UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Mémoire de Maîtrise en médecine No 836

Genetic factors of obesity and eating disorders : Copy number variations (CNV) involving *SH2B1*

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Lausanne, 14.01.2013

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Introduction:

Obesity is a growing public health issue. (1,2) It's prevalence as well as the prevalence of the comorbidities have increased.(3) The obesity epidemic is clearly associated with the recent availability of highly palatable and inexpensive caloric food. In this new "obesogenic" environment however, some individuals remain lean and others develop morbid obesity. Among the many factors underlying this variance of adiposity, genetics play a key role. Twin studies have estimated the BMI heritability (h^2) between 40 and 70%. GWAS (Genome-Wide Association Studies) have identified common variants that point towards new pathways, but their effect size are too weak to be of any use in the clinic. Studies have also focused on genes and genomic regions that are associated with very high risks of obesity. (4)

a) CNVs in DD/ID and obesity

CGH array being now used in routine clinical practice led to the identification of many new genomic disorders including several obesity-associated syndromes that are associated with DD/ID (Developmental Delay/Intellectual Disability).

Copy Number Variations (CNVs), represent a deviation from the normal number of copies. Genes are usually present in two copies (diploid) except for those encoded by the sex chromosomes. Such changes are mediated by deletions (one copy is left) or duplications (a gain resulting in three copies). CNVs may be deleterious and associated clinical manifestations. "Genomic disorders" is a term regrouping all the CNV-mediated diseases.(4)

An association has been previously shown between the recurrent interstitial ~600kb deletion in the 16p11.2 locus that predisposes to a highly penetrant form of obesity with a 43-fold increased risk of developing morbid obesity. An increase in head circumference (HC) has also been associated with the deletion, as well as a cognitive deficits and behavioral issues.

The prevalence of this deletion, defined by breakpoints 4 and 5 (BP4-BP5), is approximately 1/2000 in the general population, increasing to 0.5% in autism spectrum disorders (ASD). It is one of the most frequent single locus aetiologies of neurodevelopmental disorders and ASD.(5)

In 2010, it was associated with obesity and it's frequency in groups of patients ascertained for morbid obesity is 0.7 %.(6) Studies demonstrated the association between the reciprocal 16:29.5-30.1 duplication and being underweight (with a probable diagnosis of failure to thrive for children under the age of 5) as well as having a small head circumference. Carriers of this duplication showed significantly reduced BMI and post-natal weight, with adults duplication carriers having an 8.3 fold increased risk of being underweight. The study showed a trend towards increased severity in males. All these characteristics were associated with an unusually high frequency of selective and restrictive eating behaviors and an important reduction in head circumference. Each of the observed phenotypes is the converse of the ones reported in carriers of the deletion at this locus. Severe obesity and being underweight may represent mirror phenotypes resulting from contrasting effects on a common energy balance mechanism.(7) The latter being under the control of gene dosage at the 16p11.2 locus. To date, there are no data pointing towards a particular candidate gene.

b) CNVs involving *SH2B1*

At the same 16p11.2 locus, a second non-overlapping recurrent CNV encompassing *SH2B1,* upstream of the aforementioned rearrangement has been published.(4) (8) SNPs in this gene have been associated with BMI in GWAS studies and KO mice models confirmed this association.

Bokuchova et al have reported an association between deletions encompassing this gene and severe early onset obesity, as well as insulin resistance. SH2B1 is known to be an important factor in insulin and leptin signaling. In their study, they identified three patients who carried this 220-kb 16p11.2 deletion (28.73-28.95 Mb) and presented severe early-onset obesity. This suggested an association between this SH2B1-containing 220-kb (28.73-28.95 Mb) deletion and a severe form of obesity.(8) These patients rapidly gained weight in their first years of life and the excess weight was predominantly fat mass. Severe insulin resistance was recorded.(8) Far less is known, however, on the full spectrum of clinical symptoms associated with this CNV.(4)

This project consisted in collecting and analyzing data to fully characterize the phenotype associated with this rearrangement. We here report a large series of patients (102) with imbalances that include the SH2B1 gene region in 16p11.2. Patients were mainly ascertained from cohorts of DD/ID patients. We collected mainly anthropometric, cytomolecular and medical data. We found that deletions and duplications in this region are significantly enriched in patients ascertained for DD/ID when compared to the general population. The effect on BMI is clearly demonstrated in deletion carriers, and a mirror effect with decreased BMI is visible in males only. An effect on HC is also visible after gender stratification.

Patients and Methods

a) Patients

This study was reviewed and approved by the institutional review board of the FBM-UNIL.

Carriers were ascertained through several cohorts (Table 1).

EU Consortium: data was collected retrospectively and anonymously on questionnaires filled by physicians who prescribed the comparative genomic hybridization (CGH). These analyses were performed for patient care purpose only. Consequently, research based informed consent was not required by the institutional review board of the University of Lausanne which granted an exemption for this part of the data collection. Details on ascertainment and data collection for other cohorts have been previously published(7,9) and are presented in Supplemental Information.

b) Anthropometric measures

The accepted standard measure of overweight and obesity for children two years of age and older is the body mass index (BMI).(10) Adults with a BMI between 25 and 30 are considered overweight; those with a BMI ≥30 are considered to be obese. Unlike adults, children grow in height as well as weight. Thus, the norms for BMI in children vary with age and sex. Obesity in children is defined throughout the study by a BMI>+2SD (Standard Deviation). Z-scores were computed according to norms matching the geographic origin of the carrier (eg. for children between the ages of 2 and 20 years from the USA, BMI reference standards have been published in 2000 by the Centers for Disease Control (Figure 2A-B)).

c) Statistical analyses

Two-tailed Fisher's exact test was used to compare frequencies of the deletion and the duplication in patients and controls. Two-tailed Student's t test was performed to assess whether BMI, height, weight and HC Z scores of deletion and duplication carriers were different from zero (general population mean). Wilcoxon Rank sum test were also systematically performed to avoid issues related to non normal distribution. These tests were computed by using standard packages written in « R ».

