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(Article begins on next page)
ORIGINAL ARTICLE

A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and neurodevelopmental disorders

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ABSTRACT

Background The recurrent ~600 kb 16p11.2 BP4-BP5 deletion is among the most frequent known genetic aetiologies of autism spectrum disorder (ASD) and related neurodevelopmental disorders.

Objective To define the medical, neuropsychological, and behavioural phenotypes in carriers of this deletion.

Methods We collected clinical data on 285 deletion carriers and performed detailed evaluations on 72 carriers and 68 intrafamilial non-carrier controls.

Results When compared to intrafamilial controls, full scale intelligence quotient (FSIQ) is two standard deviations lower in carriers, and there is no difference between carriers referred for neurodevelopmental disorders and carriers identified through cascade family testing. Verbal IQ (mean 74) is lower than non-verbal IQ (mean 83) and a majority of carriers require speech therapy. Over 80% of individuals exhibit psychiatric disorders including ASD, which is present in 15% of the carriers by the age of 7 years, does not correlate with FSIQ or any behavioural trait. Seizures are present in 24% of individuals. Malformations are present in 50% of the carriers by the age of 7 years, does not correlate with FSIQ or any behavioural trait. Malformations are infrequently found, confirming only a few of the previously reported associations.

Conclusions The 16p11.2 deletion impacts in a quantitative and independent manner FSIQ, behaviour and body mass index, possibly through direct influences on neural circuitry. Although non-specific, these features are clinically significant and reproducible. Lastly, this study demonstrates the necessity of studying large patient cohorts ascertained through multiple methods to characterise the clinical consequences of rare variants involved in common diseases.

INTRODUCTION

The 16p11.2 locus (figure 1) encompasses several distinct genomic structural variants, including a recurrent interstitial deletion, of a ~600 kb region containing 29 genes.1 This deletion defined by breakpoints 4 and 5 (BP4-BP5) has a population prevalence of approximately 1/2000,2 and reaches 0.5% in autism spectrum disorders (ASD).3–7 It is one of the most frequent known single locus aetiologies of neurodevelopmental disorders and ASD.8–12

We and others have demonstrated that this deletion predisposes to a highly penetrant form of obesity with a 43-fold increased risk of developing morbid obesity.13 9 Increased head circumference (HC) has also been associated with the deletion.2 10 A mirror phenotype is observed in carriers of the reciprocal duplication who present a high risk of being underweight and microcephalic.10 11–14

The diversity of published clinical features,10–14 together with the report of asymptomatic (but not fully evaluated) transmitting parents,15 16 demonstrated the need to assess systematically the impact of the deletion on neurocognitive development and behaviour.17–19 To identify the essential clinical traits accurately and assist with genetic counselling, through a collaborative effort we have collected the largest dataset of 16p11.2 BP4-BP5~600 kb deletion carriers. It combines deletion carriers from both the 16p11.2 European and Simons Variation in Individuals Project (Simons VIP) consortia (n=116 and n=52, respectively). We present in this report the natural history, frequency, and range of phenotypes of this rearrangement. We demonstrate that the 16p11.2 deletion consistently impacts cognitive functioning, behaviour, growth, and body mass index (BMI).

PATIENTS AND METHODS

Patients

This study was reviewed and approved by the institutional review board of each site conducting the study. Signed consents were obtained from participants who underwent full assessments. For the data collected through questionnaires, information was gathered retrospectively and anonymously by
physicians who had ordered comparative genomic hybridisation (CGH) analyses performed for patient care purposes 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.

