1-BP2) did not allow for the identification of any correlations between cognitive traits and copy number state.

To date, molecular factors underlying cognitive functions have been investigated in animal models for memory. Recent data on mouse models of 16p11.2 reciprocal CNV corroborate the gene dosage effect we report for memory skills with duplicated mice performing better than wild type mice on an object recognition task (Abrogast et al.; personal communication). Also, findings on mouse model report worse performance in deleted mice compared to controls (Abrogast et al.; personal communication) (43).These observations in humans and in mice suggest that akin to other complex traits such as BMI or brain anatomy, cognitive processes may, in humans, co-vary to some extent with molecular mechanisms. Memory processes have also been linked to mechanisms regulating long-lasting synaptic potentiation and depression. These synaptic mechanisms require burst of local protein synthesis during training and stimulation (44). Both mTOR and *MAPK3* signalling regulate local synaptic protein synthesis, which in turn modulates memory performances in murine models (45, 46). The expression levels of *MAPK3,* which maps within the BP4-BP5 interval and that of mTOR pathway members are significantly altered in 16p11.2 CNV carriers (47). These are therefore good candidate genes underlying or mediating the correlation between genomic copy number and memory performances.

Both the deletion and the duplication are strongly and equally associated with ASD but our study shows that their cognitive profiles are distinct and in some cases opposed. This highlights the challenge in studying ASD as a diagnostic category. For example, there is a long-standing debate on whether ASD and specific language impairment (SLI) arise from similar genetic bases (48). This study demonstrates that the same genomic region

predisposing to ASD may or may not have a deleterious effect on structural language depending on the nature of the mutation. While language is preserved in the duplication, the deletion results in specific phonological deficits in children and partially compensated in adulthood. This dissociation between phenotypes observed in reciprocal copy number state is also corroborated by a recent study demonstrating that deletions, but not duplications encompassing ASD genes are primarily associated with impairments in language domains (49).

Special isolated skills and cognitive strengths are also features defining subgroups of ASD (50) and the profile in 16p11.2 duplications is reminiscent of enhanced memory skills also reported in ASD (51, 52). We did not identify strengths in the Block Design test, which (together with enhanced pitch discrimination not investigated in this study) is the most replicated finding in ASD. The findings on the duplication group may represent the first example of an ASD-related genetic predisposition leading to specific cognitive strengths. The absence of any specific impairment beyond the IQ shortfall in duplication carriers at risk for schizophrenia echoes the non-specific cognitive deficit pattern observed in first-episode idiopathic schizophrenia (53, 54). Studies also suggest that the risk to develop schizophrenia is less likely to appear in CNVs associated with more severe cognitive deficits (55, 56).

Our results also suggest that neuroanatomical structures previously defined based on their correlation to gene dosage at the 16p11.2 locus (16) may mediate alterations in measures of language and verbal inhibition, but not memory. These findings nicely dovetail with previously reported structural findings positing the insula as a key player in verbal inhibitory processes (57, 58), as well as the superior temporal gyrus, and caudate nucleus, implicated in SLI (59-61). Larger samples are however required to replicate these results and elucidate any specific association within groups. Interestingly, increased volume of the caudate has been observed in individuals who carry *FOXP2* mutations, which is one of the few genetic forms of

SLI studied to date. These mutations lead to severe articulation deficits in word repetition measures (62, 63). Our recent work on chromosome conformation suggests that the FOXP2 and CNTNAP2 genes are amongst the 16p11.2 chromatin interactions (Nicla et al., submitted).

One of the limitations of the study is the exclusion of carriers with moderate to severe cognitive impairment (10% of deletion and 31% of duplication carriers) unable to perform the complete assessment. Whether these low functioning carriers represent a distinct subgroup with different profiles remains therefore unknown. Although we describe a link between quantitative aspects of behavior and qualitative anatomical correlates, this study remains blind to the directionality of the covariance between genomic copies, brain structure and behavior. Our results on language assessments are largely consistent with a previous study reporting phonological deficits in the context of general language impairment (6) possibly due to the lack of adjustment for NVIQ. The other assessments in our study have not yet been reported in 16p11.2 CNV carriers and should therefore be replicated.

Conclusion

This study suggests that basic cognitive skills may be modulated in a gene dosage sensitive manner in humans. Such effects are easily clouded by the global decrease in cognitive functioning that affects both deletion and duplication carriers. The strength of this study thus lies in the administration of an extensive cognitive test battery in both CNV carrier groups and their intrafamilial controls. This allows us to precisely assess cognitive functions relative to each participant's global cognitive level. The enhanced performance in verbal memory and similar trends for inhibition skills are reminiscent of special isolated skills characterized in ASD and observed for the first time in individuals with an ASD-associated genetic variant. This is concordant with animal model data and may point towards the alteration of specific

molecular mechanism controlling memory. Further research is warranted to elucidate the contribution of specific genes within the 16p11.2 locus by studying the relationship between expression patterns of these genes and cognitive tasks, brain anatomy and brain function. These approaches may ultimately elucidate the mechanisms affecting specifically phonological, verbal memory and inhibition skills in a dose-dependent manner. This comprehensive characterization will also guide clinicians in the assessments and care of their patients with these CNVs.

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Study 3: The 16p11.2 locus modulates response to satiety before the onset obesity

The 16p11.2 locus modulates response to satiety before the onset of obesity

This article will be submitted shortly in the Amercian Journal of Clinical Nutrition
Supplemental material is presented in **Appendix 3**.

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Abstract

Background: 600kb BP4-BP5 Copy Number Variants (CNVs) at the 16p11.2 locus have been associated with a range of neurodevelopmental conditions including autism spectrum disorders and schizophrenia. The number of genomic copies in this region is inversely correlated with body mass index (BMI): The deletion is associated with a highly penetrant form of obesity (present in 50% of carriers by the age of 7 years and in 70% of adults), and the duplication with being underweight. Mechanisms underlying this energy imbalance remain unknown. **Objective:** This study aims to investigate eating behavior, cognitive traits and their relationships with BMI in carriers of 16p11.2 CNVs. **Design:** We assessed individuals carrying a 16p11.2 deletion or duplication and their intrafamilial controls using food related behavior questionnaires and cognitive measures. We also compared these carriers with cohorts of individuals presenting with obesity, binge eating disorder or bulimia. **Results:** Response to satiety is gene dosage-dependent in pediatric CNV carriers. Altered satiety response is present in young deletion carriers before the onset of obesity. It remains altered in adolescent carriers and correlates with obesity. Adult deletion carriers exhibit eating behavior similar to that seen in the obese cohort without eating disorders such as bulimia or binge eating. None of the cognitive measures are associated with eating behavior or BMI. **Conclusions:** These findings suggest that abnormal satiety response is a strong contributor to the energy imbalance in 16p11.2 CNVs carriers and, akin to other genetic forms of obesity, altered satiety responsiveness in children precedes the increase in BMI observed later in adolescence.

Introduction

Copy-number variants (CNVs) are stretches of DNA with a deletion or duplication (1). They are important contributors to mental illness and affect cognition in general population (2-4). CNVs at the 16p11.2 locus (600 kb BP4-BP5 breakpoints; 29.6-30.2 Mb; GRCh37/hg19) have been associated with neurodevelopmental and psychiatric disorders including autism and schizophrenia (5-8). We and others also demonstrated that the number of genomic copies at this locus correlates with Body Mass Index (BMI) and brain volume (5, 9-14). Specifically, while deletion carriers present a 43-fold increased risk of morbid obesity, duplication carriers have an 8-fold risk of being underweight (12-14). Murine models engineered to carry deletions and duplications that are paralogous to the 16p11.2 rearrangements show BMI phenotypes that are inverse to those observed in human with deletion and duplication mice being under-and overweight respectively (15, 16).

Obesity has been associated with cognitive as well as reward dysfunction in the literature (17). Numerous studies have investigated the relationship between cognition, behavior and BMI (18). More specifically, deficits in inhibition and decision-making have been associated with higher BMI in both children and adults (19-22). Study of these reciprocal CNVs with large effects is a unique opportunity to investigate high risk individuals before and after the onset of obesity as well as the relationships with cognitive and behavioral comorbidities.

Recent studies suggest that diminished response to satiety is a strong contributor mediating genetic forms of obesity in childhood (23-26). To date, only one study (27) reported eating behavior in 16p11.2 deletion and duplication carriers. Using a parental report questionnaire investigating aspects of disinhibited eating, the authors found that the deletion was associated with eating in the absence of hunger and that this association was primarily driven by two factors: sensitivity to external cues and boredom. Interestingly, these findings were independent of parental feeding practices.

In this study, we aim to investigate eating behavior traits associated with BMI in both adult and child carriers of the 16p11.2 BP4-BP5 CNVs. The main aims were threefold: 1/ Assess eating behavior through self- and parent-report in deletion and duplication carriers, as compared to controls, 2/ Compare deletion carriers with individuals presenting with obesity or binge eating/loss of control disorder or bulimia, 3/ Investigate the relationship between BMI and cognition.

Methods

2.1 Participants

2.1. Subjects

The study was reviewed and approved by the local Ethics committee of each site conducting the study. Informed written consents were obtained from participants or legal caregivers prior to inclusion in the study. Clinical characteristics of the adult and pediatric samples are presented in **Table 1**.

2.1.1. *Pediatric samples*

We collected data on 73 deletion carriers (59 families), 42 controls (18 intrafamilial and 24 extrafamilial) and 39 duplication carriers (31 families) ascertained through two different cohorts: 16p11.2 European Consortium and the Experiences of Children with Copy Number Variants (ECHO) study in Cardiff, UK (**Supplementary Table 1**).

Participants from the 16p11.2 European Consortium were taking part in a larger phenotyping project on the deletion/duplication of the 16p11.2 region. Most carriers were referred to the study by the clinical geneticist who had initially established the genetic diagnosis (whole genome arrays) in the context of a neurodevelopmental disorder. Inclusion criteria: presence of a 16p11.2 deletion or duplication comprising the BP4-BP5 region (29.6-30.2 – according to the human genome build GRCh37/hg19). Intrafamilial controls were non-carriers sibling from

the same family. Extrafamilial controls were recruited in the general population. Intra and extrafamilal controls have a BMI z-score between -2.5 and 2.5 standard deviation. Given the absence of significant differences between intrafamilial and extrafamilial controls, we merged both samples into a single control group for statistical analyses (**Supplementary Table 1**). Exclusion criteria: None beside age < 3 years.

Data was also available from the ECHO study which recruited through medical genetics clinics across the UK, various charities, word of mouth and the ECHO study website (http://medicine.cardiff.ac.uk/psychological-medicine-neuroscience/areas-research/copy-number-variant-research/research-projects/). Presence of the 16p11.2 CNV was confirmed from medical records and/ or by the laboratory of the Institute of Psychological Medicine and Clinical Neurosciences at Cardiff University. Intrafamilial controls were non-carrier siblings closest in age to the child with the CNV (one invited per family).

To examine the specificity of eating behavior in CNV carriers, we compared results with a group of 26 children who met criteria for loss of control over eating (LOC) according diagnosis criteria adapted for children (28). All children participated in a research project (Swiss University Study of Nutrition, SUN) at Fribourg University, Switzerland. Detailed description of the study and recruitment methods can be found in Kurz et al. (29). Children with LOC were older than deletion carriers (p= 0.005), but there were no difference in gender or BMI z-score (**Supplementary Table 2**).

2.1.2. *Adult samples*

We examined a total of 25 adult deletion carriers (21 families), 28 duplication carriers (21 families) and 38 intrafamilial controls (spouse of carriers from 26 families) from the 16p11.2

European Consortium (**Table 1**). Inclusion and exclusion criteria were the same as the ones used for the pediatric sample.

To examine the specificity of eating behavior in CNV carriers, we compared results to a group with Obesity (OB, N= 226), a group with diagnosis of Binge Eating Disorder (BED, N= 143) and a group with Bulimia Nervosa (BN, N= 241). They were diagnosed by experienced psychologists and psychiatrists according to DSM-5 criteria (30). The three cohorts were recruited from the Eating Disorder Unit in the Department of Psychiatry at the University Hospital of Bellvitge, Barcelona, Spain.

Deletion carriers were younger than the OB group but did not differ from the two other clinical groups (BN and BED). There was no difference in education level. Deletion carriers had a higher BMI z-score compared to the BN cohort but they did not significantly differ from the OB or BED groups. Finally, there was a balanced gender distribution in the deletion group whereas there were considerably more females in the two clinical and obese groups (**Supplementary Table 3**).

2.2. Anthropometric measures

BMI z-scores were computed for all data using a gender, age, and geographically matched reference population as previously described in Zufferey et al. (14).

2.3. Neuropsychological measures

Overall cognitive functioning was measured using either the Wechsler Intelligence scales for children (WISC-IV) (31), the Wechsler Intelligence scale for adults (WAIS-III) (32) or the Wechsler Abbreviated scale of Intelligence (WASI) (33). We used the Full Scale Intellectual Quotient (FSIQ) as outcome measure. Verbal inhibition skills were assessed with the Stroop

test (34) (≥ age 8) and motor inhibition with a computerized version of the Go-Nogo task (35) (≥ age 7). Raw score for errors number was used as outcome measure in both tasks.

2.4. Eating behavior assessment

2.4.1. Pediatric cohort

Parent-report was used to assess food related behavior in children or young adults (≤ 20 years) unable to complete self-report due to low cognitive level.

Child Eating Behavior Questionnaire (CEBQ, age ≥3) (36)*:* This parent report instrument (35 items) includes 4 subscales related to children's food approach behaviors - Food Responsiveness (FR), Emotional Over Eating (EOE), Enjoyment of Food (EF), and Desire to Drink (DD) - and 4 subscales assessing avoidant-type responses - Satiety Responsiveness (SR), Slowness in Eating (SE), Emotional Under Eating (EUE) and Food Fussiness (FF). Response to each question is given on a 5-point Likert scale (1 = never to 5 = always). We used the mean raw score on each subscale as the outcome measure.

2.4.2. Adult cohort

Data on eating behavior were acquired through self-reports on the following measures:

Eating Disorder Inventory-2 (EDI-2) referral form (≥ age 12) (37): This questionnaire is a self-report measure to assess symptomatology of eating problems (3 subscales) and more general psychological difficulties (5 subscales). In this study, we only used the subscales related to eating problems: Drive for Thinness (DT), Bulimia (B), and Body Dissatisfaction (BD). Responses are given using a 6-point Likert scale (1= never to 6= always). We used total raw scores of each subscale as the outcome measure.

Dutch Eating Behavior Questionnaire (DEBQ) – Externality subscale (≥ age 10) (38): This 10-item subscale assesses whether participants are attracted to food stimuli and tend to eat

regardless of the internal state of hunger or satiety. Each item consists of a 5-point Likert scale (1 = never to 5 = very often). We used the mean raw score as outcome measure.

2.5. Statistical analyses

Effect of CNVs on eating behavior traits: To estimate the effect of the 16p11.2 on eating behavior, we conducted linear mixed models to compare eating disorder behavior in the deletion versus control, deletion versus duplication and duplication versus control, while accounting for correlated measures within families (familial clustering). The group differences were systematically controlled for age, gender and FSIQ (**Supplementary Tables 4-5**) and only significant covariates were included in the final statistical model. Linear regression model was used to compare deletion carriers with clinical groups (obesity, BN and BED), while controlling for age, gender and education level **(Supplementary Table 6)**. Given the high collinearity between BMI z-score and the number of genomic copies, we subsequently added BMI z-score as an explanatory variable. Finally, we assessed the inheritance factor on eating behavior by introducing inheritance in the statistical model as a covariate (*de novo* or inherited from a parent).

Relationship between BMI, eating behavior scores and cognition within CNV groups: Pearson correlation analyses explored the relationship between BMI and cognition (FSIQ, executive functions) as well as BMI and eating behavioral traits within a group. We used similar analysis to assess the correlation between FSIQ and eating behavior scores.

Bonferroni-corrected p-values were obtained by multiplying the original p-values by the number of possible comparisons (n=3): p-values ≤ 0.016 were considered significant in the pediatric and adult cohorts when comparing CNVs carriers with controls, whereas p-values $\leq$

0.0083 were considered significant when comparing adult deletion carriers with the clinical and obese groups (n=6 comparisons). Due to missing data, the sample size might differ between outcome measures.

Statistical analyses were conducted using IBM SPSS (Version 21.0, released 2012; IBM Corp., SPSS Statistics for Windows, Armonk, New York) and R 3.1.1 (The R Project for Statistical Computing; http://www.R-project.org/).

Results

Children: Eating behavior differences between CNV carriers and controls

Child Eating Behavior Questionnaire (CEBQ) scores are described in **Supplementary Table 7**. Three out of the 9 CEBQ subscales show differences between deletion carriers and controls as well as duplication carriers (**Figure 1 A-C, Supplementary Table 8**). Deletion carriers show lower level of satiety responsiveness, increased responsiveness to food and higher sensitivity to emotional overeating compared to the two other groups. As previously reported in the literature (6), BMI z-scores are negatively correlated to the number of genomic copies (**Table 1**).