Results

102 carriers of a rearrangement overlapping the ~200 kb 16p11.2 BP2-BP3 region are included in our study. The boundaries of these rearrangements include: BP1-BP3, BP1-BP4, BP2-BP3, BP2-BP4 and exclude BP5 (Figure 1)

Ascertainment of deletion and duplication carriers is reported in Table 1. The deletion was identified in 42 out of 30'635 patients with DD/ID (Developmental Delay/Intellectual Disability), showing a frequency of ~0.1%. This is significantly higher (12 fold increase) than the frequency observed in the general population (0.01%), (OR =12; p-value = 1e-10) confirming the association between this deletion and DD/ID. Two additional carriers were identified in ASD (Autism Spectrum Disorder) and ADHD (Attention Deficit Hyperactivity Disorder) cohorts (n=510 and 591 respectively), which show similar frequencies ~0.2%.

The duplication was less frequent in the DD/ID cohort (26/30635; 0.08%) and more common in the general population resulting in a lower but significant enrichment in the DD/ID group (OR=2.6 and a *p*=5e-03) (Table 1). No duplication cases were found in the smaller ASD or ADHD cohorts.

a) Anthropometric Results: BMI

We compared available data on BMI and head circumference (HC) for 102 carriers (Figure 3). BMI Z-score were available in 71 patients (44 deletions and 27 duplications) and HC Z-score in 47 patients (29 deletions and 18 duplications).

BMI Z-scores are significantly increased in deletion carriers compared to the norm (mean BMI Z-score= 1.26, p=5.5e-07). Obesity is present in 40.9% (18/44) of deletion carriers. A non-significant decrease in BMI is observed in duplication carriers (mean BMI Z-score= -0.48, p= 0.18).

Having demonstrated an association of the deletion with increased BMI, we investigated the effect of gender and age on this phenotype (Figure 4 & 5). BMIs are similar across gender in deletion carriers: mean BMI Z-scores = 1.13 and 1.31 in females and males respectively and are significantly increased compared to the norm (p=1.9e-03 and p=1.53e-03 respectively). In the duplication group, gender stratification reveals a significant decrease in BMI of males compared to the norm; mean BMI Z-score = -1.55 (p=7.2e-03). No effect on BMI is observed in females; mean BMI Z-score = 0.36 (p=0.34).

There is no effect of age on BMI in deletion or duplication carriers (Figure 4 & 7). Obesity is observed as early as 3.67 years of age (case 488, Supplemental Information). In duplication carriers, the frequency of underweight in males is 42.9% (3/7) in children (BMI<2SD) and 16.7% (1/6) in adults (BMI <18.5).

b) Anthropometric Results : Head Circumference (HC)

Macro and microcephaly are two important phenotypes associated with the proximal 16p11.2 BP4-BP5 rearrangement. We therefore investigated the effect of SH2B1 rearrangement on Head Circumference (HC) and overall, the deletion or the duplication does not significantly impact HC. The mean Z-score is =0.28 (p=0.37) and =-0.54 (p=0.11) for the deletion and duplication carriers respectively when compared to the norm. The effect on HC becomes visible after stratifying by gender (Figure 6). Males carrying the duplication show decreased HC, mean HC Z-score =-1.88 (p=3.6e-02). No effect is present in females carrying the duplication (mean HC Z-score = -0.07; p=0.79). Gender stratification in the deletion carriers reveals no differential effect on HC (mean HC Z-score = 0.065 (p=0.9) and 0.37 (p=0.29) in females and males respectively.

c) Anthropometric Results : BMI SH2B1 VS. "Classic" 29.5-30.1

The effect of the non-overlapping 29.5-30.1 deletion and duplication have been previously published and are similar to those observed in the SH2B1 region.(7) We therefore compared the magnitude of these effects between the 2 regions (Figure 8). The effect of the SH2B1 region on BMI is slightly smaller overall, but not significantly different when compared to the 29.5-30.1 region: ΔZ-score = -0.32 for the deletion and +0.25 for the duplication.

The same comparison was performed after gender stratification (Figure 9). The SH2B1 deletion has a weaker effect across genders, but no significant difference between the 2 regions is observed: ΔZ-score = -0.54 for females and -0.2 for males. For the duplication, low BMI is observed in all subgroups except for females carrying the SH2B1 duplication resulting in a significant difference between SH2B1 and 29.5-30.1 females: ΔZ-score = +1.04 (p=0.04).

d) Medical history:

Medical history was only available for carriers ascertained on the basis of neurodevelopmental disorders. Among neurological symptoms, hypotonia is the most frequently reported symptom in 20% and 6.5% of deletion and duplication carriers respectively. Seizures were reported in 7% and 13% of deletion and duplication carriers respectively (Table 2).