Carriers were ascertained through several cohorts (table 1). Details on ascertainment and data collection for the participants have been previously published. All participants in the Simons VIP cohort were mapped using whole genome oligonucleotide arrays, and were found to have the common, literature cases (including 7 relatives) reviewed from the literature. The copy number variant detection methods used in the literature are: 19K bacterial artificial chromosome (BAC) microarray, Affymetrix 500K, Affymetrix 5.0, 44K and 105K Agilent, 44K and 105K Agilent, 105K Agilent, Affymetrix 500K, Affymetrix 6.0, Illumina HumanHap300 BeadChip, 38K BAC microarray (Swegene), 44K, 105K and 244K Agilent, Affymetrix 500K, Affymetrix 6.0, and Illumina 1M. BAC microarray, Affymetrix 6.0, Agilent 105K, NimbleGen H62, Affymetrix 6.0, Affymetrix 500K or ROMA 85K, 244K Agilent or Affymetrix 6.0. Data were available on 18/25*, 85/113** and 45/67*** deletion carriers. Due to missing data, there may be differences between the total number of cases and the sum of cases in the various columns. Statistically significant values are in bold. DD/ID, developmental disorders/intellectual disability; NA, non-available or not applicable; Simons VIP, Simons Variation in Individuals Project.

<table>
<thead>
<tr>
<th>Cohorts†</th>
<th>Mean age (y)</th>
<th>Carriers</th>
<th>M</th>
<th>F</th>
<th>Sex ratio p Value</th>
<th>Inheritance</th>
<th>Patients screened</th>
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<td>Europe‡</td>
<td>Probands</td>
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<td>85**</td>
<td>56</td>
<td>28</td>
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<td>22</td>
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<td>13</td>
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<td>Probands</td>
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<td>45***</td>
<td>26</td>
<td>19</td>
<td>0.19</td>
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<td>3</td>
<td>2</td>
<td>1</td>
<td>–</td>
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<td>DD/ID§</td>
<td>9.8</td>
<td>84</td>
<td>56</td>
<td>28</td>
<td>1.49×10⁻³</td>
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<td>18*</td>
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<td>10</td>
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<td></td>
<td>Obesity‡</td>
<td>26.5</td>
<td>15</td>
<td>4</td>
<td>11</td>
<td>0.59</td>
<td>2/3 (0:3)</td>
</tr>
<tr>
<td>Carriers TOTAL</td>
<td>–</td>
<td>285</td>
<td>166</td>
<td>117</td>
<td>2.12×10⁻³</td>
<td>92/53 (30:20)</td>
<td>107598</td>
</tr>
</tbody>
</table>

†Distribution of clinical indications for referral is detailed in the previous publication.
‡Only anthropometric data were available and previously published.
§Cases (including 7 relatives) reviewed from the literature. The copy number variant detection methods used in the literature are: 19K bacterial artificial chromosome (BAC) microarray, 44K and 105K Agilent, 44K and 105K Agilent, 105K Agilent, Affymetrix 500K, Affymetrix 6.0, Illumina HumanHap300 BeadChip, 38K BAC microarray (Swegene), 44K, 105K and 244K Agilent, Affymetrix 500K, Affymetrix 6.0, and Illumina 1M. BAC microarray, Affymetrix 6.0, Agilent 105K, NimbleGen H62, Affymetrix 6.0, Affymetrix 500K or ROMA 85K, 244K Agilent or Affymetrix 6.0. Data were available on 18/25*, 85/113** and 45/67*** deletion carriers. Due to missing data, there may be differences between the total number of cases and the sum of cases in the various columns. Statistically significant values are in bold. DD/ID, developmental disorders/intellectual disability; NA, non-available or not applicable; Simons VIP, Simons Variation in Individuals Project.
recurrent 16p11.2 BP4-BF5 deletion. Data for 96 carriers (76 probands and 20 relatives) from the European consortium were obtained by completion of a questionnaire by referring clinicians. Fifty-four probands and 18 relatives (n=72) were extensively evaluated on site by investigators of one of the consortia. Data on 117 deletion carriers ascertained for developmental disorders/intellectual disability (DD/ID) (n=64), obesity (n=15) and from the general population cohorts (n=13), were collected from our previously published studies, as well as from the literature.1 2 4 5 7 10 11 15–16 19–23

In the Simons VIP cohort, four patients were excluded based on the presence of additional pathological copy number variants (CNV), other genetic diagnoses, birth asphyxia, fetal alcohol syndrome, and/or prematurity <30 weeks. In the European series, known additional variants (three carriers with a CNV >500 kb and a pair of twins with an FGFR3 mutation) did not represent an exclusion criterion. The inclusion or exclusion of these five carriers did not have an impact on any results (supplementary table S1). They were included in the final analysis because of the arbitrariness of this filtering that only takes into account visible rearrangements and/or known point mutations. We have to assume that many additional mutational events not detectable by CGH array may be present in this dataset.