Table 1: *Clinical description of the pediatric and adult samples*

	Pediatric sample			Adult sample		
	Deletion n= 73	Control n=42	Duplication n=39	Deletion n = 25	Control n = 38	Duplication n = 28
Age in years (mean± SD)	9.6 ± 3.3	11.1 ± 3.1∞	9.1 ± 1	34.7 ± 11.9	36.6±11.4	37.5 ±12.3
Gender (M/F)	46/27	16/26†	26/13	12 / 13	16/ 22	15/13
BMI z-score (mean ± SD)	1.5 ± 1.7 ₪	0.45 ± 0.78	-0.4 ± 1.5§	2.8 ± 1.3*	0.4 ± 1.2	-0.8 ± 1.5◆
FSIQ (mean ± SD)	70 ± 15 [n=62]	104 ± 14‡ [n=18]	64± 24 [n=31]	74 ± 14	92 ± 15¶	74± 20

p-values are uncorrected

Pediatric cohort:

§ Significantly different from controls (p = 0.004)

₪ Significantly different from the controls (p =1.4e-05) and the duplication (p = 7.4 e-08)

∞ Significantly different from deletion (p = 0.014) and duplication (p = 0.015)

‡ Significantly different from deletion (p =3.8e-10) and duplication (p = 2e-09)

† Significantly different from deletion (Fisher exact test, p= 0.012) and duplication (Fisher exact test, p= 0.14)

Adult cohort:

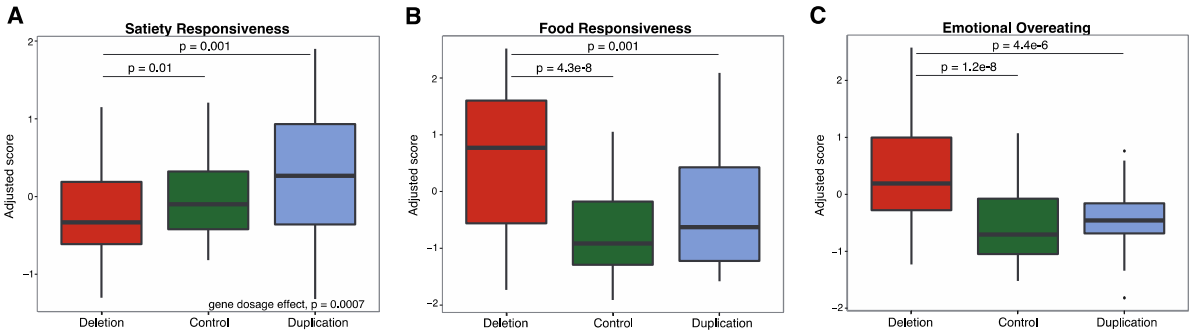
** Significant different from controls (p = 2.6e-9) and duplication (p = 4.1e-12)*

Significantly different from controls (p= 0.002)

¶ Significantly different from deletion (p = 1.7e-5) and duplication (p = 0.0004)

When BMI z-score is included in the model, differences in satiety responsiveness between groups are no longer significant (**Supplementary Table 9**). None of the other subscales (enjoyment of food, emotional undereating, food fussiness, slowness in eating, desire to drink) showed significant difference between groups **(Supplementary Figure 1 and Supplementary Table 8).**

Figure 1: Gene dosage and group comparison on the Child Eating Behavior Questionnaire (CEBQ)

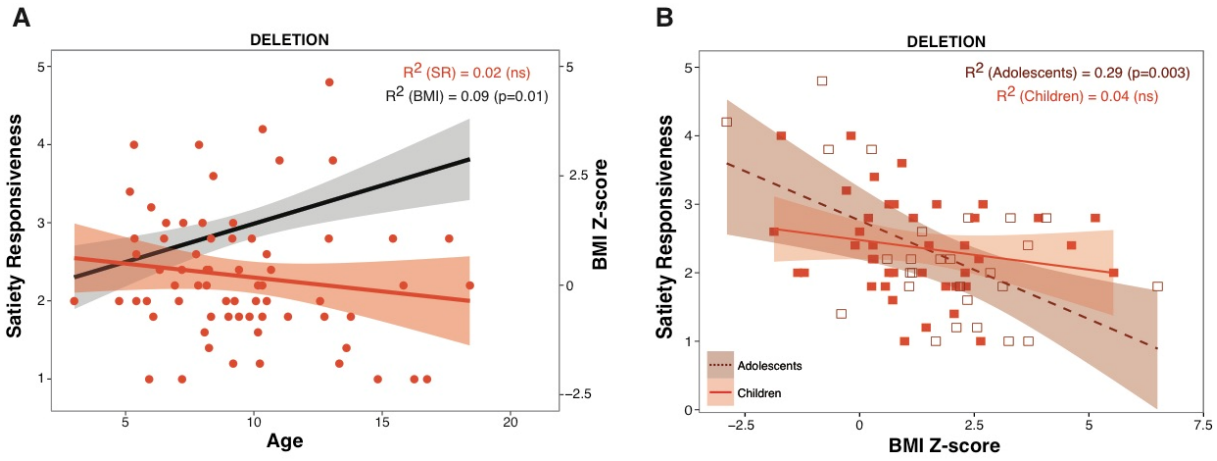


*Boxplots represent scores adjusted for age on satiety responsiveness (**A**), food responsiveness (**B**) and emotional Overeating (**C**) in deletion (red), duplication carriers (blue) and controls (green). The bold line shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whiskers end at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR). Significant group differences (p-corrected threshold = 0.01) are represented by solid lines with exact p-values above. Gene dosage effect and group contrasts are estimated in regression analyses models (linear mixed model).*

Children who carry a deletion: Relationship between eating behavior, BMI and age

Age does not affect satiety responsiveness in deletion carriers whereas BMI z-scores increase progressively with age (p= 0.003) as we previously reported (14) (**Figure 2A)**. Satiety responsiveness does not correlate with BMI z-score in children ≤10 years but a decrease in levels of satiety responsiveness is significantly associated with higher BMI z-score in adolescents > 10 years of age (p= 0.003, **Figure 2B**). The relationship between the satiety response and BMI z-score in adolescents is not specific to the deletion group and this correlation is also present in controls (p=0.002).

Figure 2: BMI z-score, age and satiety responsiveness



*Scatterplot (**A**) represent age on the X axis, satiety responsiveness score on the Y axis and BMI z-score on the Z axis for deletion group. The solid black line represents the BMI z-score over the years while the solid colored line represents the satiety responsiveness along time. Scatterplot (**B**) show the relationship between BMI z-score and satiety responsiveness for children and adolescents in deletion group. R squares (R^2) and p-values ($p \leq 0.1$, ns otherwise) are provided. Correspondent shaded areas depict the 95% confidence intervals.*

Similar analyses show that increased scores of food responsiveness and emotional overeating appear with age and do not correlate with BMI z-score in deletion carriers (**Supplementary Figure 2).** These two measures are correlated across all groups ($r^2 > 0.4$), and both negatively correlate with satiety responsiveness in deletion carriers (**Supplementary Figure 3**).

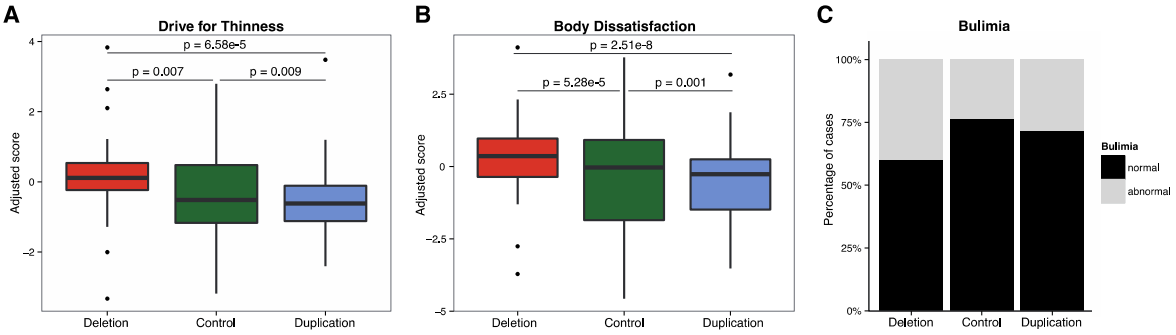
We further compared deletion carriers with a group of children with an eating disorder defined as loss of control over eating (LOC). We found no differences between these two groups on the CEBQ subscales (**Supplementary Table 10, Supplementary Figure 4**).

Adults: Body perception and eating disorder traits differences between CNV carriers and controls

Deletion carriers show increased body dissatisfaction and drive for thinness compared to controls and duplication carriers **(Figure 3A-B, Supplementary Tables 11 and 12)**. Bulimia

score **(Figure 3C)** and externality scale from the DEBQ were comparable between the three groups **(Supplementary Table 12).** When BMI z-score is included in the model, group differences are no longer present, suggesting that the above mentioned effects are mainly related to BMI **(Supplementary Table 13)**.

Figure 3: Gene dosage and group comparison on EDI-2 measures

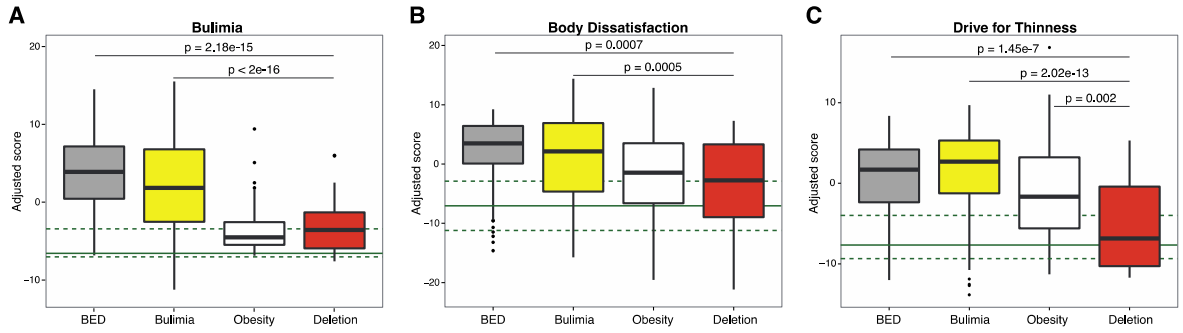


*Boxplots **(A-B)** represent scores adjusted for gender on Drive for Thinness and Body Dissatisfaction subscales (EDI-2) in deletion, duplication carriers and intrafamilial controls. The bold line shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whiskers end at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR). Significant group differences are represented by solid lines with exact p-values above. Group contrasts are estimated in regression analyses models (binomial mixed model). Stackplot (**C**) illustrates the percentage of participants with an abnormal score (> 2) on the Bulimia scale (EDI-2) in each group.*

To further understand these eating disorder traits in deletion carriers, we compared them to three other groups including individuals with obesity (OB), bulimia (BN) or binge eating disorder (BED). Bulimia and Body dissatisfaction scores are similar in deletion carriers and the OB group, and both groups score significantly lower than the BN and BED cohorts **(Figure 4A- B)**. Interestingly, deletion carriers show a significantly lower drive for thinness compared to the three other groups **(Figure 4C; Supplementary Table 14 and 15)**.

Subsequently, we investigated whether the specificity of the relationship between BMI z-score and eating disorder differs across groups (deletion carriers, BN, BED, and OB). A differential effect of BMI z-score across groups was only seen for body dissatisfaction: both eating disorder groups show a positive correlation between BMI z-score and body dissatisfaction (BN: $r^2 = 0.16$, p=6.5e-11; BED: $r^2 = 0.04$, p=0.01) whereas this relationship is not seen neither in OB nor in deletion carriers.

Figure 4: Group comparison between deletion carriers and clinical groups



*Boxplots **(A-C)** represent scores adjusted for gender and BMI z-score on Drive for Thinness, Body dissatisfaction and Bulimia subscales (EDI-2) in the deletion carriers, "constitutional" obesity (n=226), and well known eating disorders such as binge eating disorder (n=143) and bulimia (n=241). The bold line in the boxes shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whiskers end at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR). Significant group differences are represented by solid lines with exact p-values above. The solid lines flanked by the dashed lines represent the median, 1st and 3rd quartile of the extra-familial group control group (n=128).*

Global cognition, executive functions and BMI

FSIQ of CNV carriers is about 2 standard deviations lower compared to intrafamilial controls. We did not find significant relationships between BMI z-score and FSIQ. Analyses of verbal and motor inhibition also showed no relationship with BMI. There also was no correlation between cognitive scores and eating behavior scales in any of the groups.

Finally, we sought to explore the potential impact of family environment on eating behavior and obesity by comparing families where the deletion is de novo or inherited. In the latter case, transmitting parents also present cognitive impairments and obesity. The inheritance status was only available in children. Inheritance neither affects BMI z-score nor eating behavior. This analysis was not performed in the duplication due to small sample size (de novo = 7; inherited = 24, unknown = 7).

Discussion

The present study investigates the relationship between eating behavior and BMI in individuals who carry 16p11.2 BP4-BP5 CNVs, which are associated with obesity or being underweight. Children who carry a deletion present altered satiety responsiveness, which is already present before any diagnosis of obesity. The correlation between response to satiety and BMI z-score becomes significant in adolescents supporting the notion that satiety response in young deletion carriers is associated with future increase in BMI z-score. Evidence of the relevance of satiety responsiveness has been documented by longitudinal studies showing that appetitive traits in infancy are correlated with subsequent weight gain (39, 40). Our findings are also consistent with data on the relationship between satiety/satiety responsiveness and genetic factors associated with obesity (24, 26, 41). Recent studies in the general population using polygenic risk scores demonstrated that common genetic risk for higher BMI also correlates with satiety responsiveness in children even after the exclusion of *FTO* and *MC4R*, two major genes associated with obesity (25). Along with our results, this suggests that genes that are risk factors for obesity might act through appetitive mechanisms.

Although we interpret our results as a primary effect of satiety, we also show alterations in food responsiveness and emotional overeating, two components related to reward sensitivity. A previous study on 16p11.2 deletion carriers also reported subjective alteration of reward

(eating in the absence of hunger and sensitivity to boredom or external cues in deletion carriers) (27). We recently demonstrated that CNV in the 16p11.2 region is associated with altered brain structures (orbitofrontal cortex, insula, putamen and thalamus) implicated in Reward (9). However, a large body of research has demonstrated that satiety influences the subjective value of reward (42). Our current interpretation of this data is that increased responsiveness to food and emotional overeating observed in 16p11.2 deletion carriers may be the consequence of altered satiety response.

Adult deletion carriers do not present with eating disorder as defined in the DSM-5 (BN, BED). Body dissatisfaction and bulimia symptoms in deletion carriers are equal to that observed in individuals in the obese group and contrast with behaviors shown by BN and BED participants. Interestingly, deletion carriers present with significantly lower drive for thinness compared to the obese group with similar BMI. Future studies looking at personality traits known to positively correlate with drive for thinness (e.g. harm avoidance, anxiety) (43) will improve our understanding of this difference.

Although several studies have demonstrated a correlation between BMI and executive dysfunction in obesity, we show no association between BMI z-score and overall cognitive functioning. These results replicate earlier reports in 16p11.2 CNV carriers (14). The fact that LOC children and deletion carriers do not differ on any of the behavioral CEBQ dimensions suggests similar underlying mechanisms for their energy imbalance. Similarly, we did not find any relationship between BMI z-score and inhibition, corroborating previous findings in extreme weight conditions such as anorexia nervosa and obesity (20). This is analogous to findings for the 22q11.2 deletion CNV showing that psychopathology and cognitive deficits are independent sequelae (44).

One of the limitations of this work is our reliance on parent- and self-report ratings of eating behavior, which are subjective in nature. Possible under- or over-reporting on these measures would have implications for our analyses on BMI z-scores. The lack of longitudinal data prevents us from concluding how response to satiety evolves over time. Furthermore, the measures of eating behavior varied between the pediatric and adult cohorts. However, the study of a high-risk pediatric cohort offers the possibility to explore behavioral phenotypes that are not the consequence of long-standing obesity. We observed fewer significant effects of the duplication on eating behaviors that may be due to smaller sample size of the duplication group.

To conclude, our findings provide further insights into the behaviors underlying or associated with energy imbalance in 16p11.2 CNV carriers. Altered satiety response is potentially a primary mechanism contributing to later obesity in deletion carriers but co-occurring changes in the reward system may also play a role. Study of these reciprocal 16p11.2 CNVs and how they affect clinical traits is a powerful tool to shed light on common phenotypes such as obesity. For clinicians, a comprehensive characterization of eating behavior will guide care of patients presenting with this genetic disorder.

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General Discussion

1. Summary of the results

This thesis work shows that careful characterization of recurrent and reciprocal CNVs allows to investigate how a particular genomic locus may modulate cognitive, behavioral and intermediate traits in humans.

Our neuroimaging study demonstrates that gene dosage inversely correlates with global brain measures (total intracranial, grey and white matter volumes) as well as specific brain regions including reward, language and social cognition circuits. These data also show that well-established signatures of ASD and schizophrenia (such as changes in the putamen and insula) are identified in 16p11.2 CNV carriers in the absence of either an ASD or schizophrenia diagnosis.

Our investigation of cognitive traits in 16p11.2 carriers (FSIQ > 55) shows that this locus modulates specific domains including language, memory and inhibition. While the deletion is associated with deficits in language and executive functions, the neuropsychological profile of duplication carriers is devoid of specific weaknesses and even shows enhanced performances in memory with results above what is expected for their global cognitive functioning.

Investigation of eating behavior shows that the number of genomic copies at the 16p11.2 locus modulates satiety and food responsiveness. Akin to other genetic forms of obesity, abnormal satiety in children seems to reflect one of the underlying mechanisms associated with later onset of obesity.

2. Gene dosage and traits correlated to gene dosage

This project is one of the first attempts to systematically investigate the effect of gene dosage on brain structure, cognition and behavioral traits in humans. The impact of gene dosage on neuro-cognitive phenotypes has been previously hypothesized in case series (Merla et al., 2010; Van der Aa et al., 2009) but sufficiently powered cohorts of deletion and reciprocal duplication carriers were not available before the advent of genome wide analyses.

Stefansson et al. (Stefansson et al., 2013) were the first to report a negative correlation between gene dosage at the 15q11.2 BP1-BP2 locus and volume of regional structures defined by recent meta-analysis of first episode psychosis. Deletion carriers at risk for developing schizophrenia showed reduced volume in the perigenual anterior cingulate cortex, the insula and supra-marginal gyrus and in the temporal white matter. Duplication carriers showed reciprocal changes. A neuroimaging study of 16p11.2 CNV carriers (Qureshi et al., 2014)conducted in parallel with our efforts described a gene dosage effect on global brain volume in a pediatric cohort of 16p11.2 CNV carriers without clear regional effects except for opposing volume differences in the thalamus. We confirm in our MRI study that the number of genomic copies at the 16p11.2 locus modulates global brain volume of grey and white matter and further demonstrate that this genomic region modulates specific brain structures involved in reward and language circuits (Maillard et al., 2014).