Behavioral and psychiatric issues are prevalent with ASD as well as ADHD being diagnosed in approximately 1/5th of deletion carriers. Cumulatively, behavioral issues are reported in half of both deletion and duplication carriers.

Malformations were infrequent and only a few orthopedic, cardiac and digestive were reported in more than 2 carriers.

Discussion

Genomic disorders encompassing SH2B1 are less frequent (by approximately 4-5 times) than the neighboring 600kb 16p11.2 rearrangements. Very few data is therefore available for these rearrangements. We show in this large series that both the deletion and the duplication are significantly enriched in groups of patients ascertained for DD/ID. When compared to the 600kb BP4-5 rearrangements, the neurodevelopmental impact of the "SH2B1 deletion" is similar or higher, but the effect of the "SH2B1 duplication" is lower (Table 1).

We confirm the increase of BMI (+1.26 SD) associated the SH2B1 deletion, obesity being present in 40.9% of carriers. Strikingly, the reciprocal duplication shows a mirror effect on BMI only after gender stratification. Male carriers but not females present a decrease in BMI (-1.55 SD). This represents to our knowledge the second case of a correlation between gene dosage and BMI. It is unclear why the mirror effect on BMI is not observed in female but intriguingly, a similar gender effect was observed in males carrying the BP4-5 600kb who presented lower BMIs and higher risk of being underweight when compared to females.

When looking at HC results, we could not find anything confirming the previously published association between deletion and increased HC and its reciprocal mirror effect with the duplication.(7) The only thing concomitant with this association appeared after gender stratification, with the males carrying the duplication showing reduced HC (-1.88 SD).

The SH2B1 genomic disorder is similar in many aspects to the BP4-5 rearrangements. This is true for BMI, for HC as well as the neuropsychiatric symptoms. For SH2B1 deletion carriers, the frequency of seizures/epilepsy (7%) is lower to those reported in carriers of the BP4-5 deletion carriers (24%) but ASD frequency is similar (18% and 15% respectively). Detailed phenotyping is required to evaluate the finer similarities between the 2 genomic disorders. The deletion as well as the duplication do not appear to be "malformation syndromes". We did not observe recurrent anomalies and did not confirm the association with kidney and urinary tracts malformations previously reported.(11)

Several hypothesis may be formulated to explain the striking phenotypic concordance between these 2 non-overlapping regions:

i) A gene or a series of genes involved in the same neurodevelopmental and energy balance mechanism are present in 2 independent contiguous regions at the 16p11.2 locus.

ii) These 2 regions are dependent and one region may control the expression of genes in the other region.

Considering these 2 hypotheses, we investigated the possibility of a cumulative effect on the aforementioned traits, in carriers of CNVs overlapping both regions. We identified 3 deletions and 5 duplications carriers. BMI effects of both rearrangements are confirmed, with deletions cases having all increased BMI (mean = 3.9 SD, average = 3.44 SD), and duplication cases having the opposite mirror effect (mean = -2.74 SD, average = -2.8 SD). These effects seem stronger than what is observed in CNVs covering each region separately, but larger sample size is required.

Regarding HC, we observed a trend towards lower Z-score in duplication carriers, as described in previous papers (7) (mean = -1.07 SD, average = -1.15 SD). No data was available for deletion carriers concerning Head Circumference.

While the BP4-5 and the SH2B1 genomic disorders may be similar at the phenotypic level, they differ at the genetic level. There is a 3-fold difference in size and number of genes (8:30). In the BP4-5 region, 4 genes have already been linked to phenotypes (KTCD13/ALDOA/MAPK3 for head circumference and PRRT2 for epilepsy) (12,13). Regarding the effect on BMI, SH2B1 has been the sole candidate gene for the distal 200kb region (14). Intriguingly, point mutations of

SH2B1 have recently been reported in 5 patients with obesity as well as neurocognitive and language disorder suggesting that SH2B1 may also have an effect on neurodevelopment.

The same study reported(14)impaired NGF-induced neuronal differentiation in vitro related to SH2B1 loss of function. This observation also suggests that many deleterious mutations impacting central regulation of energy balance may also impact neurodevelopment on a broader scale, leading to the frequently reported association between developmental delay and obesity. (15,16)

Conclusions :

This study confirms significant increase of BMI in SH2B1 deletions carriers, which was suggested by previous studies. This highlights again the importance of rare variants in common disorders. Intriguingly, a mirror effect of the duplication on BMI is visible in males only. This is also true for the decrease in HC in males carrying the duplications. In addition to energy balance and anthropometric effects, cognitive and behavioral phenotypes are also reminiscent of the BP4-5 600kb deletion. This raises the question of a possible relationship between the 2 loci.

Personal thoughts on this Master Project

From a personal point of view, this project has brought me a lot. When I chose this subject, I had almost no idea on what it meant and moreover on research itself. Having done this work helped me understand better the significance and importance of medical genetics in the modern practice of medicine. It also contributed to open my mind to the scientific side of my future job, as well as giving me tools and ideas for further research projects.

I sincerely hope that this work will lead to new research projects and ideas, to help understand the complexity of this topic and improve medical practice, as well as medical care for patients concerned with these issues.