In the VIP cohort, ethnicity was 75% Caucasian, 5.8% African American, 1.9% Native American, 7.7% other (mixed ancestry), 9.6% unknown (adopted). For the 72 patients evaluated on site in the European cohort, all carriers were of European descent.

All available data on patients ascertained for DD/ID from the 16p11.2 European cohort, Simons VIP and the literature (n=285) were pooled for statistical analysis. Due to missing data for some phenotypes, the denominator changes, which is specified throughout the text. Data collected through questionnaires or by direct assessment are presented separately in supplementary table S1. They were included in the final analysis because of the arbitrariness of this filtering that only takes into account visible rearrangements and/or known point mutations. We have to assume that many additional mutational events not detectable by CGH array may be present in this dataset.

In the VIP cohort, ethnicity was 75% Caucasian, 5.8% African American, 1.9% Native American, 7.7% other (mixed ancestry), 9.6% unknown (adopted). For the 72 patients evaluated on site in the European cohort, all carriers were of European descent.

Cognitive functioning, psychiatric and behavioural assessment

Overall cognitive functioning was assessed using either the Mullen Scales of Early Learning, the Differential Ability Scales—Second Edition (Early Years and School Age, DAS-II), the Wechsler Intelligence Scales (WPPSI-III; WISC-IV; WAIS-III) or the Wechsler Abbreviated Scales of Intelligence, depending on the age and ability level of the individual.25 Vineland Adaptive Behaviour Scales (VABS)26 were used to measure adaptive skills in daily life. Intellectual disability (ID) was diagnosed as having an FSIQ of 70 or below on a standardised IQ test as well as concurrent deficits in adaptive functioning as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision (DSM-IV-TR) criteria.27

Different standardised neuropsychological measures were used to assess global cognitive functioning of carriers in the literature.5 11 15 16 23 For the purpose of this study, throughout the text we use the term FSIQ to refer to normalised cognitive levels (general population mean=100; standard deviation (SD)=15). ASD was diagnosed by experienced, research-reliable clinicians using the Autism Diagnostic Interview—Revised (ADI-R)28 and the Autism Diagnostic Observation Schedule (ADOS).29 The Diagnostic Interview for Genetic Studies (DIGS)30 was performed for adults, while children’s behavioural and emotional problems were assessed with parent report questionnaires.31–33 Additional DSM-IV-TR diagnoses were made by licensed psychologists and psychiatrists using history, parent report and in-person interview. For all aforementioned evaluations, the clinicians were not blinded to the genetic status of the participants.

Statistical analyses

To prevent bias due to intrafamilial correlations, deletion-carrying relatives of probands were excluded as required to avoid including more than one member of the same family in a single analysis. Obesity and morbid obesity in adults are defined throughout the study as BMI ≥30 and 40 kg/m², respectively. Z scores were computed for all data using gender, age, and geographically matched reference populations as previously described.1 Obesity in children and macrocephaly were defined as BMI and HC Z score ≥2, respectively.

One-tailed Fisher’s exact test was used to compare frequencies of the deletion in patients and controls. Two-tailed Student’s t test was performed to assess whether BMI, height, weight, and HC Z scores of deletion carriers were different from zero (general population mean). Correlation between these features and FSIQ were examined using Spearman correlation. The binomial test was performed to test for gender bias.

A linear model was applied to correct HC Z scores for BMI, age, and gender effects, using Matlab function regress. A two-tailed Student t test was performed to test the residual effects being different from zero. Linear mixed effect model was used to analyse the longitudinal and cross-sectional data and properly handle autocorrelations. Model fitting was performed to obtain the mean Z score and the corresponding p value for a given time window. The calculations were done using the lme function from the R package nlme.