The same gene dosage approach was applied by Stefansson et al. (Stefansson et al., 2013) to cognitive traits in carriers of 15q11.2 BP1-BP2 and 16p11.2 CNVs but either the small sample size (for 16p11.2) or the small effect size (for 15q11.2) did not allow to identify any correlations between cognitive traits and copy number state. To our knowledge, our neurocognitive study demonstrates for the first time that cognitive and behavioral traits may be under gene dosage dependence. Our findings are corroborated by studies in mice models of

16p11.2 CNVs. Recent data on mouse models of 16p11.2 reciprocal CNV (Abrogast et al., submitted) corroborate the gene dosage effect found for memory skills in our 16p11.2 carriers' cohort with duplication mice performing better than wild type and deletion mice in an object recognition task.

Molecular factors underlying the neurobiology of cognitive functions have been studied in model organisms (Costa-Mattioli and Monteggia, 2013). In particular, memory has been linked to long-lasting synaptic potentiation and depression and these synaptic mechanisms require burst of local protein synthesis during training and stimulation. Both mTOR and Erk1 (*MAPK3*) signalling regulates local synaptic protein synthesis which in turn modulates memory performances in murine models (Costa-Mattioli and Monteggia, 2013; Stoica et al., 2011). *MAPK3,* which maps within the BP4-BP5 interval and mTOR pathway members, which expression levels are altered by 16p11.2 CNVs (Migliavacca et al., submitted) are candidates genes underlying the correlation between memory tasks and genomic copy number.

3. Elucidating contribution of individual genes in CNVs

The contribution of individual genes to cognitive and behavioral phenotypes is still unresolved in 16p11.2 rearrangements as in many CNVs, including 22q11.2 and Williams syndrome, suggesting that CNVs may be complex/oligogenic neurodevelopmental disorders in and of themselves (Jarvinen-Pasley et al., 2008; Squarcione et al., 2013). To investigate the contribution of promising candidates gene in the 16p11.2 region, we tested if changes in mRNA levels of 18 candidates genes measured in lymphoblastic cell lines mediated the effect of gene dosage on brain anatomy (Maillard et al., 2014). Because gene expression was highly correlated to gene dosage (70% of the variance of mRNA levels is explained by copy number) we were not able to identify the contribution of individual genes or sets of genes to specific anatomical patterns. However, this approach remains promising provided that genes under

investigation are expressed in peripheral tissue and larger datasets are assembled. Future studies might use a similar approach to further investigate the individual contributions of genes within this region.

4. Genetic forms of obesity and eating behavior traits

Obesity is a highly heritable trait and many genes have been associated with BMI (Speliotes et al., 2010). Alterations of satiety have been described in genetic forms of obesity (Acosta et al., 2014; Llewellyn et al., 2014; Wardle et al., 2008). A study conducted in 10 year old children of the general population has shown that a polygenic risk score including the top 28 common SNPs validated in genome-wide association studies was correlated, as expected, to BMI and also satiety responsiveness (Llewellyn et al., 2014). This correlation with satiety was also present even after the exclusion of *FTO* and *MC4R* (two genes previously associated with obesity) from the score, indicating that this relationship may be a general phenomenon among genes involved in obesity. Our findings support this notion by showing a 16p11.2 gene dosage effect on satiety responsiveness in children. Remarkably, altered measures of response to satiety are present in young children before the onset of obesity suggesting that altered satiety response may mediate the association between the deletion and obesity. This has been observed in longitudinal studies showing that appetitive traits in infancy were associated with weight gain in early childhood (Parkinson et al., 2010; Van Jaarsveld et al., 2011).

However, while these data strongly point towards dysregulation of appetitive mechanisms, it could also reflect disruption of any peripheral or central nodes of this energy balance loop. Our understanding of the mechanisms underlying obesity in 16p11.2 deletion carriers remains extremely limited. Mechanisms could implicate any central or peripheral primary alteration as well as secondary aggravating effect such as alterations of the gut microbiota, which we are currently investigating.

5. Shared genetic factors between mental disorders

Genome wide association studies investigating rare and common variants in ASD and schizophrenia have documented shared genetic factors between these two mental disorders (Doherty and Owen, 2014; Malhotra and Sebat, 2012) although these two conditions do not segregate in the same families (Crespi and Badcock, 2008). Interestingly, at least four different loci (1q21.1, 16p11.2, 22q11.2, 22q13.3) show a reciprocal association with ASD and schizophrenia (Malhotra and Sebat, 2012). Namely, if the deletion is associated with one of the two conditions, the duplication is preferentially associated with the other. In addition, any given CNV is rarely associated with both ASD and schizophrenia. Recently, data has shown that the 22q11.2 duplication, which is associated with ASD also protects against schizophrenia (Rees et al., 2014). This has led to the hypothesis that these two conditions may be manifestations of opposite alterations of the same mechanism (Crespi and Crofts, 2012). Consistent with this notion, we show in our MRI study a significant overlap between the pattern of brain anatomy modifications seen in 16p11.2 CNVs with well-established structural signatures of ASD and schizophrenia. Notably, opposing alterations in the reward system structures, influenced by 16p11.2 gene dosage, are also the main structural changes shared by these disorders (Adolphs, 2003; Cheung et al., 2010; Nickl-Jockschat et al., 2012).

6. Obesity and mental disorders

Obesity is a frequent comorbidity of neuropsychiatric disorders (Chen et al., 2010) including ASD, ADHD and DD/ID. This association occurs independently of socio-economic factors suggesting potential common underlying mechanisms in these diseases (Broder-Fingert et al., 2014; Chen et al., 2010; Cortese et al., 2008; Curtin et al., 2010; Doody and Doody, 2012; Pagoto et al., 2009). It has been hypothesized that the reward system, and the striatum in particular, may underlie the co-occurrence of metabolic and psychiatric manifestations (Grimm et al., 2014; Kenny, 2011; Lopresti and Drummond, 2013; Pannacciulli et al., 2006).

Notably, dysfunction in the reward system and dopaminergic circuits have been found in obesity, ASD and schizophrenia (Avena and Bocarsly, 2012; Dichter et al., 2012; Fagundo et al., 2012). Consistently, our MRI study demonstrates that gene dosage at the 16p11.2 locus modulates neural circuitry and in particular the reward system. Portmann et al. (Portmann et al., 2014) recently provided further evidence for the involvement of the basal ganglia, striatum and dopaminergic circuitries in a mouse model of 16p11.2 deletion syndrome. Changes in reward-related behavior have not yet been investigated in 16p11.2 CNV carriers and will be the focus of future studies.

7. Predictive value of the 16p11.2 CNVs on the clinical phenotype

The predictive value of a genetic variant is important for clinicians to provide appropriate counseling. Ultimately, relevant genetic variants will also be actionable and will guide decision in medical care. One of the most challenging aspects of these newly identified CNVs is the degree of phenotypic variability. While the 16p11.2 deletion carriers' profile is fairly consistent, the duplication carriers' profile is more variable and complex, which is in accordance with the large phenotypic variance reported in duplications of most genomic regions (Malhotra and Sebat, 2012). We have observed the same phenomenon in our cohort: While deletion carriers present with a decrease in FSIQ distribution without any change in the variance (a simple shift of the mean), the distribution of FSIQ in duplication carriers demonstrates increased numbers at both extremes of the FSIQ distribution. There is a 19.4-fold increase in duplication carriers with very low FSIQ ≤ 40 compared to 1/200 in deletion carriers as well as a 2.0-fold enrichment of duplication carriers above population average (FSIQ > 100) versus 20/200 of the deletion carriers (d'Angelo, submitted). Through our studies we were able to describe the neuropsychological and eating behavior phenotypes in 16p11.2 CNV carriers showing increased cognitive variability in duplication along with an increased risk for language disorder in deletion carriers that can guide clinicians with the

counseling.

8. Limitations

Proband participants were recruited through a network of geneticists who saw the patients in the context of a neurodevelopmental disorder. Participants were thus symptomatic and may not fully represent the whole range of phenotype associated with 16p11.2 rearrangements. In contrast, the adults recruited in our studies are mostly transmitting parents which is a specific criteria selecting against significant neuropsychiatric disorders. Recruiting adult participants with psychiatric disorders was particularly difficult, as these patients are not referred to geneticists for etiological investigations. Other recruitement strategies (web-based recruitment method) used by our collaborators at the Simons' Foundation for the Simon's VIP cohort (USA) was associated with specific biases and selected strongly against disabilities in parents. As a result, they were unable to ascertain families where the deletion was inherited (Zufferey et al., 2012). Using multiple ascertainment methods will allow us to provide a comprehensive cognitive and behavioral characterization of the 16p11.2 population. Although some phenotypes (e.g. cognition) are sensitive to the ascertainment method, others seem more robust across cohorts such as the effect on BMI z-score and head circumference (Zufferey et al., 2012).

Another anticipated limitation in the studies was the sample size. We were unable to acquire MRI data on participants with significant behavioral deficits and BMI/waist circumference beyond MRI scanning safety standards meaning that we were unable to scan participants with morbid obesity. In the cognitive study we were only able to collect data on a small group of severely affected individuals (mostly duplication carriers) impeding us from running statistical analyses on this subset of participants. Expanding the 16p11.2 deletion and duplication cohorts would allow more statistical power to study subgroups based on variables such as severity of the symptoms or kinship. Finally, we only have cross-section data on the

participants. Longitudinal studies would be of great interest to follow the natural history of brain development and clinical traits.

Conclusion

Study of reciprocal CNVs is a powerful method to investigate gene dosage effects on brain structure and clinical traits as well as to explore phenotypes commonly associated in neurodevelopmental disorders. Careful investigation of deletion and reciprocal duplication allowed us to demonstrate that global brain volumes and specific brain regions were modulated by gene dosage, further supporting what had previously been shown with measure of head circumference. We also reported for the first time a gene dosage effect on behavior and specific cognitive measures.

This novel approach allows for understanding of how a trait may be modulated by a molecular mechanism and is relevant for neuropsychiatric disorders, which in this context may be studied as a set of dimensional measures. Future studies including transcription and biomarker data in larger samples will help understand the underlying mechanisms and whether these are specific to the 16p11.2 or shared by other genetic etiologies associated with ASD and schizophrenia. On the more practical side, our comprehensive characterization of the differential neuropsychological profile and food related behaviors of the duplication and deletion carriers will guide clinicians in the assessments and care of their patients presenting with these CNVs.

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Appendix

Appendix 1

Supplemental Material : The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity

Supplementary Table 1: DSM-IV-TR diagnoses

	Deletion N= 14	**Duplication** N=17
Communication disorder (315.31; 315.32; 315.39)	6	0
Intellectual disability (317; 318)	4	3
Depression (296.3)	2	5
Posttraumatic stress disorder (309.81)	1	0
Disruptive behavior disorder NOS (312.9)	1	0
Pathological gambling (312.31)	0	1
ASD (299)	0	2
Social phobia (300.23)	0	2
Bipolar disorder (296.6)	0	1
Anxiety disorder (300.01; 300.22)	0	4

DSM-IV-TR [1] diagnosis code is given in parentheses.

Supplementary Table 2: Neuroradiological findings

	Deletion N= 14	**Controls** N=23	**Duplication** N=17
Arnold Chiari malformation, type 1	3	0	0
Posterior fossa arachnoïd cyst	0	1	1
Enlarged cisterna magna	0	2	0
Corpus callosum hypoplasia	0	0	1
Ventricular asymmetry	1	0	2
Enlarged ventricles	0	0	1

Legend: A senior board certified neuroradiologist reviewed all MRI images for brain abnormalities

Supplementary Table 3: Global brain metrics: adjusted mean values for age and gender

	Deletion N=14	**Controls** N=23	**Duplication** N=17	**Gene dosage effect**
	Mean ± SD			
HC z-score	0.73 ± 1 ¶	-.38 ± .96	-.73 ± 1.4	p= 0.0001
GM (mm^3)	792 ± 32 ♦	762 ± 46	743 ± 69	p= 0.009
WM (mm^3)	468 ± 41 ♦	454 ± 40	412 ± 46§	p< 0.0001
CSF (mm^3)	289 ± 30	285 ± 34	274 ± 40	ns
TIV (mm^3)	1563 ± 50 ♦	1534 ± 97	1471 ± 127	p= 0.004
CT (mm)	2.47 ± 0.07§	2.53 ± 0.08	2.48 ± 0.07	ns
CS (m^2)	0.21 ± 0.01♦	0.21 ± 0.01	0.19 ± 0.014§	p=0.009

Legend: Gene dosage effect tested with a linear regression analysis using the number of copies as a numerical variable. Results are presented uncorrected for multiple testing.

¶ significantly different from the two other groups, $p < 0.05$
§ significantly different from the control group, $p < 0.05$
♦ significantly different from duplication carriers, $p < 0.05$

HC - head circumference; **GM** - gray matter; **WM** - white matter; **CSF** - cerebrospinal fluid; **TIV** - total intracranial volume; **CT** - cortical thickness, **CS** - cortical surface area.

Supplementary Table 4: Correlations between BMI z-score and global metrics

	R	**p-value**
CS	0.165	0.236
CT	-0.103	0.465
GM	0.367	**0.008**
WM	0.233	0.094
TIV	0.323	**0.019**
CSF	0.118	0.403

Legend: Correlations between BMI z-scores and global metrics adjusted for age and gender on the whole sample (n=54). Uncorrected p-values < 0.05 are highlighted in bold.
CS - cortical surface area; **CT** - cortical thickness; **GM** - gray matter; **WM** - white matter; **TIV** - total intracranial volume; **CSF** - cerebrospinal fluid.

Supplementary Table 5: Voxel-based morphometry results

Regions	Side	MNI coordinates			Peak T-value
		x	y	z	
GRAY MATTER[1]					
DEL > CTRL > DUP					
Anterior insula, putamen, caudate	L	-29	9	12	6.98
	R	30	9	10	8.03
Superior temporal gyrus & supramarginal gyrus	L	-38	-34	22	5.60
	R	37	-27	9	8.03
Fusiform gyrus	L	-14	-57	1	5.35
Orbit-frontal cortex	L	-21	17	-17	5.83
	R	18	18	-21	5.37
Medio-dorsal thalamus	L	-9	-24	0	4.80
	R	6	-25	1	5.53
Calcarine & lingual sulcus	L	-23	-60	10	4.71
	R	18	-67	3	4.95
Inferior, middle and superior occipital gyrus	R	21.0	-97.5	7.5	5.74
DEL > CTRL					
Anterior insula, putamen, caudate* (*L: p=0.054)	L	-32	9	15	5.09
	R	31	12	15	6.40
Superior temporal gyrus, supramarginal gyrus	L	-38	-34	24	8.66
	R	42	-28	25	7.94
Area triangularis	L	-51	20	-5	5.96
Calcarine sulcus, cuneus, inferior and middle occipital gyrus, lingual gyrus	R	20	-93.0	10	5.16
CTRL > DUP					
Caudate	L	-20	14	10	3.76
	R	17	14	19	4.58
Putamen	L	-27	8	10	3.60
	R	30	8	9	4.31
DEL < CTRL < DUP					
Lobule VIII of cerebellar hemisphere, crus II of cerebellar hemisphere, flocculus	R	24	-50	-62	5.23
Middle temporal gyrus	L	-51	-36	4	7.42
Supramarginal gyrus	L	-44	-51	22	5.58
Lobule VIII of cerebellar hemisphere, lobule VIIb of cerebellar hemisphere	L	-33	-69	-55	4.76
Lateral fusiform gyrus	L	-39	-52	-14	5.03
DEL < CTRL					
Lobule VIII of cerebellar hemisphere, crus II of cerebellar hemisphere	L	-27	-66	-57	4.56
	R	29	-72	-57	4.86

Middle temporal gyrus	L	-51	-36	3	7.25
	R	48	-30	-8	5.76
WHITE MATTER[2]					
DEL > CTRL > DUP					
Inferior fronto-occipital fasciculus, forceps minor, anterior thalamic radiation, superior longitudinal fasciculus, cingulate	L	-40	25	4	7.36
	R	42	38	-6	4.72
Forceps major, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, anterior thalamic radiation	L	-39	-30	7	6.77
	R	42	-42	10	5.01
Inferior and superior longitudinal fasciculus	L	-27	-70	16	5.32
	R	27	-61	19	5.79
Inferior fronto-occipital fasciculus, uncinate fasciculus, cingulate, anterior thalamic radiation	L	-20	25	-12	5.32
	R	26	27	-12	4.77
DEL > CTRL					
Forceps major, inferior and superior longitudinal fasciculus, anterior thalamic radiation, cingulate	L	-20	-76	12	5.88
	R	27	-60	19	5.82
FRACTIONAL ANISOTROPY IN GRAY MATTER[3]					
DEL > CTRL > DUP					
Middle and superior temporal gyrus	L	-56	-36	-1	6.27
	R	60	-39	4	4.49
Lateral fusiform gyrus	L	-39	-49	-14	5.54
	R	39	-49	-11	5.14
Middle occipital gyrus	L	-24	-90	7	5.54
DEL > CTRL					
Middle temporal gyrus	L	-56	-36	-2	6.89
(*: p=0.067)	R*	58	-37	4	4.18
Lateral fusiform gyrus	L	-41	-49	-14	5.77
MEAN DIFFUSIVITY IN GRAY MATTER[4]					
DEL < CTRL < DUP					
Caudate*(L p=.052, R p=0.88)	L*	-18	23	0	5.77
	R*	15	21	-2	5.20
Middle and transverse temporal gyrus	L	-60	-30	-7	5.10
	R	57	-13	-12	5.37
Anterior cingulate gyrus	L/R	0	39	0	4.43
CTRL < DUP					
Caudate	L	-15	21	-2	5.36
	R	12	21	-2	5.66

Legend: Significant anatomical structures included in the statistical maps (FWE correction, cluster level, $p<0.05$). Trend ($p< 0.09$) are indicated by a *.

[1] No significant result for the pairwise comparison [CTRL< DUP].

[2] No significant result for the following pairwise comparisons: [CTRL > DUP] or [DEL < CTRL < DUP] or [DEL < CTRL] or [CTRL < DUP].