Acknowledgments:

I would first like to thank Dr. Sébastien Jaquemont for his guidance, help and constant availability all throughout this work. His motivation and scientific point of view have been very handful. Then to Dr. Jacques Beckmann, who gave me the opportunity to work and got me started on this project. Many thanks go to Jonathan Sobel as well, who moreover to be a good and close friend of mine, accepted to help me with all the statistics and figures required to elaborate my work. More globally, I would also like to thank the entire department of medical genetics of the CHUV, for being so friendly and nice to work with. At last, I thank all the participants to this study, patients and their families, for contributing to the advancement of science, research and medicine.

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Tables and Figures:

Table 1 Ascertainment of deletion and duplication carriers

Deletions							
Ascertainment	**Origin**	**Mean age (y)**	**Del.**	**%; OR; *p***	**M/F**	**De novo/inherited* (mother:father)**	**Patients screened**
DD/ID	EU Consort.	8.6	42	0.14; 12; 1e-10	20/22	7/17 (9:8)	30635
	Literature	27.6	11	NA	4/1	2/3 (0:3)	NA
ASD	Literature	6	1	0.2; 18; 0.07	1/0	0/0	510
ADHD	Literature	8	1	0.2; 15; 0.08	1/0	0/0	591
General population	Literature (Decode)	50.1	4	0.01; -; -	1/3	0/0	36301
Carriers TOTAL		**-**	**59**	**-**	**27/26**	**9/20 (9:11)**	**67767**

Duplications							
Ascertainment	**Origin**	**Mean age (y)**	**Dup.**	**%; OR; *p***	**M/F**	**De novo/inherited* (mother:father)**	**Patients screened**
DD/ID	EU Consort.	7.92	26	0.08, 2.6, 5e-03	13/13	1/13 (9:4)	30635
	Literature	NA	5	NA	NA	1/1 (1:0)	NA
ASD	Literature	NA	0	NA	0/0	0/0	510
ADHD	Literature	NA	0	NA	0/0	0/0	591
General population	Literature (Decode)	51.9	12	0.03; -; -	4/8	0/0	36301
Carriers TOTAL		**-**	**43**	**-**	**17/21**	**2/14 (10:4)**	**67767**

*: cases where data was missing for inheritance are not included, % : frequency of carriers, OR : Odds Ratio, *p* : p-value, enrichment of the CNV compared to the frequency in the general population (Fischer's exact test), DD/ID : Developmental Delay/Intellectual Disability, ASD : Autism Spectrum Disorder, ADHD : Attention Deficit Hyperactivity Disorder, NA : non-available or not applicable

Table 2 Medical and behavioral symptoms in deletion and duplication carriers

Pathology	Del	Dup
Neurologic :	16/55 (29.1%)	7/31 (22.6%)
Epilepsy	4/55 (7.3%)	4/31 (12.9%)
Hypotonia	11/55 (20%)	2/31 (6.5%)
Ataxia	1/55 (1.8%)	1/31 (3.2%)
Behavorial :	32/55 (58.2%)	17/31 (54.8%)
ASD/PDD	10/55 (18.2%)	5/31 (16.1%)
ADHD/ADD	11/55 (20%)	4/31 (12.9%)
Other	11/55 (20%)	8/31 (25.8%)

Tables and Figures

Figure 1

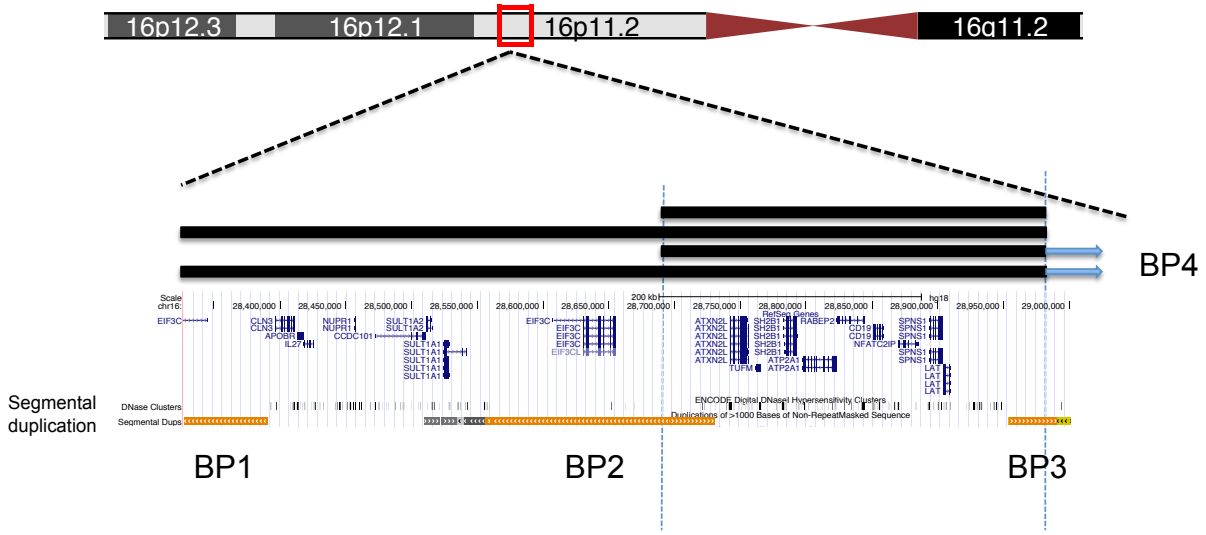
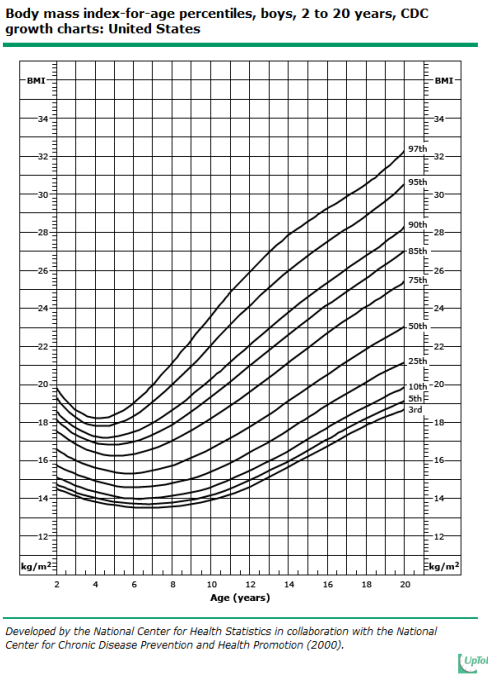