For each of the W×age windows the mean Z scores were computed for each of the P patients resulting in a W×P matrix. Since W tests were carried out, we applied a multiple testing correction that takes into account the correlation structure of the test statistics. As a first step, the effective number of tests (W_eff) was derived based on the Pearson’s correlation matrix of the W×P dataset.34 We then applied Bonferroni correction of the linear mixed effect model p values, but using W_eff instead of W tests to correct for multiple testing.

Breakpoint mapping with short arm of chromosome 16 custom array CGH

To confirm 16p11.2 deletions and ensure that the breakpoints are within the BP4 and BF5 low copy repeats (figure 1), we hybridised Cy5-labelled DNA of the European patients to custom made Nimblegen arrays. These arrays contained 71 000 probes spread across the short arm of chromosome 16 from 22.0 to 32.7 Mb (at a median space of 45 bp between 27.5 and 31.0 Mb) and 1000 control probes situated in invariable region of the X chromosome.5 Cy5-labelled DNA from the GM12042 CEPH (Centre d’Etude du Polymorphisme Humain) cell line was invariably used as reference. DNA labelling, hybridisation and washing were performed according to Nimblegen protocols. Scanning was performed using an Agilent C2565BA Microarray Scanner. Image processing, quality control, and data extraction were performed using the NimbleScan software V2.5.
RESULTS
The extent of the 16p11.2 rearrangements was assessed by aCGH through standard medical diagnostic procedures and confirmed by a high density custom made array as published (see Methods). Only carriers of the ∼600 kb 16p11.2 BP4-BP5 deletion were considered for further phenotyping; we gathered clinical information on 285 such carriers (figure 1). Cognitive functioning was evaluated in 71 carriers (mean FSIQ=76.1; SD=16.4; figure 2A) and was on average 32 points lower in de novo carriers when compared to their non-carrier family members (∼−2 SD, p=3.96×10−27; table 2A). There is a trend towards higher FSIQ in de novo (n=32) versus inherited (n=14) carriers (FSIQ 83 vs 74; p=0.13; table 2A and supplementary table S1). Of note, parents of de novo deletion carriers who directly enrol their child through a web based questionnaire (VIP cohort) have a higher IQ than the mean of the general population. Among carriers, 20% met DSM-IV-TR criteria for ID (65% mild FSIQ 55–70 and 35% moderate FSIQ 40–55). There are no differences in FSIQ between probands referred for neurodevelopmental disorders and carriers who were not medically ascertained (relatives who carry the deletion) (table 2, supplementary table S1). Carriers’ verbal IQ (mean=74; SD=17.5; range 25–107; n=42) is significantly lower than non-verbal IQ (mean=83.3; SD=18.0; range 47–160; n=45) (p=0.02). Of the 24 carriers evaluated in Europe, 20 (83%) had speech and language therapy during childhood.

The 16p11.2 BP4-BF5 deletion has been repeatedly associated with ASD, and is one of its most frequent known aetiologies. Of the fully assessed carriers, ∼15% (8/55) of the children and no adults met criteria for ASD by ADOS and ADI-R. More than 70% (51/70) of non-ASD carriers were found to have other DSM-IV-TR diagnoses including attention deficit and disruptive behaviour disorders, anxiety disorders, mood disorders, and substance related disorders (table 2B). There is a significant excess of males among carriers ascertained for neurodevelopmental disorders (138 M/75 F; p=2.4×10−4) in contrast to other criteria (table 1). Gender, however, is not a significant covariate of FSIQ, adaptive level (Vineland), behavioural scores (Social Responsiveness Scale, SRS), neurological symptoms or any other trait (supplementary table S1).