[3] No significant result for the following pairwise comparisons: [CTRL>DUP] or [DEL < CTRL < DUP]
[4] No significant result for the following pairwise comparisons: [DEL< CTRL] or [DEL > CTRL > DUP].
Additionally, there are no significant results for FA or MD in white matter.
DEL – deletion carriers; **CTRL** – intrafamilial controls; **DUP** – duplication carriers.

Supplementary Table 6: Surface-based results

Regions	Side	Cluster extent (nb vertices)	Cluster p-value	VBM overlap
CORTICAL THICKNESS[1]				
DEL > CTRL > DUP				
Insula and Supramarginal gyrus	R	4451	< 0.001	+
Inferior pre- and postcentral gyrus	R	4659	< 0.001	
Superior postcentral gyrus	R	3053	0.005	
Supramarginal gyrus	L	2273	0.03	+
DEL > CTRL				
Inferior pre- and postcentral gyrus and supramarginal gyrus	R	3136	0.007	+
Superior parietal gyrus	L	2084	< 0.001	
Superior postcentral gyrus	L	1876	0.04	
DEL < CTRL < DUP				
Orbitofrontal and rostral middle frontal gyrus	R	5193	< 0.001	
Middle temporal and fusiform gyrus	L	3330	0.002	+
Orbitofrontal cortex	L	3180	0.002	
Inferior temporal cortex	R	2696	0.005	
Superior precentral and prefrontal gyrus	R	2913	0.008	
Lateral occipital cortex	L	2191	0.04	
DEL < CTRL				
Middle temporal and fusiform gyrus	L	4115	< 0.001	+
CTRL < DUP				
Medial obitofrontal cortex	L	2778	0.02	
SURFACE AREA[2]				
CTRL < DUP				
Rostral middle frontal gyrus and frontal pole	R	2976	< 0.001	
Insula	L	2256	0.002	+

Legend: Significant cortical structures for cortical thickness and surface area (p value after FWE correction, cluster level)
[1] For the pairwise comparison [CTRL > DUP], there are no significant results surviving FWE correction.

[2] For the following pairwise comparisons: [DEL > CTRL > DUP] or [DEL > CTRL] or [CTRL > DUP] or [DEL < CTRL < DUP] or [DEL < CTRL], there are no significant results surviving FWE correction.
DEL – deletion carriers; **CTRL** – intrafamilial controls; **DUP** – duplication carriers.

Supplementary Table 7: Shared brain structure alterations in ASD, SZ and 16p11.2

Side	Region	MNI coordinates			ASD	SZ	Overlap with 16p11.2 CNV carriers
		X	Y	Z	%	%	
Decrease in Gray Matter							
L	**Putamen**	-24	4	1	99.8	0.2	**Yes**
R	Parahippocampal Gyrus	31	-15	-20	42.9	57.1	No
R	**Posterior Cingulate**	24	-57	16	41.1	58.9	**Yes**
R	**Putamen**	31	2	1	38.9	61.1	**Yes**
R	Insula (anterior)	43	-20	-8	23.1	76.9	No
L	**Thalamus**	-6	-19	9	23.1	76.9	**Yes**
R	**Insula** (posterior)	36	-15	13	22.6	77.4	**Yes**
L	Precuneus/Cingulate	2	-43	36	0.4	99.6	No
R	Cingulate Gyrus	12	27	28	0.2	99.8	No
L	Insula/Inferior Frontal Gyrus	-40	25	-6	0.1	99.9	No
L	Superior Frontal Gyrus	-1	41	51	0.1	99.9	No
L	Caudate (Head/Body)	3	15	1	0	100	No
L	Temporal Gyrus	-64	-23	12	0	100	No
L	Uncus/Amygdala	-17	-3	-28	0	100	No
R	Middle Frontal Gyrus	48	39	14	0	100	No
Increase in Gray Matter							
L	**Superior temporal gyrus**	-35	-51	8	7.8	92.2	**Yes**
L	**Putamen**	-23	3	9	5.6	94.4	**Yes**

Legend: Overlap between brain structural alterations identified in a meta-analysis of 25 autism spectrum disorders and schizophrenia studies [2] and our analysis of 16p11.2 CNV carriers. Talairach coordinates provided in the meta-analysis by Cheung et al. [2] were transformed into MNI space using the method described in the manuscript. The ASD column shows, in percentage, the clusters contributed by ASD studies. The SZ column shows in percentage the clusters contributed by SZ studies [2]. Blue and green colors represent a contribution > 1% for ASD and SZ respectively.
The last column "overlap" indicates whether regions of the statistical maps defined by gene dosage of the 16p11.2 locus (GM) overlap with regions involved in ASD/SZ with Euclidean distance < 2cm. Directionality was not taken into account.

Supplementary Table 8: Correlation between brain global metrics and the 18 genes within BP4-BP5 region as well as *SH2B1*

	TIV		GM		WM		CSF		CS		CT	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
QPRT	-0.5	**0.008**	-0.4	**0.02**	-0.4	**0.032**	-0.2	0.255	-0.3	0.107	0.2	0.314
KIF22	-0.6	**0.001**	-0.6	**0.003**	-0.6	**0.002**	-0.3	0.076	-0.5	0.011	0.3	0.125
MAZ	-0.6	**0.001**	-0.6	**0.003**	-0.6	**0.002**	-0.3	0.078	-0.4	0.034	0.3	0.147
PRRT2	-0.4	**0.042**	-0.3	0.088	-0.4	0.073	-0.5	0.014	-0.1	0.701	0.1	0.588
C16ORF53	-0.5	**0.003**	-0.5	**0.01**	-0.5	**0.013**	-0.4	0.036	-0.2	0.267	0.3	0.167
MVP	-0.6	**0.001**	-0.6	**0.002**	-0.5	**0.003**	-0.5	0.018	-0.3	0.106	-0.1	0.798
CDIPT	-0.7	**6.87E-05**	-0.6	**0.001**	-0.7	**1.25E-04**	-0.4	0.05	-0.4	0.021	0.2	0.347
KCTD13	-0.6	**0.001**	-0.6	**0.002**	-0.6	**0.002**	-0.4	0.05	-0.3	0.1	0.2	0.395
TMEM219	-0.5	**0.004**	-0.5	**0.008**	-0.5	**0.005**	-0.4	0.063	-0.3	0.143	0.2	0.348
TAOK2	-0.6	**4.15E-04**	-0.5	**0.01**	-0.6	**0.001**	-0.6	0.003	-0.4	0.073	0.1	0.529
HIRIP3	-0.7	**1.08E-04**	-0.6	**0.001**	-0.7	**2.20E-04**	-0.5	0.018	-0.5	0.01	0.2	0.252
INO80E	-0.7	**1.14E-04**	-0.6	**0.001**	-0.6	**2.71E-04**	-0.4	0.057	-0.4	0.025	0.1	0.468
ALDOA	-0.5	**0.005**	-0.5	**0.011**	-0.5	**0.005**	-0.3	0.157	-0.3	0.173	0.2	0.386
PPP4C	-0.6	**0.001**	-0.6	**0.002**	-0.6	**0.001**	-0.3	0.089	-0.4	0.056	0.2	0.241
YPEL3	-0.5	**0.01**	-0.5	**0.012**	-0.6	**0.002**	-0.3	0.088	-0.4	0.073	-0.1	0.695
GDPD3	-0.3	0.114	-0.2	0.329	-0.4	0.067	-0.2	0.22	-0.2	0.451	0.3	0.111
MAPK3	-0.5	**0.009**	-0.5	**0.012**	-0.5	**0.005**	-0.3	0.146	-0.3	0.147	0.1	0.719
CORO1A	-0.6	**0.001**	-0.6	**0.002**	-0.5	**0.004**	-0.2	0.217	-0.4	0.044	0.3	0.206
SH2B1	-0.1	0.5	0.0	1	0.1	0.8	-0.3	0.1	0.0	0.9	0.4	0.02

Legend: Uncorrected p-values from Pearson correlation tests assessing the relationship between global brain metrics and 18 genes within BP4-BP5 region as well as one gene (*SH2B1*) outside the interval. P-values in bold are those surviving Benjamini & Hochberg FDR correction. After adjusting for the number of genomic copies, none of the marginally significant correlations between expression levels and global metrics survive FDR correction.
TIV – total intracranial volume; **GM** - gray matter **WM** - white matter; **CSF** - cerebrospinal fluid; **CS** - cortical surface area; **CT** - cortical thickness.

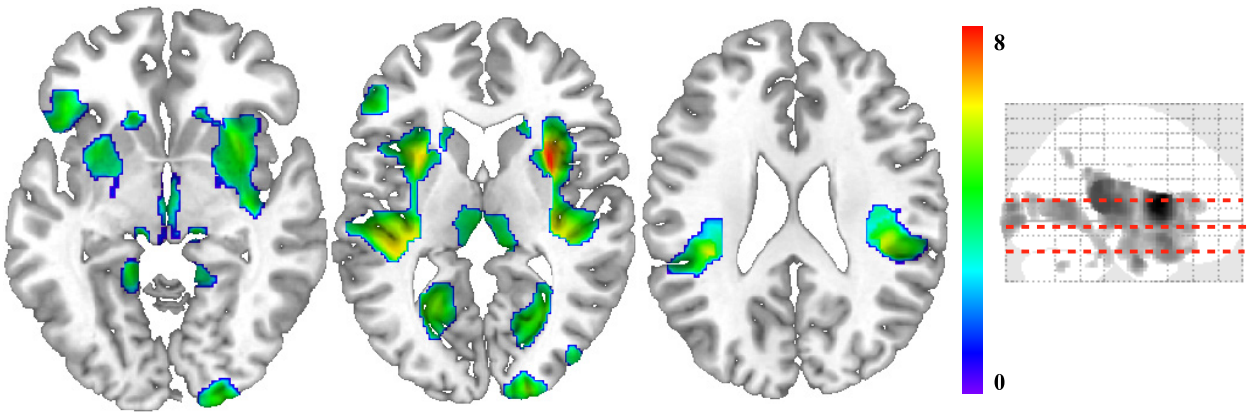
Supplementary Table 9: Relationship between mRNA levels of genes within the BP4-BP5 interval and ROI identified in the first eigenimage

	Right putamen	Left insula	Left STS	Right STS
MAPK3	0.043	0.069	0.265	0.056
KCTD13	0.021	0.034	0.140	0.059
ALDOA	0.011	0.032	0.096	0.023
MVP	0.492	0.363	0.860	0.569
CDIPT	0.059	0.041	0.104	0.031
PPP4C	0.052	0.027	0.090	0.016
TAOK2	0.183	0.260	0.288	0.179
INO80E	0.062	0.049	0.165	0.026
KIF22	0.110	0.040	0.130	**0.008**
TMEM219	0.020	0.027	0.059	0.034
MAZ	0.078	0.023	0.120	**0.007**
PRRT2	0.291	0.623	0.588	0.947
YPEL3	0.318	0.111	0.218	0.234
CORO1A	0.139	0.073	0.255	**0.010**
GDPD3	**0.003**	0.019	0.012	**0.011**
QPRT	**0.003**	0.008	0.012	**0.006**
C16ORF53	0.019	0.031	0.082	0.026
HIRIP3	0.350	0.118	0.318	0.054
SH2B1	0.091	0.937	0.797	0.481

Legend: Results show uncorrected p–values resulting from the univariate analysis testing the relationship between mRNA levels and ROI extracted from the first eigenimage (shown in Figure 4) of the MLM analysis. Significant p-values surviving Benjamini & Hochberg FDR correction are highlighted in bold.
After adjusting for the number of genomic copies, none of the correlations between expression levels and ROI survive FDR correction.
STS – superior temporal sulcus

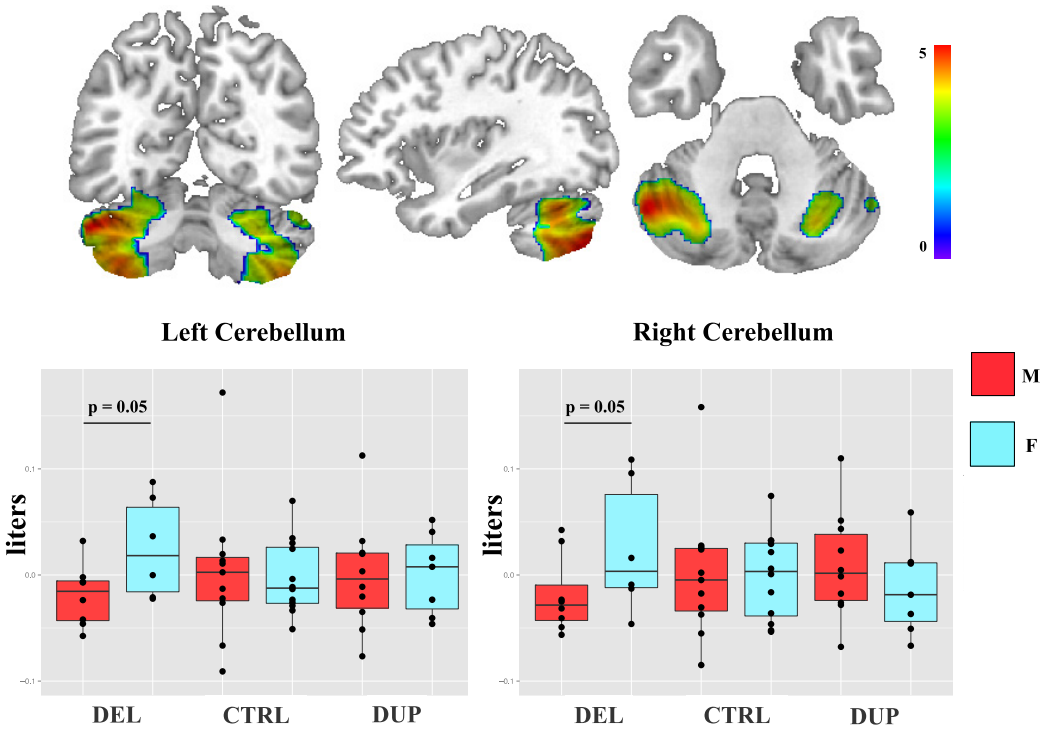
Figures

Supplementary Figure 1: Effect of Gene dosage adjusting for FSIQ



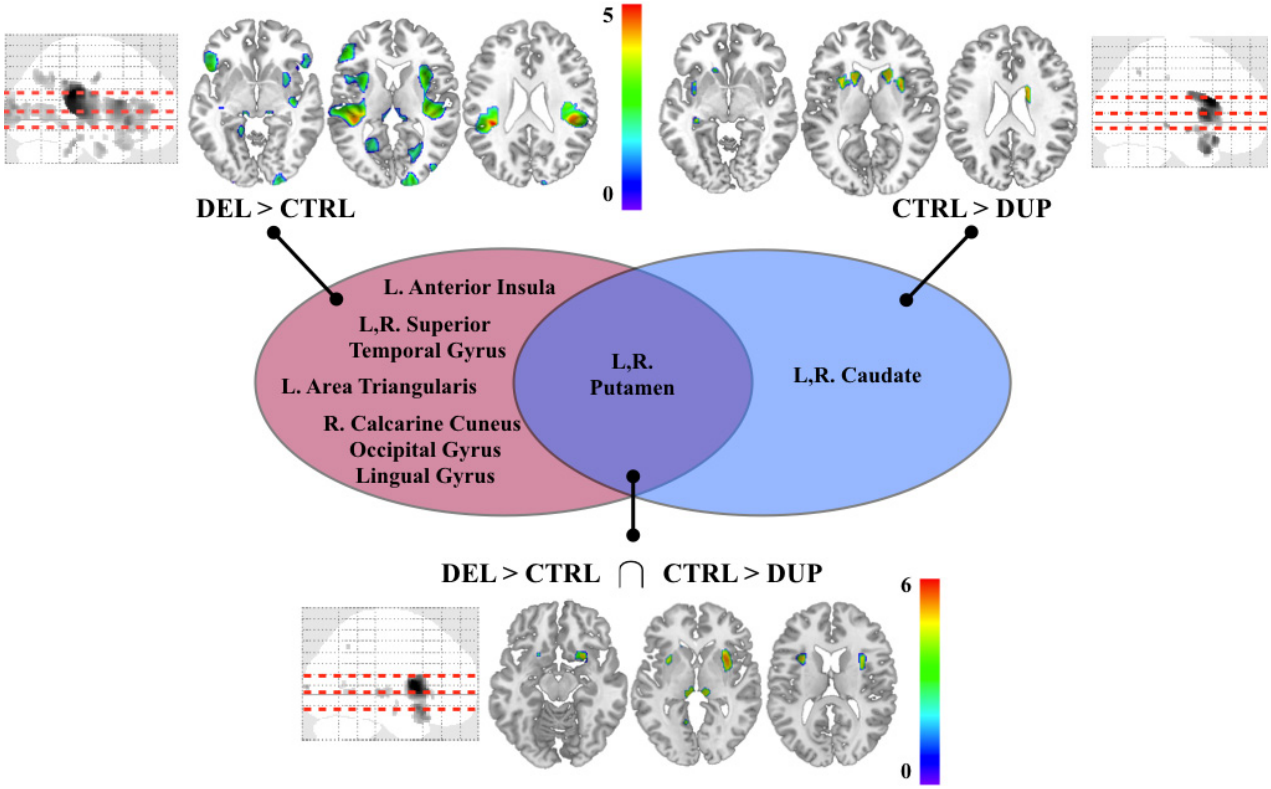
Legend: Whole brain analysis using gene dosage (number of copies) as the main explanatory variable and FSIQ as a covariate. The color bar represents T scores. The statistical map overlaps with the patterns of the analysis presented in Fig 2. For presentation purposes results are displayed at significance threshold of $p<0.001$ uncorrected at voxel level

Supplementary Figure 2: Interaction between CNV and gender



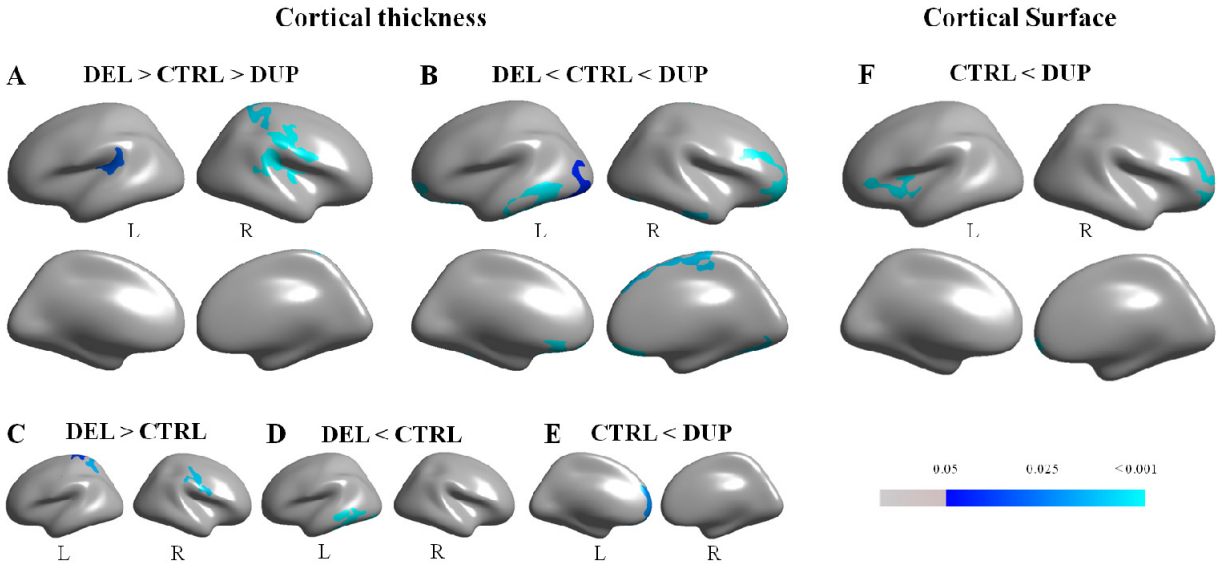
Legend: Whole brain analysis identifies a gender-by-CNV interaction in the Crus I and lobules VI, VIIb, VIII of the cerebellar hemisphere bilaterally. Male deletion carriers show significantly less GM volume in the cerebellum bilaterally compared to their female counterparts as opposed to the usually observed sexual dimorphism with larger cerebellums in males[3,4]. The color bar represents T scores. For representation purposes results are displayed at significance threshold of $p<0.001$ uncorrected at voxel level.
DEL - deletion carriers; **CTRL** - intrafamilial controls; **DUP** – duplication carriers.