Figure 1 The 16p11.2 locus. Highly homologous blocks of low copy repeats (LCRs) may act as a substrate for non-allelic homologous recombination, predisposing to genomic disorders. Five LCRs have been defined as mediators of recurrent and clinically relevant imbalances within the 16p11.2 chromosomal band. To clarify the terminology, we propose to number these "recombination hotspots" from telomere to centromere as breakpoints BP1 to BP5. The current study describes only features associated with the distal 220 kb recurrent deletion or duplication, containing the *SH2B1* gene, delineated by BP2 and BP3 at genome sequence coordinates 28.7-28.9 Mb, respectively.

Figure 2A-B

A



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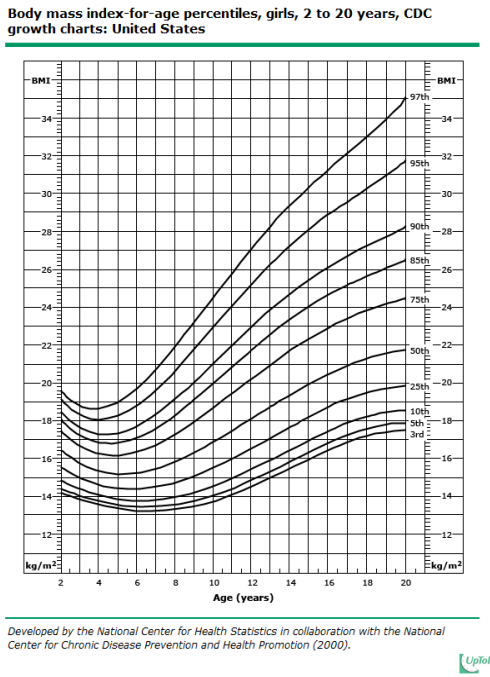


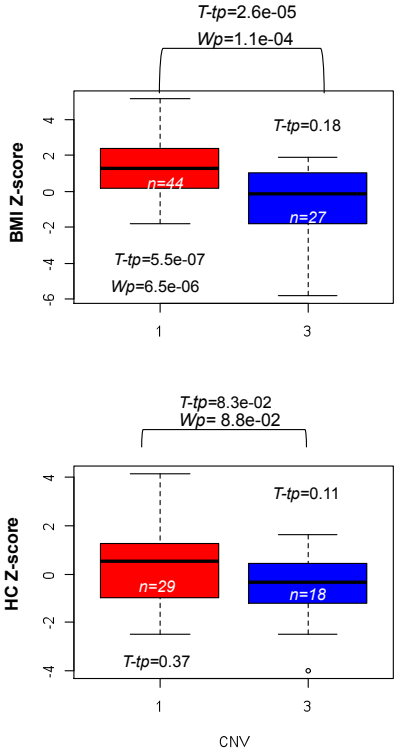


Figure 3. BMI Z-score and HC Z-score distribution in deletion and duplication carriers.

Red and blue boxplots represent deletion and duplication data respectively. Two-tailed Student's T tests comparing the distribution between groups and the norm (T-t p value presented above or below the boxplot) and between deletion and duplication groups (p value presented in the brakets) were performed. For all significant p values, a Wilcoxon ranksum test was also performed to avoid issues related to non normal distribution (*Wp).*

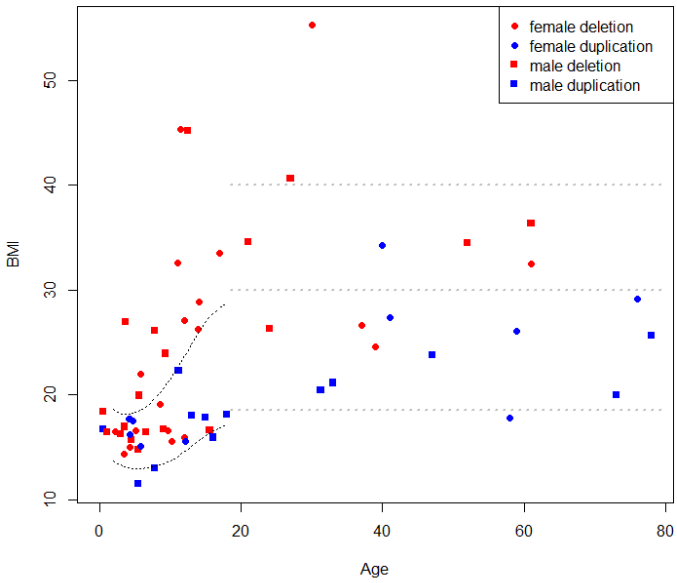


Figure 4. BMI in function of age

Figure 4 shows the cross sectional distribution of BMI in carriers. The dashed lines represent the 3rd and 97th Swiss Growth Charts centile, while the dotted lines represent the thresholds for underweight (BMI=18.5), obesity (30) and morbid obesity (40).