![Figure 2](image-url) Distribution of full scale intelligence quotient (FSIQ) and body mass index (BMI) in deletion carriers. (A) Distribution of FSIQ of 16p11.2 BP4-BF5 deletion carriers (grey bars), intrafamilial non-carrier relatives (control, blue bars) and general population (blue bell curve). The red dashed vertical line represents the FSIQ threshold (70) for intellectual disability (ID). FSIQ is on average 32 points lower in carriers (n=71; mean=76.1; SD=18.4) when compared to their relatives who did not carry the deletion (n=68; mean=108.3; SD=10.9). SD in carriers is similar to that of the reference population (mean=100; SD=15). Bin size was calculated to obtain 10 equal sized bins. (B) Cross-sectional distribution of BMI in carriers (circles: female; open squares: male). BMI progressively increases throughout childhood and adulthood. 70% of the adult carriers are obese (BMI ≥30). The dashed lines represent the 3rd and 97th Center for Disease Control and Prevention (CDC) centile, while the dotted lines pinpoint the thresholds for underweight (BMI =18.5), obesity (30), and morbid obesity (40).

### Table 2 FSIQ (A), behavioural and psychiatric features (B) in deletion carriers and intrafamilial controls

<table>
<thead>
<tr>
<th>Carriers</th>
<th>Probands</th>
<th>Relatives</th>
<th>Non-carriers</th>
<th>Inherited</th>
<th>De novo</th>
<th>Unknown</th>
<th>Parents</th>
<th>Siblings</th>
<th>Parents</th>
<th>Siblings</th>
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<tbody>
<tr>
<td>Mean age (years)</td>
<td>14.7</td>
<td>10.9</td>
<td>15.7</td>
<td>36.8</td>
<td>10.2</td>
<td>38.6</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean FSIQ</td>
<td>74</td>
<td>82.7</td>
<td>64.9</td>
<td>78.6</td>
<td>65.3</td>
<td>109.1</td>
<td>106.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cases</td>
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<td>32*</td>
<td>14</td>
<td>7</td>
<td>4</td>
<td>46</td>
<td>22</td>
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<table>
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<tr>
<th>Carriers</th>
<th>16p11 Europe</th>
<th>Simons VIP</th>
<th>Total cohorts</th>
</tr>
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<tr>
<td>DSM-IV-TR diagnosis</td>
<td>Children</td>
<td>Adults</td>
<td>Children</td>
</tr>
<tr>
<td>ASD†</td>
<td>1 (12.5)</td>
<td>0</td>
<td>7 (15)</td>
</tr>
<tr>
<td>Any DSM-IV-TR diagnosis other than ASD</td>
<td>4 (50)§</td>
<td>6 (50)§</td>
<td>39 (83)</td>
</tr>
<tr>
<td>No diagnosis</td>
<td>3 (37.5)</td>
<td>6 (50)</td>
<td>1 (2)</td>
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<tr>
<td>Total</td>
<td>8</td>
<td>12</td>
<td>47</td>
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</table>

*Two mosaic cases. †10 probands and 10 relatives in the 16p11.2 European cohort and 44 probands and 6 relatives in Simons VIP. §ADOS and Autism Diagnostic Interview criteria. Include attention deficit and disruptive behaviour disorders (n=4), anxiety disorders (n=5), mood disorders (n=3) and substance related disorders (n=2). Patients can have more than one diagnosis. ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision; FSIQ, full scale intelligence quotient.
We observed characteristic anthropometric patterns in deletion carriers. Birth weight is below average (n=124, Z score=−0.61; supplementary table S4), whereas Z scores for BMI are significantly higher by the age of 3.5 (Z score=1.01, p=0.05) (figure 3B). Longitudinal data show that this increase in BMI can be sudden and dramatic (figure 3D, supplementary figure S1). By the age of 7 years, obesity is a major comorbidity present in more than 50% of the carriers (supplementary figure S2). Excluding carriers ascertained for obesity, the frequency reaches 75% in adults (36/48), and among all adult obese patients 45% are morbidly obese (figure 2B). The variance of BMI in deletion carriers is higher than in the general population. Panel B: BMI is significantly lower in the obese group (triangles). Height is significantly increased in prepubertal obese carriers (figure 3B). Longitudinal data (figure 3B,C, supplementary figure S4) show that HC, which is lower at birth by 0.57 Z scores (n=58), increases during infancy (+1.74 Z scores, p=4.8×10⁻⁶), several years before the elevation of BMI Z scores. In the Simon Simplex Collection of ASD children, 41.3% of patients with obesity have macrocephaly when compared to 12.5% in non-obese patients (p=5.4×10⁻²⁶) (supplementary figure S5).