Supplementary Figure 3: Differential and overlapping contributions of 16p11.2 deletion and duplication to brain anatomy.



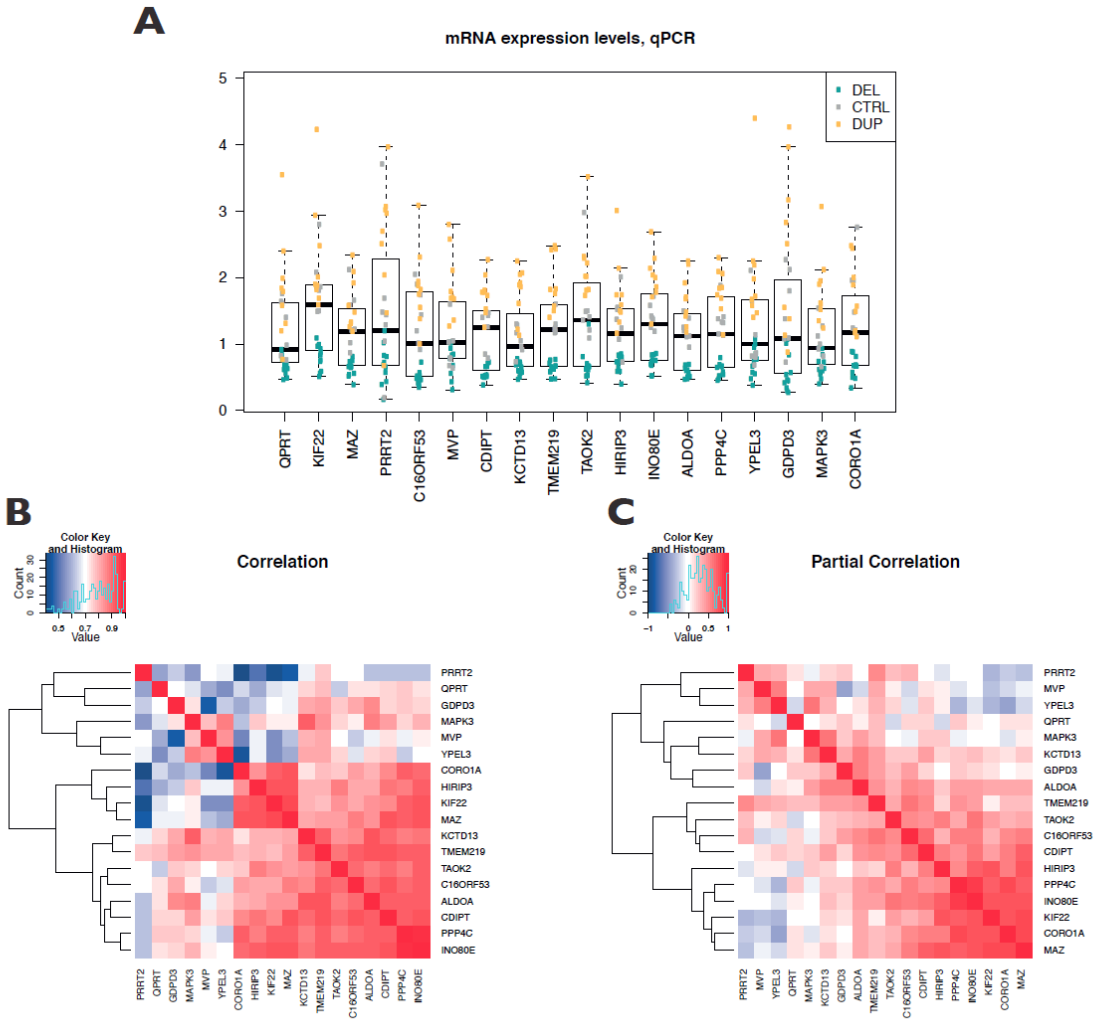
Legend: The intersection represents the conjunction analysis looking at the intersection between between the two differential contrasts: DEL > CTRL and CTRL > DUP. Results show significant volume changes in the right putamen (p_{FWE} =0.019). The comparison DEL > CTRL shows that deletion carriers have significantly more GM volume (FWE, $p < 0.05$) in the superior temporal region, supramarginal gyrus, putamen, insula bilaterally and left area triangularis compared to controls. The comparison CTRL > DUP shows that DUP have significant less GM volume in the caudate nucleus and putamen compared to CTRL. The color bar represents T scores. For presentation purposes, results are displayed at $p<0.001$ uncorrected at voxel level.
DEL – deletion carriers; **CTRL** – intrafamilial controls; **DUP** – duplication carriers.

Supplementary Figure 4: Effects of gene dosage and group comparisons analysis for regional cortical thickness and surface area



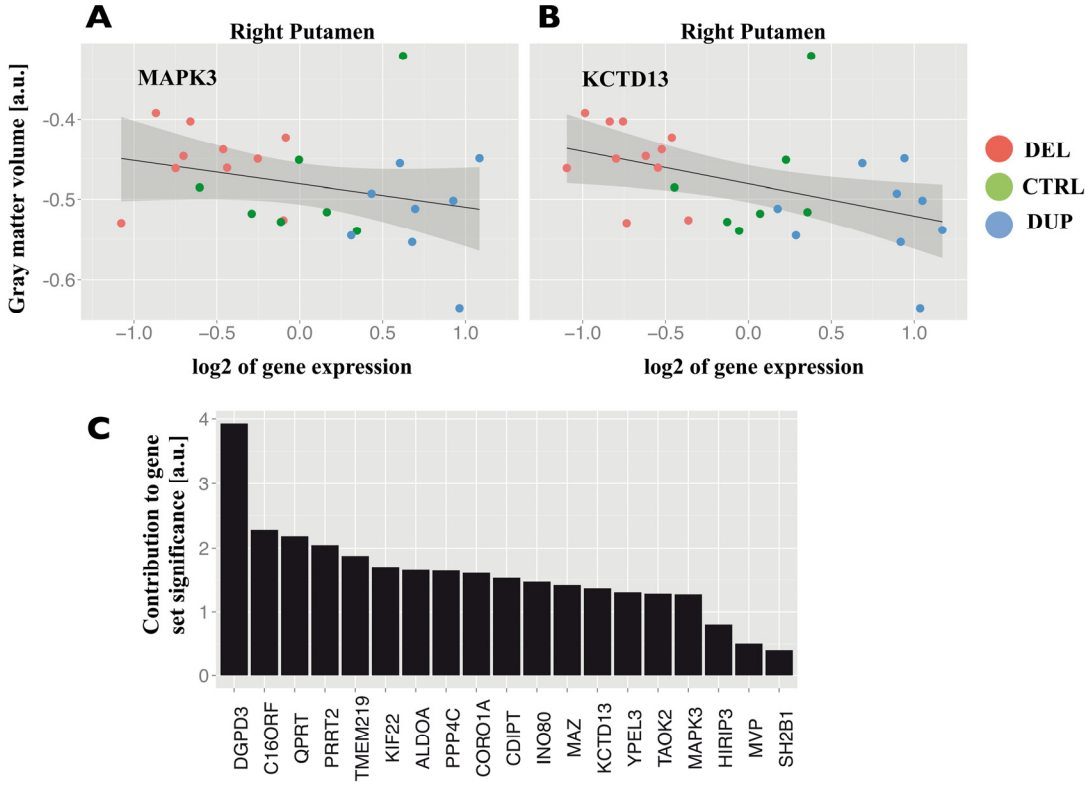
Legend: Gene dosage and group comparisons on cortical thickness (**A-E**) and surface area (**F**). (**A**) Gene dosage negatively correlates with regional cortical thickness changes in the left insula, inferior pre/postcentral gyrus, superior postcentral gyrus and supramarginal gyrus bilaterally. (**B**) Gene dosage positively correlates with regional cortical thickness changes in the right rostral middle frontal gyrus, inferior temporal cortex, superior precentral and prefrontal gyri, left middle temporal and lateral occipital cortices and the orbitofrontal cortex bilaterally. (**C - D**) group comparison DEL > CTRL with increased cortical thickness in the DEL in the left superior postcentral and parietal gyrus, right inferior pre-and postcentral gyrus in addition to the supramarginal gyrus. There is also a decrease in cortical thickness for the DEL in the left middle temporal and fusiform gyrus. (**E**) group comparison CTRL<DUP with increased cortical thickness in the DUP in the medial orbitofrontal gyrus. (**F**) group comparison CTRL< DUP with cortical surface in DUP in the left insula, right rostral middle frontal gyrus and frontal pole.
Only clusters surviving family wise error correction (cluster level) are presented. The color bar represents the family wise error corrected p-values. If no clusters are identified, the gene dosage or contrast analysis is not presented.
DEL – deletion carriers; **CTRL** – intrafamilial controls; **DUP** – duplication carriers.

Supplementary Figure 5: Expression levels of 18 genes at the 16p11.2 locus



Legend: (A) Boxplots of the gene expression levels measured by qPCR in lymphoblastoid cell lines of 18 genes located on Chr16 (BP4-BP5) and ordered according to their chromosomal coordinates. Strip charts were overlaid to the boxplots: each dot represents the expression level of a sample and it is colored according to the CNVs. (B): Heat map representing the Pearson correlation coefficients among the gene expression levels of the same 18 genes. (C): Heat map representing the Pearson partial correlation coefficients adjusting for copy number. Corresponding color keys are reported.

Supplementary Figure 6: Contribution of individual genes to anatomical pattern



Legend: (A-B) Correlation between the log2 mRNA levels and relative volume of the right putamen (MAPK3: r = 0.15, p = 0.04; KCTD13: r = 0.2, p = 0.02). Correlations between the relative volumes of other anatomical structures and mRNA levels are detailed in Supplementary Table 8. **(C)** Relative contribution of individual expression levels to the first eigenimage (Fig.4 main text). High rates of shared variance between mRNA levels of genes within the16p11.2 BP4-BP5 interval result in similar contributions of most genes to the first eigenimage.

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Appendix 2

Supplemental Material Study 2: Gene dosage at the 16p11.2 locus modulates language, verbal memory and inhibition

Supplementary Table 1: Characteristics of the participants excluded from the cognitive analyses

	Deletion N = 6	**Duplication** N = 12
Mean age ± SD [range]	13 ± 4.8 [7.8-18.4]	13.2 ± 8.7 [3.8-31.5]
Gender (M/F)	6 / 0	8 / 4
Handedness: R/L/Un	1 /5/ 0	5 / 5 / 2
Inheritance: N / In / U	2 / 4/ 0	2 / 9 / 1
Kinship (proband / relative)	4 / 2	11 / 1
ASD	2	8
Schizophrenia	0	0
Mean FSIQ ± SD [range]	47 ± 4 (n=4) [42-51]	37 ± 6.7 (n=11) [30-45]
Mean NVIQ ± SD [range]	56 ± 9 (n=5) [47-67]	40 ± 9.9 (n=11) [30-54]
Mean VIQ ± SD [range]	57 ± 7.6 (n=3) [45-59]	38 ± 8.9 (n=10) [30-51]

SD - standard deviation; **M** - male; **F** -female; **L** - left; **R -** right; **Un** - undefined; **N** - de novo; **In** - inherited; **U** - unknown; **ASD**, Autism Spectrum Disorder; **FSIQ** - full scale IQ (standard score); **NVIQ** -non verbal IQ (standard score); **VIQ** - verbal IQ (standard score).

Supplementary Table 2: Additional deleterious CNVs in 16p11.2 carriers

16p11.2 carriers	Case	Chr	Boundaries (HG19) Primary	Distal	CNV	Size (Kb)	Genes	Gene symbols
Duplication	1*	16	28491925	29040000	16	548	18	IL27,APOBR,NURP1, CCDC101,SULT1A2,SULT1A1,NPIPB8,EIF3C,NPIPB9,ATXN2L,TUFM,SH2B1,ATP2A1, RABEP2,CD19,NFATC2IP,SPNS1,LAT
	2*	16	28492125	29040000	16	548	18	IL27,APOBR,NURP1, CCDC101,SULT1A2,SULT1A1,NPIPB8,EIF3C,NPIPB9,ATXN2L,TUFM,SH2B1,ATP2A1, RABEP2,CD19,NFATC2IP,SPNS1,LAT
	3*	16	28492125	29040000	16	548	18	IL27,APOBR,NURP1, CCDC101,SULT1A2,SULT1A1,NPIPB8,EIF3C,NPIPB9,ATXN2L,TUFM,SH2B1,ATP2A1, RABEP2,CD19,NFATC2IP,SPNS1,LAT
	4	X	31574736	31843530	1	268	1	DMD
	5	15	30938215	32438943	1	1,500	10	ARHGAP11B, FAN1, MTMR10, TRPM1, KLF13, OTUD7A, CHRNA7, HERC2P10, MIR211, RP11-16E12.1
Deletion	1	7	72740000	74140000	3	1,400	29	ZNF658B, FAM74A3, LOC101927448, LOC101927475, KGFLP2, FAM95B1, LOC101930147, LOC100133920, RP11-327I22.6, RP11-475I24.8, FAM27E, KGFLP1, RP11-475I24.8, MGC21881, LOC100126582, CNTNAP3, SPATA31A1, SPATA31A2, SPATA31A3, ZNF658, SPATA31A4, SPATA31A5, ANKRD20A2, CBWD7, FOXD4L2, ANKRD20A3, SPATA31A6, CNTNAP3, LOC101928102

* For case 1, 2 and 3, patients have a larger duplication including the BP1-BP3 500kb region containing the SH2B1 region. In these cases, the region extending beyond the BP4-BP5 interval is presented as the equivalent of an additional deleterious CNV.

Supplementary Table 3: Characteristics of the participants performing the MRI

	Deletion N = 14	**Controls** N = 23	**Duplication** N=17
Age in years (mean± SD)	24.6 ± 13.3	33 ±12.3	35.7 ±12.5
Gender (M/F)	8 / 6	11/ 12	10 / 7
Handedness (R/L)	11 / 3	23 / 0	14 / 3
FSIQ (mean± SD)	74 ± 14	98 ± 13[§]	72 ± 16
NVIQ (mean±SD)	79 ± 11	102 ± 13[§]	73 ± 15

SD, standard deviation; **M**, male; **F**, female; **R**, right; **L**, left; **FSIQ**, full scale intellectual quotient (standard score); **NVIQ**, non verbal intellectual quotient (standard score).
[§] significantly different from the two other groups, ANCOVA, post-hoc group comparisons, $p < 0.05$ Bonferroni corrected.