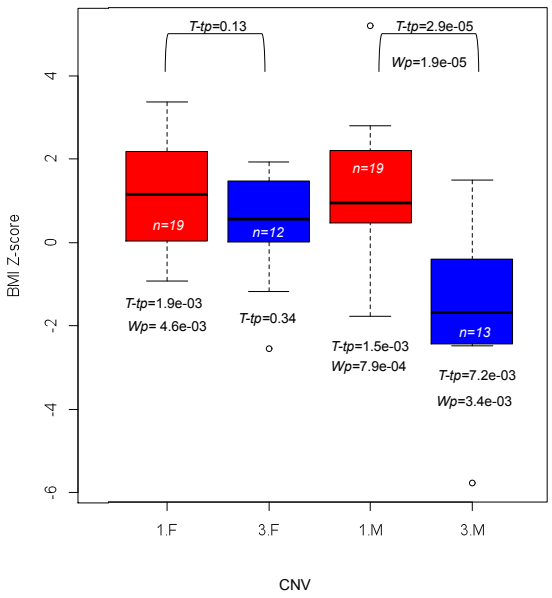


Figure 5. BMI Z-score stratified by gender and copy number.

Red and blue boxplots represent deletion and duplication data respectively. Two-tailed Student's T tests comparing the distribution between groups and the norm (T-t p value presented above or below the boxplot) and between deletion and duplication groups (p value presented in the brackets) were performed. For all significant p values, a Wilcoxon ranksum test was also performed to avoid issues related to non normal distribution (*Wp).*

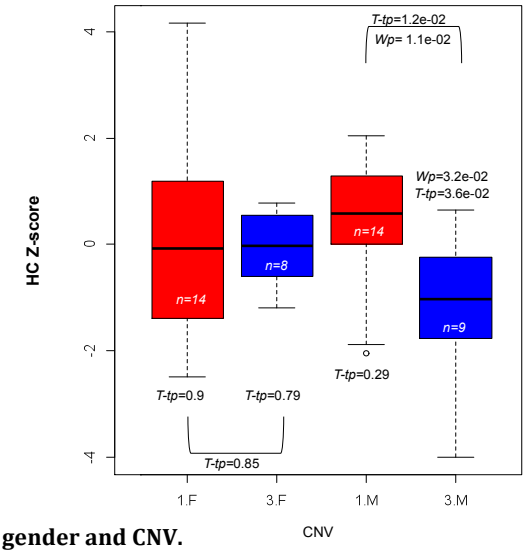


Figure 6. HC Z-score stratified by gender and CNV.

Red and blue boxplots represent deletion and duplication data respectively. Two-tailed Student's T tests comparing the distribution between groups and the norm (T-t p value presented above or below the boxplot) and between deletion and duplication groups (p value presented in the brakets) were performed. For all significant p values, a Wilcoxon ranksum test was also performed to avoid issues related to non normal distribution (*Wp).*

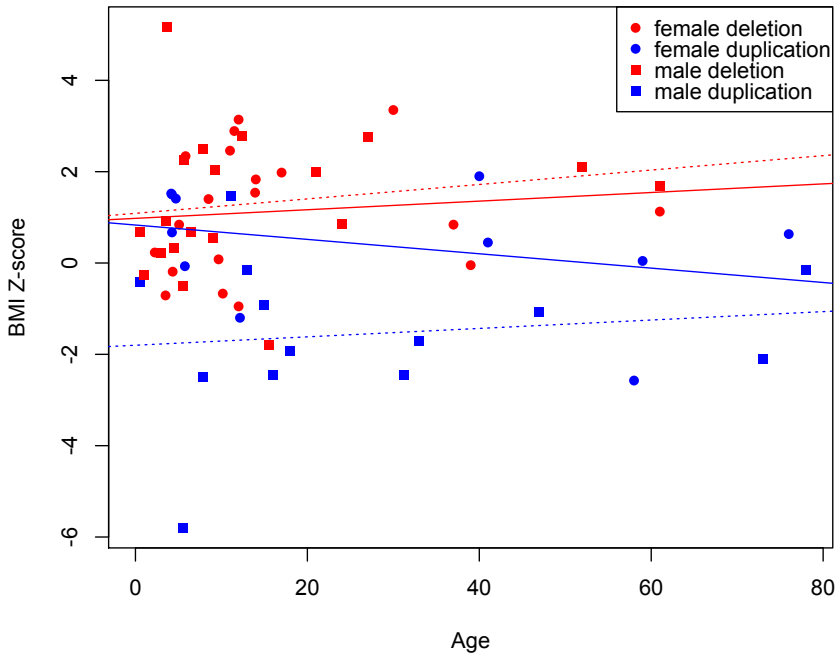


Figure 7. BMI Z-score in function of age

Age was not a significant covariate in any of the four linear models represented on the graph.