Neurological features observed in patients referred for DD/ID include a wide range of findings (supplementary table S2). Gross motor delay was reported in 57.6% of the patients (52/85 ascertained for DD/ID in the European cohorts), which is consistent with previous series, and mean age of walking is significantly delayed (mean=20.5 months, SD=8.6, n=50, p=8.63×10⁻⁶). Epilepsy is a frequent feature reported in 47/195 (24%) of the probands. While this frequency is not significantly different in non-medically ascertained carriers (5/38, 13%, p=0.2), it is higher in the fully assessed probands (22/54, 41%, p=1.6×10⁻⁴). A small fraction of carriers (12/233) received a diagnosis of paroxysmal dyskinesia syndrome (OMIM 128200). MRI performed in a subset of carriers (n=65) showed mostly mild and non-recurrent features. Posterior fossa

Figure 3 Height, body mass index (BMI), and head circumference (HC) in 16p11.2 BP4-BP5 deletion carriers through development. Height (panel A), BMI (panel B) and HC (panel C) mean Z scores (and corresponding p values in red) for each age window were computed using a mixed effect model to analyse longitudinal and cross-sectional data together. p Values are derived from a two-sided t test of the fixed effects estimates probing whether they are significantly different from 0. Full red dots are p values surviving multiple testing correction (significance’s threshold at 6.3×10⁻⁴) for height in both obese and non-obese, at 5.6×10⁻³ for BMI, and at 7.1×10⁻³ for HC) as opposed to empty red dots. Number of cases N is indicated for each age category. Panel A: Deletion carriers were classified in two groups; either the ‘obese group’ (squares) if they presented obesity at least once during their development, or the non-obese group (triangles). Height is significantly increased in prepubertal obese carriers while non-obese children remain slightly shorter than the general population. Panel B: BMI is significantly elevated by 3.5 years of age. Panel C: HC follows a rapid increase (+1.74 Z score, p=4.8×10⁻⁴) during infancy, and remains high throughout life. Panel D: Longitudinal measures of BMI in a subset of 12 carriers illustrating different age onsets of BMI acceleration. The grey area specifies the interval between the 3rd and 97th centile as defined by the WHO data (http://www.who.int/childgrowth/en) between 0–2 years and the Centre for Disease Control and Prevention data above 2 years of age. The white line marks the 50th centile. All available longitudinal data are included in supplementary figure S2.
and/or craniocervical junction related abnormalities (eg, Chiari
1 malformation, cerebellar tonsillar ectopia, platybasia) were
present in 19 of 41 fully reviewed MRIs.

Malformations or major medical problems are present in
76/130 (58%) of the probands from both cohorts, of which 53
have only a single pathological feature (supplementary tables
S3 and S6). In non-medically ascertained carriers identified in
the families through cascade testing, this frequency is much
lower (10/38, 26.3%, \( p = 7.6 \times 10^{-4} \)). No specific recurrent mal-
formation sequence or multisystemic involvement is observed
(supplementary table S6). There are, however, features likely
to be associated with the deletion which, in most cases, do not
require treatment such as vertebral abnormalities (hemiverteb-
rae or kyphoscoliosis affect ∼20% of carriers). Facial dys-
morphia was visible in 69/150 (46%) carriers, yet no recurrent
facial gestalt was observed in either cohort.