Supplementary Table 4: Cognitive results for each of the groups

Cognitive variables	Deletion mean ± SD (n)	Control mean ± SD (n)	Duplication mean ± SD (n)
Overall cognitive functioning			
FSIQ (standard score)	71.5±13.4 (51)	95.9 ±14.1 (45)	74.7 ±18.7 (38)
NVIQ (standard score)	77.9 ±11.7 (55)	98.2 ±14.3 (45)	76 ±15.9 (38)
VIQ (standard score)	73.3 ±14.2 (40)	94.9 ±17.9 (21)	82.9 ±18.7 (23)
Language			
Phonological skills			
Non-word repetition (z-score)	-3.5 ±2.7 (48)	-0.8 ±1.4 (41)	-1.5 ±2 (26)
Oromotor sequences (z-score)	-3.9 ±2.4 (43)	-0.7 ±1.4 (43)	-2.1 ±2.8 (27)
Phonological processing (z-score)	-2.3 ±2.5 (33)	-0.4 ±1.2 (31)	-2 ±2.3 (21)
Sentence Repetition (age adjusted [§])*	-1 [-1.7 / -0.7] (22)	0.8 [0.3 / 1.4] (35)	0 [-1.1 / 0.9] (14)
Lexical skills			
Word definition (z-score)	-1.6 ±1 (51)	-0.3 ±0.9 (43)	-1.2 ±1.1 (38)
Word comprehension (z-score)	-0.4 ±1.2 (28)	0.8 ±1 (19)	-0.6 ±1.2 (17)
Semantic fluency (age adjusted [§])	-2.2 ±6.1 (40)	3.3 ±6.7 (33)	-0.8 ±6.9 (28)
Phonemic fluency (age adjusted [§])	-1.9 ±4.6 (32)	2.7 ±4 (31)	-1.1 ±3.5 (23)
Comprehension and verbal skills			
Syntax comprehension (age adjusted [§])*	-0.8 [-1.3 / 0.3] (19)	0.9 [0.7-2.3] (26)	-0.8 [-1.9 / -0.5] (14)
Verbal reasoning (z-score)	-1.3 ±1 (44)	-0.3 ±1.1 (42)	-1.1 ±1.2 (33)
Written language			
Reading fluency (z-score)	-1.8 ±2.2 (24)	0.4 ±1.2 (30)	-0.5 ±1.9 (17)
Reading comprehension (z-score)	-0.9 ±1.5 (16)	0.4 ±1.1 (21)	-1.3 ±1.9 (15)
Spelling (age and gender adjusted [§])*	-0.2 [-1.3 / 0.4] (27)	0.6 [-0.8 / 1.4] (32)	0 [-0.3 /0.4] (15)
Memory			
Verbal short-term memory			
Forward digit span (age adjusted [§])	-1 ±1.3 (45)	1 ±2.2 (37)	0.3 ±2 (34)
Verbal long-term memory			
CVLT encoding (gender adjusted z-score)	-0.7 ±1.2 (23)	0.3 ±0.9 (27)	0.5 ±1.5 (15)
CVLT delayed recall (gender adjusted z-score)	-0.7 ±1.2 (23)	0.2 ±1.2 (27)	0.6 ±1.3 (15)
Visuo-spatial short-term memory			
Forward spatial span (age adjusted [§])	-0.8 ±2.2 (29)	1.6 ±1.9 (23)	-0.6 ±2 (22)
Visuo-spatial long-term memory			
ROCF immediate recall (z-score)	-1.7 ±1.2 (19)	-0.4 ±1.5 (22)	-1.7 ±1.1 (17)
ROCF delayed recall (z-score)	-1.8 ±1.2 (18)	-0.5 ±1.4 (21)	-1.9 ±1 (17)
Executive functions			
Working memory			
Backward digit span (age adjusted [§])	-0.4 ±2.1 (43)	0.9 ±2.4 (35)	-0.5 ±1.7 (29)
Backward spatial span (age adjusted [§])	-0.5 ±2 (28)	1.4 ±2.6 (23)	-0.9 ±2.2 (20)
Planning			
ToL total correct (z-score)	-0.3 ± 0.9 (26)	0.3 ± 1.1 (21)	-0.6 ± 0.7 (21)
ToL total move (z-score)	-0.9 ± 1.1 (26)	0.2 ± 1.1 (21)	-0.8 ± 1.1 (21)
Inhibition			
Stroop response time (z-score)	0.2 ± 1.6 (36)	0.5 ± 1.1 (37)	0.2 ± 1.8 (28)
Stroop number of success (age adjusted [§])*	-0.3 [-2.3 / 1] (36)	1.4 [-0.2 / 2.4] (37)	1.1 [-0.1 / 1.9] (29)
GoNogo response time (z-score)	0.6 ± 0.9 (26)	0.3 ± 1 (27)	-0.3 ± 1.2 (23)
GoNogo number of success (age adjusted [§])*	-0.6 [-1.7 / 1.5] (27)	1.1 [0 / 1.4] (27)	1 [0.1 / 1.6] (23)
Fine motor skills			
Purdue dominant hand (z-score)	-1.5 ±1.2 (30)	0.1 ±1.1 (26)	-1 ±0.9 (24)
Purdue non dominant hand (z-score)	-1.5 ±1.2 (30)	0.2 ±0.8 (26)	-1 ±0.8 (25)
Purdue bimanual (z-score)	-1.5 ±0.9 (29)	0 ±1 (26)	-1.5 ±1.3 (23)
Purdue assembly (z-score)	-2.4 ±1.3 (29)	-1 ±1.3 (26)	-2.5 ±1.1 (22)

FSIQ - full scale IQ; **NVIQ** - non verbal IQ; **VIQ** - verbal IQ; **CVLT** - California Verbal Learning Test; **ROCF** - Rey-Osterrieth Complex Figure; **ToL** - Tower of London.

* median [25^{th} – 75^{th} percentile] presented for non linear models
§ raw score

Supplementary Table 5: Group contrasts for cognitive measures showing no significant gene dosage effect and/or group differences

	Deletion versus Duplication		Deletion versus Control		Duplication versus Control		Gene dosage
Cognitive variables	Estimate* (Std. Err)	*P*-value	Estimate* (Std. Err)	*P*-value	Estimate* (Std. Err)	*P*-value	Estimate (*P*-value)
Language							
Phonological skills							
Phonological processing (z-score) [a]	0.62 (0.57)	0.28	-0.68 (0.61)	0.27	-0.06 (0.71)	0.93	0.34 (0.23)
Lexical skills							
Word comprehension (z-score) [a]	-0.16 (0.34)	0.63	-0.37 (0.41)	0.36	-0.54 (0.46)	0.24	-0.07 (0.68)
Semantic fluency (age adjusted) [a]	2.11 (1.71)	0.22	-3.26 (1.9)	0.09	-1.15 (2.13)	0.59	1.21 (0.16)
Phonemic fluency (age adjusted) [a]	0.76 (1.21)	0.53	-2.24 (1.23)	0.07	-1.47 (1.4)	0.29	0.51 (0.41)
Comprehension and verbal skills							
Verbal reasoning (z-score) [a]	0.06 (0.23)	0.79	-0.24 (0.23)	0.31	-0.18 (0.26)	0.48	0.05 (0.68)
Written language							
Reading comprehension (z-score) [b]	-0.38 (0.48)	0.42	-0.23 (0.54)	0.66	-0.63 (0.54)	0.25	-0.19 (0.42)
Spelling (age and gender adjusted)[c]	0.71 (0.36)	0.05	-0.21 (0.27)	0.42	0.49 (0.38)	0.19	0.32 (0.057)
Memory							
Visuo-spatial short-term memory							
Forward spatial span (age adjusted) [a]	0.23 (0.59)	0.7	-1.5 (0.76)	0.047	-1.3 (0.74)	0.08	0.09 (0.77)
Visuo-spatial long-term memory							
ROCF immediate recall (z-score) [b]	0.07 (0.37)	0.84	-0.17 (0.42)	0.68	-0.09 (0.44)	0.83	0.04 (0.82)
ROCF delayed recall (z-score) [b]	-0.09 (0.34)	0.78	-0.19 (0.42)	0.64	-0.29 (0.43)	0.52	-0.04 (0.81)
Executive functions							
Working memory							
Backward digit span (age adjusted) [a]	0.24 (0.46)	0.6	0.11 (0.53)	0.83	0.36 (0.58)	0.54	0.11 (0.64)
Backward spatial span (age adjusted)[a]	-0.55 (0.62)	0.37	-0.03 (0.76)	0.97	-0.58 (0.75)	0.44	-0.23 (0.34)
Planning							
ToL total correct (z-score) [a]	-0.35 (0.25)	0.17	0.05 (0.34)	0.87	-0.29 (0.34)	0.39	-0.17 (0.16)
ToL total move (z-score) [a]	0.07 (0.31)	0.81	-0.41 (0.43)	0.34	-0.33 (0.42)	0.44	0.04 (0.81)
Inhibition							
Stroop response time (z-score) [a, §]	-0.06 (0.4)	0.87	-0.17 (0.34)	0.63	-0.23 (0.38)	0.5	-0.02 (0.91)
Fine motor skills							
Purdue dominant hand (z-score) [a]	0.55 (0.27)	0.048	-0.86 (0.31)	0.008	-0.3 (0.33)	0.35	0.29 (0.043)
Purdue bimanual (z-score) [a]	0.009 (0.29)	0.97	-0.72 (0.33)	0.036	-0.73 (0.34)	0.037	0.03 (0.86)
Purdue assembly (z-score) [a]	-0.15 (0.33)	0.66	-0.69 (0.39)	0.08	-0.84 (0.39)	0.037	-0.06 (0.71)

Std. Err - Standard Error; **ROCF -** Rey-Osterrieth Complex Figure; **ToL -** Tower of London.
[a] Linear mixed model; [b] Linear model; [c] Generalized linear mixed model
[§] model not adjusted for IQ.
˜positive estimate: Deletion carriers' score < duplication.

Supplementary Table 6: Group contrasts on cognitive measures not adjusting for IQ.

	Deletion versus Duplication		Deletion versus Control		Duplication versus Control	
Cognitive variables	Estimate[ε]	*P*-value	Estimate[ε]	*P*-value	Estimate[ε]	*P*-value
Overall cognitive functioning						
FSIQ (z-score)	3.5	0.33	-25.7	6.8e-14	-22.2	1.6e-09
NVIQ (z-score)	-2.01	0.51	-21.34	7.7e-13	-23.4	3.9e-12
VIQ (z-score)	6.03	0.2	-18.76	4.7e-06	-12.73	0.008
Language						
Phonological skills						
Non-word repetition (z-score)	1.87	0.0016	-2.33	1.8e-06	-0.46	0.42
Oromotor sequences (z-score)	1.78	0.003	-3.01	1.6e-09	-1.28	0.02
Phonological processing (z-score)	0.37	0.55	-2	0.0002	-1.67	0.007
Sentence Repetition (age adjusted [§])	0.64	0.008	-1.23	7.9e-10	-0.59	0.009
Lexical skills						
Word definition (z-score)	0.47	0.046	-1.3	1.5e-09	-0.83	0.0003
Word comprehension (z-score)	-0.18	0.62	-1.4	7.8e-05	-1.56	9e-05
Semantic fluency (age adjusted [§])	1.56	0.37	-5.59	0.0009	-4	0.024
Verbal fluency (age adjusted [§])	0.39	0.75	-3.83	0.0007	-3.43	0.005
Comprehension and verbal skills						
Syntax comprehension (age adjusted[§])	-0.18	0.29	-1.33	2.9e-10	-1.52	3.4e-12
Verbal reasoning (z-score)	-0.05	0.83	-0.86	0.0001	-0.91	0.0003
Written language						
Reading fluency (z-score)	1.35	0.017	-2.21	1.8e-05	-0.88	0.11
Reading comprehension (z-score)	-0.35	0.51	-1.27	0.01	-1.63	0.001
Spelling (age and gender adjusted [§])	0.55	0.11	-0.76	0.0014	-0.2	0.56
Memory						
Verbal short-term memory						
Forward digit span (age adjusted [§])	1.44	0.002	-1.94	1.7e-05	-0.49	0.27
Verbal long-term memory						
CVLT encoding (gender adjusted z-score)	1.26	0.002	-1.07	0.002	0.19	0.61
CVLT delayed recall (gender adjusted z-score)	1.24	0.004	-0.88	0.015	0.36	0.38
Visuo-spatial short-term memory						
Forward spatial span (age adjusted [§])	0.36	0.55	-2.51	0.0001	-2.15	0.001
Visuo-spatial long-term memory						
ROCF immediate recall (z-score)	0	0.98	-1.28	0.003	-1.29	0.003
ROCF delayed recall (z-score)	-0.16	0.69	-1.3	0.002	-1.46	0.0006
Executive functions						
Working memory						
Backward digit span (age adjusted [§])	0.04	0.93	-1.38	0.005	-1.33	0.01
Backward spatial span (age adjusted[§])	-0.31	0.64	-2.01	0.003	-2.32	0.001
Planning						
ToL total correct (z-score)	-0.29	0.29	-0.66	0.017	-0.95	0.001
ToL total move (z-score)	0.13	0.69	-1.05	0.001	-0.93	0.007
Inhibition						
Stroop response time (z-score)	-0.06	0.87	-0.17	0.63	-0.23	0.5
Stroop number of success (age adjusted [§])	0.52	0.04	-1.12	4.8e-08	-0.6	0.02
GoNogo response time (z-score)	-0.94	0.003	0.31	0.28	-0.63	0.042
GoNogo number of success (age adjusted [§])	0.99	0.007	-0.69	0.012	0.29	0.45

Fine motor skills						
Purdue dominant hand (z-score)	0.58	0.056	-1.62	4.4e-07	-1.04	0.001
Purdue non dominant hand (z-score)	0.49	0.07	-1.68	2.5e-08	-1.2	8.3e-05
Purdue bimanual (z-score)	0.05	0.87	-1.55	5.6e-06	-1.49	6.3e-05
Purdue assembly (z-score)	-0.09	0.79	-1.42	0.0001	-1.51	0.0002

* negative estimate: Deletion or Duplication carriers' score < controls.

FSIQ - full scale IQ; **NVIQ** - non verbal IQ; **VIQ** - verbal IQ; **CVLT** - California Verbal Learning Test; **ROCF** - Rey-Osterrieth Complex Figure; **ToL** - Tower of London.

˜positive estimate: Deletion carriers' score < duplication.

*negative estimate: Deletion or Duplication carriers' score < controls.

§ raw score

Supplementary Table 7: IQ has a main positive impact on most of the cognitive variables while the effect of gender is significant in verbal long term and spelling variables, revealing a female advantage

	IQ		Gender	
Cognitive variables	Estimate	*P*-value	Estimate	*P*-value
Overall cognitive functioning				
FSIQ	NA	NA	-3.07	ns
NVIQ	NA	NA	-4.5	ns
VIQ	NA	NA	-2.47	ns
Language				
Phonological skills				
Non-word repetition (z-score)	0.04	**0.0004**	0.05	ns
Oromotor sequences (z-score)	0.06	**1.1e-05**	0.86	0.02[§]
Phonological processing (z-score)	0.07	**3.4e-07**	0.18	ns
Sentence Repetition (raw score)	0.03	**2.4e-09**	0.16	ns
Lexical skills				
Word definition (z-score)	0.04	**1.3e-12**	0.08	ns
Word comprehension (z-score)	0.04	**5.3e-07**	0.21	ns
Semantic fluency (raw score)	0.15	**0.0001**	1.13	ns
Phonemic fluency (raw score)	0.11	**4.7e-05**	0.79	ns
Comprehension and verbal skills				
Syntax comprehension (raw score)	0.04	**4.5e-15**	0.15	ns
Verbal reasoning (z-score)	0.04	**3.2e-11**	-0.09	ns
Written language				
Reading fluency (z-score)	0.04	**0.0014**	0.63	ns
Reading comprehension (z-score)	0.05	**6.5e-06**	0.87	0.02[§]
Spelling (raw score)	0.04	**1.5e-07**	0.96	**1.2e-07**[†]
Memory				
Verbal short-term memory				
Forward digit span(raw score)	0.05	**8.5e-08**	0.17	ns
Verbal long-term memory				
CVLT encoding (z-score)	0.04	**1.3e-06**	0.8	**0.0014**[†]
CVLT delayed recall (z-score)	0.04	**3.3e-05**	0.97	**0.0007**[†]
Visuo-spatial short-term memory				
Forward spatial span (raw score) [a]	0.06	**7.9e-05**	-0.04	ns
Visuo-spatial long-term memory				
ROCF immediate recall (z-score) [a]	0.05	**4.6e-08**	-0.1	ns
ROCF delayed recall (z-score) [a]	0.05	**2.8e-08**	0.39	ns
Executive functions				
Working memory				
Backward digit span (raw score)	0.07	**2.2e-08**	-0.55	ns
Backward spatial span (raw score) [a]	0.08	**1.6e-07**	-0.38	ns
Planning				
ToL total correct (z-score) [a]	0.03	**1.1e-05**	-0.22	ns
ToL total move (z-score) [a]	0.03	**0.0001**	-0.2	ns
Inhibition				
Stroop response time	0.001	ns	0.032	ns
Stroop number of success (raw score)	0.02	**1.6e-05**	-0.15	ns
GoNogo response time (z-score) [a]	0.007	ns	0.01	ns
GoNogo number of success (raw score) [a]	0.01	0.09	-0.13	ns
Fine motor skills				
Purdue dominant hand (z-score) [a]	0.04	**3.3e-09**	0.19	ns
Purdue non dominant hand (z-score)	0.04	**1.6[e]-08**	0.1	ns
Purdue bimanual (z-score)	0.05	**2.5e-09**	0.16	ns
Purdue assembly(z-score) [a]	0.04	**3.8e-07**	0.34	ns

FSIQ - full scale IQ; **NVIQ** - non verbal IQ; **VIQ** - verbal IQ **CVLT** - California Verbal Learning Test; **ROCF-** Rey-Osterrieth Complex Figure; **ToL** -Tower of London. **NA -** not applicable; **ns -** result not significant (p-value ≥ 0.1).
Significant p-values (corrected threshold, $p = 0.005$) are highlighted in bold.
[§] Females > males, predictor not significant in the final model.
[†] Females > males.
[a] Main effect of FSIQ, if not specified NVIQ.