Red dotted line : male deletion group, red filled line : female deletion group, blue filled line : female duplication group, blue dotted line : male duplication group. The effect of age was not significant.

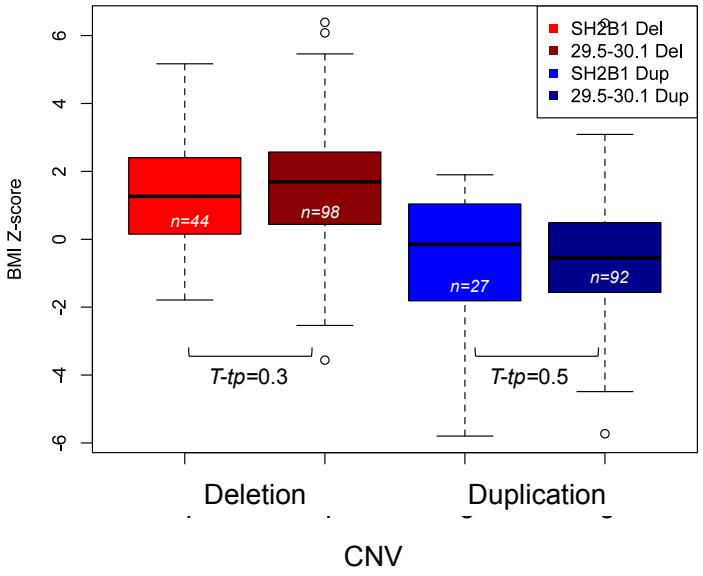


Figure 8. BMI Z-score stratified by 16p11.2 loci and copy number

Figure 8 shows BMI Z-score in function of CNV. Darkred and darkblue boxplots, showing respectively deletions and duplications carriers of the "classic" region (29.5-30.1). Lightred and lightblue : deletions and duplications carriers of the SH2B1 region.

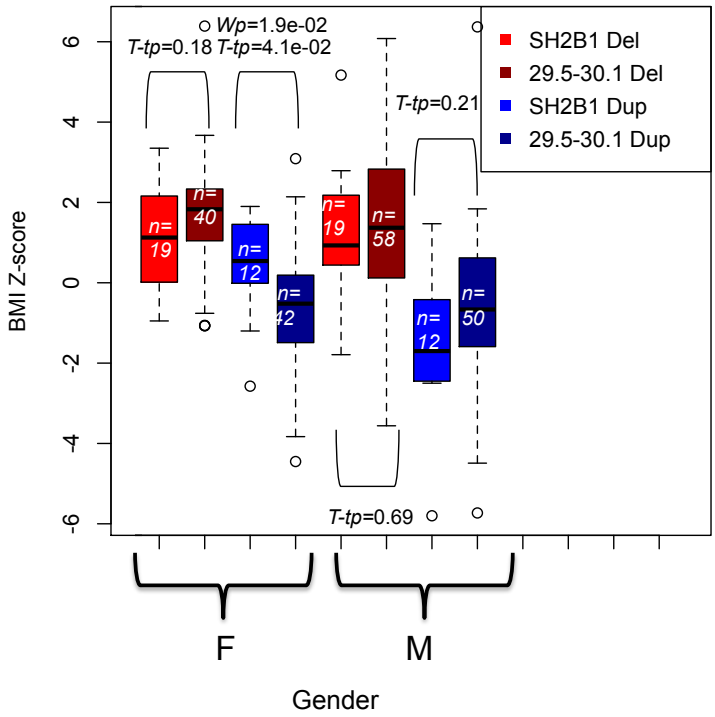


Figure 9. BMI Z-score stratified by 16p11.2 loci, gender and copy number.

BMI Z-score stratified by gender and 16p11.2. Again, darkred and darkblue boxplots showing respectively deletions and duplications carriers of the "classic" region (29.5-30.1).

Supplemental Information

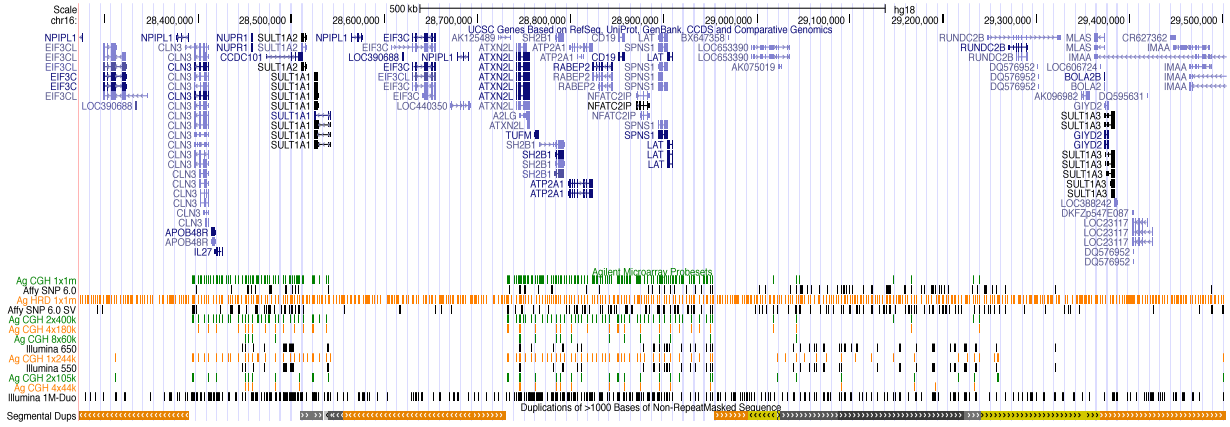
Cohorts :