The frequency of this BP4-BPS deletion (table 1) among
patients referred for clinical chromosome hybridisation micro-
array is similar for both consortiums (0.25%). Though the two
cohorts were assembled in different contexts and healthcare
systems, as well as using different ascertainment criteria, they
share striking similarities with the exception of the rate of
inherited cases. Among Simons VIP patients, the deletion arose
almost exclusively de novo (table 1). Globally, for participants
with available parental data (51%), the deletion occurred de
novo in 92/145 cases (including two mosaic cases) (64%) and
was inherited in the remaining cases (36%). Assuming a stable
prevalence of the deletion, this high rate of de novo events
implies decreased fitness of this deletion (0.52 and 0.20 in the
European and Simons VIP cohorts, respectively).

DISCUSSION

Our study demonstrates that the recurrent 600 kb BP4-BPS
deletion, which has a prevalence of ∼0.05% in the general
population,\(^7\) has a consistent impact on traits affecting adap-
tive skills. FSIQ is decreased by ∼2 SD in carriers of both
genders, about 20% of them meeting criteria for ID. Since the
FSIQ variance remains identical to that of the general popula-
tion \((p=0.25)\), approximately 30% of deletion carriers fall
within the normal range \((FSIQ \geq 85)\). Bias in these estimates is
unlikely since there were no differences in FSIQ scores of pro-
bands and non-medically ascertained carriers.

FSIQ does not capture the full range of disabilities experi-
enced by deletion carriers. A history of speech therapy is fre-
quent (33%) and psychiatric comorbidities affect >80% of
 carriers. The penetrance of ASD is 15% in our cohorts
(table 2B), supporting the association of this deletion with
ASD.\(^8\) Increased growth velocity of HC during infancy recap-
tilates the well documented pattern in idiopathic ASD. This
was inherited in the remaining cases (36%). Assuming a stable
prevalence of the deletion, this high rate of de novo events
implies decreased fitness of this deletion (0.52 and 0.20 in the
European and Simons VIP cohorts, respectively).

and/or craniocervical junction related abnormalities (eg, Chiari
1 malformation, cerebellar tonsillar ectopia, platybasia) were
present in 19 of 41 fully reviewed MRIs.

Malformations or major medical problems are present in
76/130 (58%) of the probands from both cohorts, of which 53
have only a single pathological feature (supplementary tables
S3 and S6). In non-medically ascertained carriers identified in
the families through cascade testing, this frequency is much
lower (10/38, 26.3%, \( p = 7.6 \times 10^{-4} \)). No specific recurrent mal-
formation sequence or multisystemic involvement is observed
(supplementary table S6). There are, however, features likely
to be associated with the deletion which, in most cases, do not
require treatment such as vertebral abnormalities (hemiverteb-
rae or kyphoscoliosis affect ∼20% of carriers). Facial dys-
morphia was visible in 69/150 (46%) carriers, yet no recurrent
facial gestalt was observed in either cohort.

The frequency of this BP4-BPS deletion (table 1) among
patients referred for clinical chromosome hybridisation micro-
array is similar for both consortiums (0.25%). Though the two
cohorts were assembled in different contexts and healthcare
systems, as well as using different ascertainment criteria, they
share striking similarities with the exception of the rate of
inherited cases. Among Simons VIP patients, the deletion arose
almost exclusively de novo (table 1). Globally, for participants
with available parental data (51%), the deletion occurred de
novo in 92/145 cases (including two mosaic cases) (64%) and
was inherited in the remaining cases (36%). Assuming a stable
prevalence of the deletion, this high rate of de novo events
implies decreased fitness of this deletion (0.52 and 0.20 in the
European and Simons VIP cohorts, respectively).

DISCUSSION

Our study demonstrates that the recurrent 600 kb BP4-BPS
deletion, which has a prevalence of ∼0.05% in the general
population,\(^7\) has a consistent impact on traits affecting adap-
tive skills. FSIQ is decreased by ∼2 SD in carriers of both
genders, about 20% of them meeting criteria for ID. Since the
FSIQ variance remains identical to that of the general popula-
tion \((p=0.25)\), approximately 30% of deletion carriers fall
within the normal range \((FSIQ \geq 85)\). Bias in these estimates is
unlikely since there were no differences in FSIQ scores of pro-
bands and non-medically ascertained carriers.