Supplementary Table 8: Group contrasts taking out additional deleterious CNVs in duplication (n=5) and deletion (n=1) carriers. Results are adjusted for IQ and we only show variables with reported group differences

	Deletion versus Duplication		Deletion versus Control		Duplication versus Control	
Cognitive variables	Estimate[˜]	*P*-value	Estimate[*]	*P*-value	Estimate[˜]	*P*-value
Overall cognitive functioning						
FSIQ (z-score)	4	0.29	-25.4	**2.7e-13**	-21.4	**2e-08**
NVIQ (z-score)	-1.5	0.64	-20.83	**3.4e-12**	-22.34	**1e-10**
VIQ (z-score)	5.42	0.28	-18.16	**1.3e-05**	-12.74	0.011
Language						
Phonological skills						
Non-word repetition (z-score)	2	**0.0014**	-1.82	**0.0013**	0.19	0.78
Oromotor sequences (z-score)	2.24	**0.0002**	-2.78	**1.1e-06**	-0.54	0.39
Sentence Repetition (age adjusted[§])	0.69	0.013	-0.77	**0.0014**	-0.075	0.81
Lexical skills						
Word definition (z-score)	0.56	0.009	-0.72	**0.0012**	-0.17	0.48
Comprehension and verbal skills						
Syntax comprehension (age adjusted[§])	-0.12	0.52	-0.77	**0.003**	-0.89	**0.0013**
Written language						
Reading fluency (z-score)	1.17	0.051	-1.69	**0.003**	-0.52	0.39
Memory						
Verbal short-term memory						
Forward digit span (age adjusted[§])	1.47	**0.0009**	-0.77	0.1	0.69	0.17
Verbal long-term memory						
CVLT encoding (gender adjusted z-score)	1.3	**0.0001**	0.09	0.76	1.4	**0.0003**
CVLT delayed recall (gender adjusted z-score)	1.31	**0.0005**	0.31	0.38	1.62	**0.0002**
Visuo-spatial short-term memory						
Forward spatial span (age adjusted[§])	-0.11	0.86	-1.21	0.11	-1.32	0.09
Visuo-spatial long-term memory						
Executive functions						
Inhibition						
Stroop number of success (age adjusted[§])	0.45	0.083	-0.84	**0.002**	-0.38	0.21
GoNogo response time (z-score)[♦]	-0.87	0.009	0.31	0.28	-0.56	0.089
GoNogo number of success (age adjusted)[§ ♦]	0.96	0.024	-0.72	0.01	0.24	0.58
Fine motor skills						
Purdue dominant hand (z-score)	0.60	0.046	-0.88	0.009	-0.27	0.43
Purdue non dominant hand (z-score)	0.44	0.095	-1.15	**0.0003**	-0.7	0.031
Purdue bimanual (z-score)	0.009	0.97	-0.73	0.025	-0.72	0.037
Purdue assembly (z-score)	-0.2	0.57	-0.69	0.08	-0.9	0.036

FSIQ - full scale IQ; **NVIQ** - non verbal IQ; **VIQ** - verbal IQ; **CVLT** - California Verbal Learning Test.

Significant p-values (corrected threshold, $p = 0.005$) are highlighted in bold.

♦ model not adjusted for IQ.

§ raw score

˜positive estimate: Deletion carriers' score < duplication.

*negative estimate: Deletion or Duplication carriers' score < controls.

Supplementary Table 9: Analyses of de novo versus inherited deletion carriers adjusting for IQ. This analysis could not be performed in duplication carriers due to insufficient sample size

	Deletion carriers De novo versus Inherited		
Cognitive variables	N De novo / Inherited	Estimate	p-value
Overall cognitive functioning			
FSIQ (z-score)	9 / 25	0.72	ns
NVIQ (z-score)	11 / 26	2.2	ns
VIQ (z-score)	8 / 22	-6.97	ns
Language			
Phonological skills			
Non-word repetition (z-score)	8 / 25	-0.45	ns
Oromotor sequences (z-score)	8 / 23	0.32	ns
Phonological processing (z-score)	8 / 16	0.67	ns
Sentence Repetition (age adjusted)§	2 / 8	NA	NA
Lexical skills			
Word definition (z-score)	9 / 25	0.14	ns
Word comprehension (z-score)	7 / 16	0.06	ns
Semantic fluency (age adjusted) §	9 / 18	0.5	ns
Phonemic fluency (age adjusted) §	6 / 13	0.56	ns
Comprehension and verbal skills			
Syntax comprehension (age adjusted) §	2 / 7	NA	NA
Verbal reasoning (z-score)	7 / 20	0.006	ns
Written language			
Reading fluency (z-score)	2 / 10	NA	NA
Reading comprehension (z-score)	2 / 6	NA	NA
Spelling (age and gender adjusted)§	3 / 12	NA	NA
Memory			
Verbal short-term memory			
Forward digit span (age adjusted)§	7 / 22	-0.56	ns
Verbal long-term memory			
CVLT encoding (gender adjusted z-score)	1 / 9	NA	NA
CVLT delayed recall (gender adjusted z.score)	1 / 9	NA	NA
Visuo-spatial short-term memory			
Forward spatial span (age adjusted)§	8 / 12	-1.1	ns
Visuo-spatial long-term memory			
ROCF immediate recall (z-score)	5 /6	NA	NA
ROCF delayed recall (z-score)	4 /6	NA	NA
Executive functions			
Working memory			
Backward digit span (age adjusted)§	7 / 20	-1.16	ns
Backward spatial span (age adjusted)§	7 / 12	-1.19	ns
Planning			
ToL total correct (z-score)	6 / 12	-0.26	ns
ToL total move (z-score)	6 / 12	0.19	ns
Inhibition			
Stroop response time (z-score)	4 /18	NA	NA
Stroop number of success (age adjusted)§	4 /18	NA	NA
GoNogo response time (z-score) ♦	7 /11	0.43	ns
GoNogo number of success (age adjusted)§♦	6 / 12	-0.08	ns
Fine motor skills			
Purdue dominant hand (z-score)	6 /16	0.33	ns

Purdue non dominant hand (z-score)	6 /16	0.9	ns
Purdue bimanual (z-score)	6 /15	0.45	ns
Purdue assembly (z-score)	6 /15	0.24	ns

FSIQ - full scale IQ; **NVIQ** - non verbal IQ; **VIQ** - verbal IQ **CVLT** - California Verbal Learning Test; **ROCF-** Rey-Osterrieth Complex Figure; **ToL** -Tower of London. **NA -** not applicable due to small numbers.

♦ model not adjusted for IQ.

§ raw score

Supplementary Table 10: GM Voxel-based morphometry results for brain structure-behavior correlations

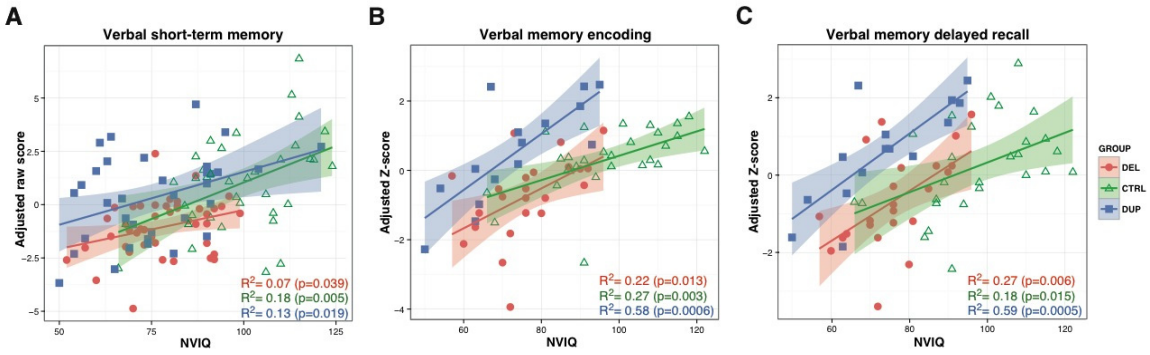
Regions	Side	MNI coordinates			Peak T-value
		x	y	z	
Verbal inhibition (negative correlation)					
Anterior insula & Opercular part of the inferior frontal gyrus[§]	L	-36	8	4	6.25
	R	31	-19	19	5.33
Transverse temporal gyri[§]	R	42	-21	15	5.87
Rolandic operculum*	L	-42	-18	12	5.20
Non-word repetition (negative correlation)					
Caudate*	R	18	24	4	5.04

L – left hemisphere**; R –** right hemisphere

[§] Anatomical structures which survive family-wise error correction at a cluster level $p<0.05$, marginal p-value ($p<0.09$) are indicated by a *.

Figures

Supplementary Figure 1: Verbal memory measures as a function of Non Verbal IQ



Association between verbal short-term memory (**A**), verbal memory encoding (**B**) or verbal memory delayed recall (**C**) measures and Non verbal IQ. All scores are presented adjusted for age. Panel **B** and **C** are also adjusted for gender. Red dots represent the deletion carriers, blue squares the duplication carriers and green triangles represent the intrafamilial controls. Colored solid lines represent the regression slopes between the memory tasks scores and NVIQ. The correspondent shaded areas depict the 95% confidence intervals. R squares (R^2) and p-values ($p \leq 0.1$, ns otherwise) are given for each group.

NVIQ - non verbal IQ; **DEL** - deletion carriers; **CTRL** - intrafamilial controls; **DUP** - duplication carriers.

Appendix 3

Supplemental Material Study 3: 16p11.2 locus modulates response to satiety before the onset of obesity

Supplementary Table 1: Ascertainment of pediatric CNV 16p11.2 carriers and controls

	16p11.2 EUROPEAN CONSORTIUM	**CARDIFF UNIVERSITY ECHO STUDY**
Deletion (N)	**43**	**30**
Mean age ± SD	9.19 ± 3.51	10.17 ± 3.02
Gender (M/F)	27 / 16	19 / 11
Inheritance: Dn / In / U	19 / 21 / 3	12 / 12 / 6
Mean BMI z-score ± SD	1.66 ± 1.76	1.35 ± 1.54
Duplication (N)	**27**	**12**
Mean age ± SD	8.26 ± 4.19	11.01 ± 3.39*
Gender (M/F)	17 / 10	9 / 3
Inheritance: Dn / In / U	7 / 17 / 3	0 / 8 / 4
Mean BMI z-score ± SD	-0.49 ± 1.64	-0.11 ± 1.27
Intrafamilial controls (N)	**10**	**8**
Mean age ± SD	9.67 ± 4.89	12.12 ± 3.2
Gender (M/F)	7 / 3	3/5
Mean BMI z-score ± SD	0.34 ± 0.96	0.94 ± 0.61
Extrafamilial controls (N)	**24**	-
Mean age ± SD	11.45 ± 1.87	-
Gender (M/F)	6 / 18	-
Mean BMI z-score ± SD	0.35 ± 0.73	-

SD - standard deviation; **M** - male; **F** -female; **Dn** - de novo; **In** - inherited; **U** – unknown

* Duplication carriers from Cardiff ECHO study are slightly older than the ones recruited through the 16p11.2 European consortium (t= -2.16, p=0.039)

Intrafamilial and extrafamilial controls do not differ in terms of age, BMI z-score and gender distribution.

Supplementary Table 2: Pediatric clinical cohort characteristics

	Deletion n = 73	**LOC** n = 26
Age in years (mean ± SD)	9.59 ± 3.33	10.96 ± 1.42♦
Gender (M/F)	47/ 26	12/14
BMI z-score (mean ± SD)	1.53 ± 1.67	1.52 ± 1.32

LOC-Loss of Control eating group

♦ Significant difference (p = 0.005)
There are 5 missing values for BMI z-score in the deletion carriers (n=68)

Supplementary Table 3: Adult clinical cohort characteristics

	Deletion n = 25	**OB** n=226	**BN** n=241	**BED** n=143
Age in years (mean ± SD)	34.7 ± 11.9*	40.7 ± 10.6	34 ± 7.5	33.9 ± 9.7
Gender (M/F)	12 / 13♦	42/190	13/261	6/148
BMI z-score (mean ± SD)	2.8 ± 1.3†	3.4 ± 1.1	0.6 ± 1.4	2.4 ± 0.9
Education years (≤12/ >13)$^{\infty}$	18/7	186/36	190/78	112/36

OB –obesity; **BN** –bulimia nervosa; **BED** - binge eating disorder; **BMI** –body mass index.

*Significantly different from OB cohort (p=0.01)
♦Significantly different from OB (Fisher exact test, p=0.001), BN (p=1.8e-08) and BED (p=3.95e-08) cohorts
†Significantly different from BN (p<2e-16)

Supplementary Table 4: Main effect of age, gender and BMI z-score for deletion, controls and duplication carriers on the Child Eating Behavior Questionnaire (CEBQ)

	Age		**Gender**		**BMI z-score**		**Group**		**FSIQ***	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
DD	0.026	0.22	-0.282	*0.060*	0.116	**0.011**	-0.079	.398	-0.008	*0.029*
EF	0.025	0.256	-0.071	0.662	0.154	**0.001**	-0.215	*0.025*	-0.012	**0.003**
EOE	0.063	**0.003**	-0.188	0.208	0.219	**2.6e-06**	-0.455	**7.6^{e}-07**	-0.005	0.118
EUE	3.03^{e}-03	0.896	-0.198	0.211	-0.119	*0.018*	-0.127	0.216	7.7^{e}-04	0.852
FF	-0.046	*0.078*	-0.14	0.444	-0.035	0.55	0.046	0.69	8.8^{e}-03	**0.005**
FR	0.039	0.172	-0.221	0.278	0.281	**3.7e-06**	-0.445	**0.0002**	-0.009	*0.073*
SE	-0.049	*0.042*	-0.118	0.497	-0.196	**0.0002**	0.212	*0.049*	7.6^{e}-03	0.11
SR	-0.023	0.259	0.058	0.687	-0.204	**5.7e-06**	0.346	**8.06^{e}-05**	5.7^{e}-03	0.157

DD-desire to drink; **EF**-enjoyment of food; **EOE**-emotional overeating; **EUE**-emotional undereating; **FF**-food fussiness; **FR**-food responsiveness; **SE**-slowness in eating; **SR**-satiety responsiveness.

*Sample size: deletion n= 62, intrafamilial controls n= 18, duplication n= 31

Significant p-values surviving correction for multiple comparisons are highlighted in bold.

Significant p-values or trends that do not survive correction for multiple comparisons are in italic.

Supplementary Table 5: Adult questionnaires : deletion, intrafamilial controls and duplication cohorts

	Age		Gender		FSIQ		Group	
	Odds	*p-value*	*odds*	*p-value*	*odds*	*p-value*	*odds*	*p-value*
EDI-2 B [1]	1.049	0.461	3.197	0.448	1.053	0.389	-0.977	0.987
	Estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
EDI-2 DT [2]	-0.007	0.386	1.161	**2.15 e-11**	-0.009	0.167	-0.776	**1.35e-05**
EDI-2 BD [2]	0.003	0.595	1.562	**<2e-16**	0.001	0.732	-0.681	**3.82e-08**
DEBQ-E[3]	-0.005	0.387	-0.137	0.345	0.003	0.444	0.0023	0.842

[1] Logistic mixed model; [2] Binomial mixed model; [3]Linear mixed model
EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction; **DEBQ-E,** Externality scale
Significant p-values surviving correction for multiple comparisons are highlighted in bold.
Significant p-values or trends that do not survive correction for multiple comparisons are in italic.

Supplementary Table 6: Adult questionnaires: deletion, BED, BN and OB cohorts

	Age		Gender		Education		BMI z-score	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
EDI-2 DT	0.012	0.576	1.833	*0.011*	-0.193	0.162	0.265	0.124
EDI-2 B	-0.021	0.200	0.547	0.359	0.018	0.863	0.259	*0.064*
EDI-2 BD	-0.051	*0.073*	6.113*	**7.44 e-10**	-0.001	0.997	1.785	**2.48e-14**

* Female > Male
Significant p-values surviving correction for multiple comparisons are highlighted in bold.
Significant p-values or trends that do not survive correction for multiple comparisons are in italic.

Supplementary Table 7: Mean raw scores and standard deviation on the CEBQ

	Deletion n = 73	**Controls** n = 42	**Duplication** n = 39	**LOC** n=26
	Mean ± SD			
DD	2.50 ± 0.99	1.97 ± 0.73	2.50 ± 1.01	2.60 ± 0.97
EF	3.95 ± 0.96	3.39 ± 0.86	3.68 ± 1.15	4.09 ± 0.59
EOE	2.36 ± 1.10	1.56 ± 0.70	1.63 ± 0.86	2.26 ± 0.89
EUE	2.62 ± 1.03	2.38 ± 0.95	2.55 ± 1.04	2.69 ± 0.65
FF	2.91 ± 1.18	2.59 ± 0.89	3.14 ± 1.23	2.41 ± 0.81
FR	3.14 ± 1.28	1.98 ± 0.78	2.39 ± 1.19	3.01 ± 0.90
SE	2.39 ± 1.01	2.46 ± 0.85	2.87 ± 1.31	2.00 ± 0.81
SR	2.35 ± 0.85	2.62 ± 0.74	2.96 ± 1.07	2.20 ± 0.60

LOC-Loss of Control eating group

DD-desire to drink; **EF**-enjoyment of food; **EOE**-emotional overeating; **EUE**-emotional undereating; **FF**-food fussiness; **FR**-food responsiveness; **SE**-slowness in eating; **SR**-satiety responsiveness.

Supplementary Table 8: Group contrasts for the CEBQ controlling for age and family

	Deletion versus Duplication		**Deletion versus Control**		**Control versus Duplication**	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
DD	-0.101	0.593	-0.060	**0.001**[a]	0.499	*0.018*
EF	-0.338	*0.074*	-0.706	**0.0002** [a]	0.368	*0.086*
EOE	-0.797	**4.44**[e]**-06**[b]	-0.939	**1.21e-08**[a]	0.142	0.436
EUE	-0.184	0.378	-0.287	0.128	-0.01	0.642
FF	0.186	0.423	-0.351	0.108	0.537	*0.035*
FR	-0.741	**0.001**[b]	-1.288	**4.26 e-08**[a]	0.517	*0.035*
SE	0.465	*0.026*	0.035	0.864	0.430	*0.073*
SR	0.688	**0.001**[d]	0.298	**0.011**[c]	0.287	0.136

DD-desire to drink; **EF**-enjoyment of food; **EOE**-emotional overeating; **EUE**-emotional undereating; **FF**-food fussiness; **FR**-food responsiveness; **SE**-slowness in eating; **SR**-satiety responsiveness.

[a] Deletion > controls ; [b] deletion > duplication; [c] Deletion < controls ; [d] Deletion < duplication.

Significant p-values surviving correction for multiple comparisons are highlighted in bold.

Significant p-values or trends that do not survive correction for multiple comparisons are in italic.