Concerning the general population cohort, and more specifically *deCODE :*

Patients and controls were all Icelandic and were recruited from all over Iceland. All participants with ADHD met DSM-IV criteria for ADHD (477 combined type, 250 inattentive type, 58 hyperactive-impulsive type, 40 unspecified). ADHD subjects were recruited from outpatient pediatric, child, and adult psychiatry clinics in Iceland, and diagnoses had been made on the basis of standardized diagnostic assessments and had been reviewed by experienced clinicians. Autistic individuals (n=351) met Autism Diagnostic Interview Revised (ADI-R) criteria and were ascertained through the State Diagnostic Counselling Center and the Department of Child and Adolescent Psychiatry in Iceland. schizophrenia diagnoses were assigned according to Research Diagnostic Criteria (RDC) through the use of the Schedule for Affective Disorders and Schizophrenia Lifetime Version (SADS-L). Schizophrenia patients (n=657) were recruited from outpatient pediatric, child, and adult psychiatry clinics, and diagnoses had been made on the basis of standardized diagnostic assessments and had been reviewed by experienced clinicians. Recruitment of the psychiatric patients at deCODE has been described in more details elsewhere, ADHD, ASD schizophrenia and the control population. All participants, cases and controls, returned signed informed consents prior to participation in the study. All personal identifiers associated with medical information, questionnaire results, and blood samples were encrypted according to the standards set by the Data Protection Committee of Iceland. All procedures related to this study have been approved by the Data Protection Authority and National Bioethics Committee of Iceland.

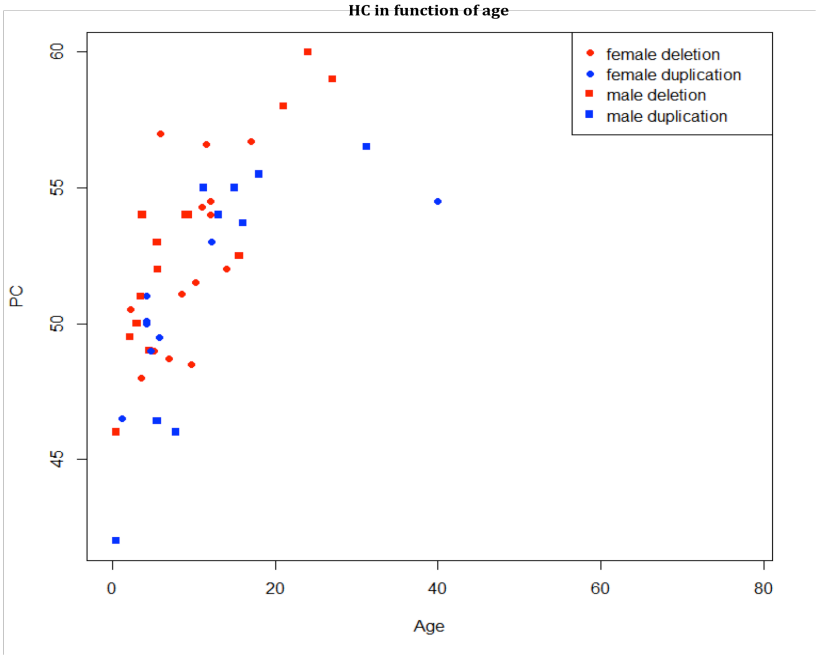
Figures :

Supplemental Figure 1



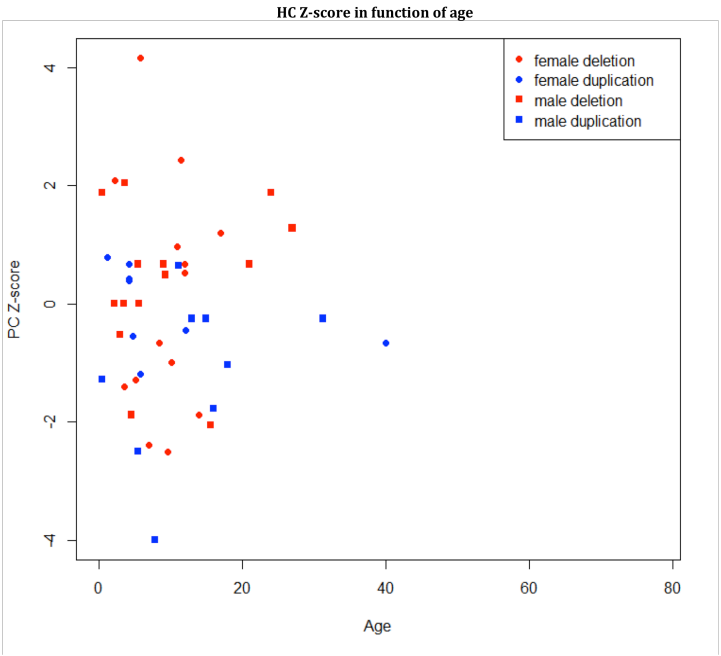
Coverage of the 16: 28.3-29.5 region by several array CGH and SNP array platforms

Supplemental Figure 2



Supplemental Figure 2 shows HC in function of age.

Supplemental Figure 3



Supplemental Figure 3 shows HC Z-score in function of age.