Supplementary Table 9: Group contrasts for the Child Eating Behavior Questionnaire controlling for BMI z-score, age and family

	Deletion versus Duplication		**Deletion versus Control**		**Control versus Duplication**	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
DD	0.0002	0.999	-0.525	**0.007**[a]	0.525	*0.014*[c]
EF	-0.202	0.356	-0.548	**0.007** [a]	0.346	0.116
EOE	-0.644	**0.001**[b]	-0.842	**1.5e-06**[a]	0.197	0.286
EUE	-0.438	*0.056*	-0.349	*0.075*	-0.089	0.681
FF	0.304	0.25	-0.305	0.186	0.609	*0.017*
FR	-0.476	*0.066*	-0.105	**2e-05**[a]	0.573	0.029
SE	0.315	0.187	-0.123	0.575	0.438	*0.07*
SR	0.474	*0.018*	0.233	0.198	0.241	0.222

DD-desire to drink; **EF**-enjoyment of food; **EOE**-emotional overeating; **EUE**-emotional undereating; **FF**-food fussiness; **FR**-food responsiveness; **SE**-slowness in eating; **SR**-satiety responsiveness.

[a] Deletion > controls ; [b] deletion > duplication; [c] Controls < duplication.

Significant p-values surviving correction for multiple comparisons are highlighted in bold.

Significant p-values or trends that do not survive correction for multiple comparisons are in italic.

Supplementary Table 10: Group contrasts on the CEBQ controlling for age and BMI z-score

	Deletion vs LOC	
	estimate	*p-value*
DD	-0.008	0.91
EF	0.079	0.631
EOE	0.404	0.852
EUE	0.073	0.689
FF	0.0001	0.999
FR	0.0001	0.999
SE	-0.091	0.679
SR	0.033	0.833

LOC-Loss of Control eating group

DD-desire to drink; **EF**-enjoyment of food; **EOE**-emotional overeating; **EUE**-emotional undereating; **FF**-food fussiness; **FR**-food responsiveness; **SE**-slowness in eating; **SR**-satiety responsiveness.

Supplementary Table 11: Descriptive results on Eating Disorder Inventory 2 (EDI-2) and Dutch Eating Behavior Questionnaire – externality scale (DEBQ-E) in the adult cohort

	Deletion n = 25	**Intrafamilial Controls** n = 38	**Duplication** n = 28
EDI-2 DT [b] median [1st -3rd quartile]	5 [2-8]	2 [0-7]	1 [0-2]
EDI-2 B [c] (> clinical threshold %)	28	7.8	14.3
EDI-2 BD [b] median [1st -3rd quartile]	13 [6 - 21]	8.5 [2 - 15.7]	5 [1 - 6.5]
DEBQ-E [a] mean± SD	2.37 ± 0.73 [n=19]	2.63 ± 0.57	2.49 ± 0.82 [n=20]

[a] Linear mixed model; [b] Binomial mixed model; [c] Logistic mixed model

EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction; **DEBQ-E** Externality scale

Supplementary Table 12: Group contrasts for the EDI-2 subscales and the DEBQ-E scale controlling for gender

	Deletion versus Duplication		**Deletion versus Control**		**Control versus Duplication**	
	estimate	*p*-value	estimate	*p*-value	estimate	*p*-value
EDI-2 B [1]	-0.626	0.806	-0.672	0.733	0.045	0.985
EDI-2 DT [2]	-1.676 [a]	**6.58e-05**	-0.642	**0.007**	-1.033 [b]	**0.009**
EDI-2 BD [2]	-1.445[a]	**2.51e-08**	-0.669 [c]	**5.28e-05**	-0.775 [b]	**0.001**
DEBQ-E[3]	0.029	0.901	0.256	0.180	-0.227	0.259

[1] Logistic mixed model; [2] Binomial mixed model; [3]Linear mixed model

[a] Duplication < Deletion; [b] Duplication < Control ; [c] Control < Deletion, p-values are uncorrected for multiple testing. Values highlighted in bold are those surviving multiple testing correction.

EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction; **DEBQ-E,** Externality scale

Supplementary Table 13: Group contrasts for the EDI-2 and DEBQ-E controlling for gender and BMI z-score

	Deletion versus Duplication		**Deletion versus Control**		**Control versus Duplication**	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
EDI-2 B [1]	1.176	0.345	-0.072	0.946	1.249	0.197
EDI-2 DT [2]	-0.358	0.485	0.197	0.531	-0.555	0.158
EDI-2 BD [2]	0.211	0.535	0.433	0.050	-0.222	0.367
DEBQ-E[3]	0.308	0.323	0.458	0.057	0.150	0.198

[1] Logistic mixed model; [2] Binomial mixed model; [3]Linear mixed model

EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction; **DEBQ-E,** Externality scale

Supplementary Table 14: EDI-2 mean raw scores for deletion carriers, clinical (BN, BED) and obese cohorts

	Deletion n = 25	**OB** n=232	**BN** n=273	**BED** n= 154
	Mean ± standard deviation			
EDI-2 DT	5.7 ± 5.1	9.7 ± 5.1	14.4 ± 5.2	12.5 ± 4.8
EDI-2 B	1.9 ± 2.6	0.7 ±1.1	10.4 ± 5.4	9.7 ± 4.3
EDI-2 BD	13.2 ± 8.6	17.1 ± 6.9	18 ± 7.4	21.2 ± 5.9

OB –obesity; **BN** –bulimia nervosa; **BED** - binge eating disorder

EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction

Supplementary Table 15: EDI-2 linear regression models controlling for gender and BMI z-score

	Deletion versus OB		**Deletion versus BN**		**Deletion versus BED**	
	estimate	*p-value*	*estimate*	*p-value*	*estimate*	*p-value*
EDI-2 DT	3.296[a]	**0.002**	8.562 [b]	**2.02e-13**	6.044 [c]	**1.45e-07**
EDI-2 B	-1.558	0.092	9.043 [b]	**<2e-16**	7.780 [c]	**2.18e-15**
EDI-2 BD	0.271	0.857	5.486 [b]	**0.0005**	5.271 [c]	**0.0007**

OB –obesity; **BN** –bulimia nervosa; **BED** - binge eating disorder

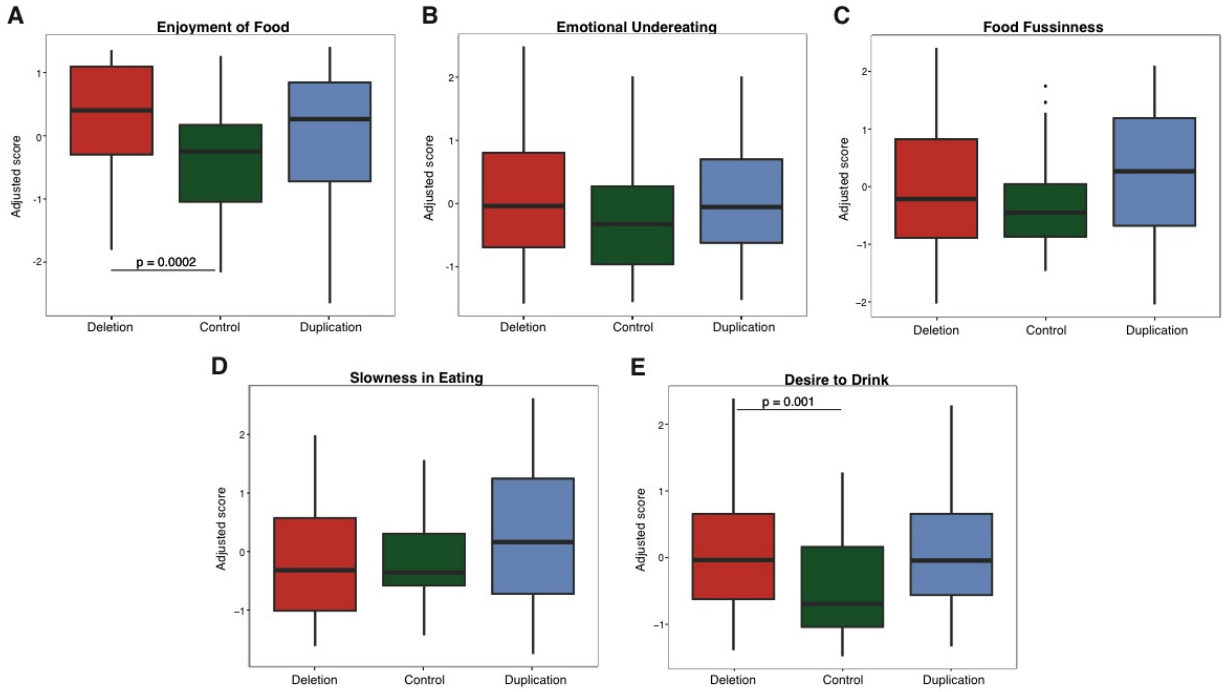
[a] Deletion < OB; [b] Deletion < BN; [c] Deletion < BED

Significant p-values surviving correction for multiple comparisons are highlighted in bold.

EDI-2 DT, Drive for Thinness; **EDI-2 B**, Bulimia; **EDI-2 BD**, Body dissatisfaction.

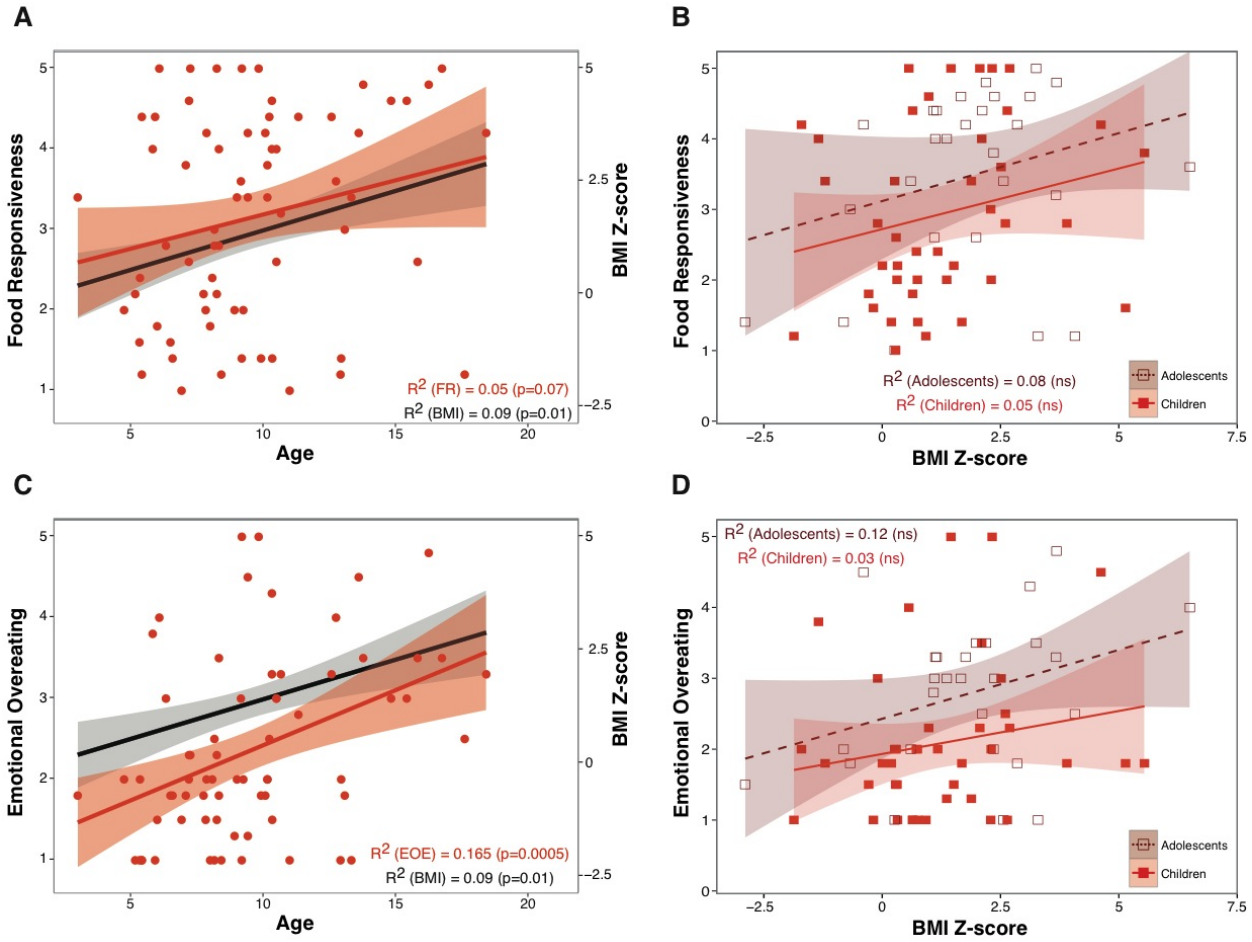
Supplementary Figures

Supplementary Figure 1: Group comparisons on the Child Eating Behavior Questionnaire subscales in deletion carriers, controls and duplication carriers



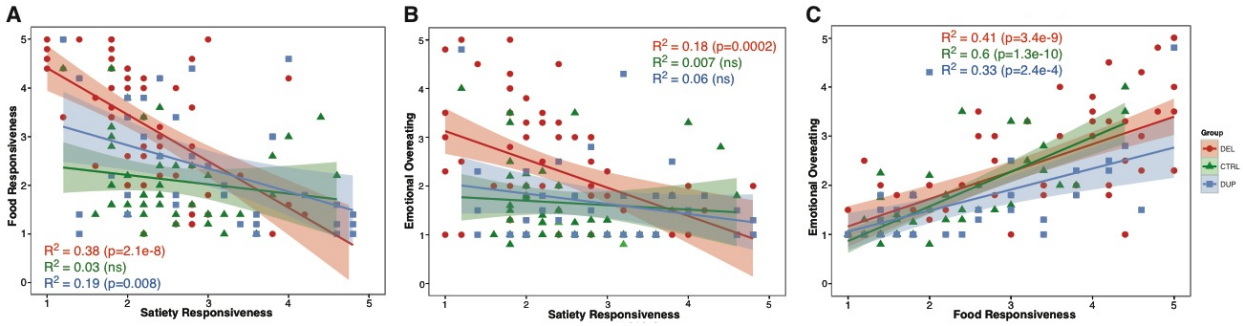
The boxplots show adjusted scores for age on enjoyment of food (**A**), emotional undereating (**B**), food fussiness (**C**), slowness in eating (**D**) and desire to drink (**E**). The bold line shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whiskers end at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR). We represent significant group differences by solid lines with exact p-values above.

Supplementary Figure 2: Relationship between food responsiveness **(A-B)** and emotional overeating **(C-D)**, with BMI z-score and age in deletion carriers



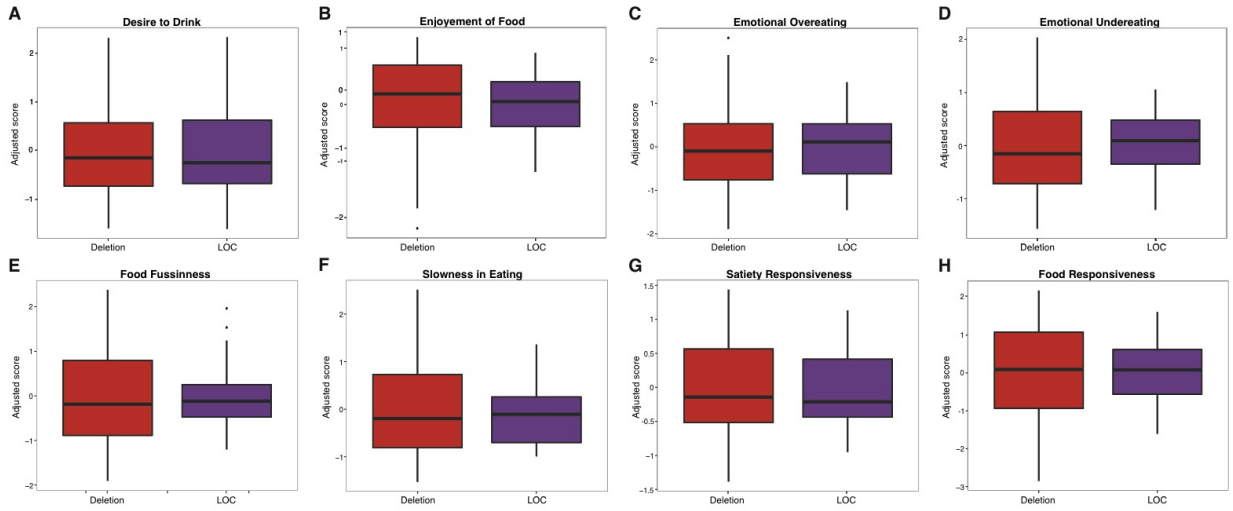
Left Scatterplots (**A and C**) represent age on the X axis, food responsiveness (**A**)/emotional overeating (**C**) score on the Y axis and BMI z-score on the Z axis for deletion carriers. The solid black line represents the BMI z-score over the years while the solid colored line represents food responsiveness and emotional overeating along time, respectively. Right Scatterplots (**B-D)** show the relationship between food responsiveness (**B**) or emotional overeating (**D**) and BMI z-score for children and adolescents in deletion group. R squares (R^2) and p-values ($p \leq 0.1$, ns otherwise) are given for each group. Shaded areas depict the 95% confidence intervals.

Supplementary Figure 3: Cross correlations per group between food and satiety responsiveness and emotional overeating in deletion carriers, controls and duplication carriers.



Scatterplots showing correlation between satiety responsiveness and food responsiveness (**A**), satiety responsiveness with emotional overeating (**B**), and correlation between food responsiveness and emotional overeating (**C**). R squares (R^2) and p-values ($p \leq 0.1$, ns otherwise) are given for each group. Red dots represent the deletion carriers, the blue squares represent duplication carriers and the green triangle the control group. Colored solid lines represent the regression slopes whereas the correspondent shaded areas depict the 95% confidence intervals.

Supplementary Figure 4: Differences between deletion carriers and LOC (loss of control over eating) group on the subscales of the CEBQ



The boxplots show adjusted scores for age on each of the eight CEBQ subscales. The bold line shows the median, the bottom and top of the box, the 25th (Q1) and the 75th (Q3) percentile, respectively. The upper whisker ends at highest observed data value within the span from Q3 to Q3 + 1.5 times the interquartile range (IQR; Q3-Q1), lower whiskers end at lowest observed data value within the span for Q1 to Q1 – (1.5*IQR).