FSIQ does not capture the full range of disabilities experi-
enced by deletion carriers. A history of speech therapy is fre-
quent (33%) and psychiatric comorbidities affect >80% of
 carriers. The penetrance of ASD is 15% in our cohorts
(table 2B), supporting the association of this deletion with
ASD.\(^8\) Increased growth velocity of HC during infancy recap-
tilates the well documented pattern in idiopathic ASD. This
shared head growth pattern, which has attracted considerable
attention as a marker of abnormal brain development, has been
linked to an increase in white matter volume, brain weight and
numbers of neurones in the prefrontal cortex of ASD
patients.\(^9\)

Obesity is a major comorbidity of 16p11.2 deletion carriers,
with a penetrance among adults of >70%. Durable weight loss
in adolescents or adults has not yet been documented: (1) two
adults treated by bariatric surgery relapsed several years later;
and (2) an adolescent dropped from 39.5 to 27.7 kg/m\(^2\) by
dieting and engaging in intense physical activity, but returned
to his initial weight 2 years after discontinuing this regimen.
Weight control is therefore recommended although no data are
available on the efficacy of early intervention in deletion car-
riers. The reported association of ID with obesity\(^10\) has led to
the proposal that impaired cognition may result in abnormal
eating behaviour and obesity.\(^2\) In 16p11.2 deletion carriers,
however, obesity occurs independently of cognitive function,
adaptive or social behaviour scores.

We hypothesise that the deletion directly affects the neural
circuitry involved in all these phenotypes, including energy
balance. The early increase in HC precedes the onset of obesity
(supplementary figure S4). This increase in HC is also observed
later in childhood and adolescence (figure 3C) when correlation
with brain volume is lower and contribution of skull thickness
higher.\(^42,43\) Furthermore we demonstrate that childhood
obesity in 16p11.2 deletion carriers, as well as in patients with
autism (SSC cohort), is a confounding factor contributing to
increased HC (supplementary figure S5). This deletion lowers
final adult height by 1 SD (supplementary figure S3), though the
well documented association between idiopathic childhood
obesity and prepubertal height acceleration\(^36\) is maintained in
obese children carrying the deletion (figure 3A,B, supplemen-
tary figure S5).

Epilepsy is the most frequent neurological disorder observed
in deletion carriers, and electroencephalogram (EEG) evaluation
should be prescribed if abnormal movements or behaviours sus-
picious for seizures are observed. A smaller and possibly under-
estimated fraction of carriers have paroxysmal dyskinesia
syndrome. We failed to observe a difference in the presence or
absence of epilepsy when stratifying for either cognitive func-
tioning, BMI or HC (supplementary table S1). It is possible
that epilepsy and the latter phenotypes are related to haploin-
sufficiency of distinct genes (figure 1B). Mutations in PRRT2, a
gene mapping to the deleted interval, were recently identified in
patients diagnosed with epilepsy and paroxysmal dyskinesia.\(^44\)
whereas a 118kb deletion that encompasses MVP, CDIPT, SEZ6L2, ASPHD1, and KCTD13 segregated in a three-
generation pedigree with ASD and other neurodevelopmental
abnormalities but not epilepsy.\(^45\) Of note, morpholino-driven
reduction of the expression level of the KCTD13 ortholog
resulted in macrocephaly in zebrafish, while its deletion in the
brain of mouse embryos resulted in an increase of proliferating
cells.\(^38\)

Although we report a large spectrum of malformations (sup-
plementary table S3), the 16p11.2 deletion should not be
regarded primarily as a malformation syndrome. Indeed, the
majority of these abnormalities are infrequent, suggesting
either fortuitous associations or low penetrance (eg, holoprosa-
my/microphthalmia) possibly through unmasking of recessive
mutations. Vertebral and spinal related anomalies (∼20%, sup-
plementary table S3) seem, however, to be strongly associated
with the deletion, suggesting that spine x-ray and orthopaedic
evaluations should be routinely performed in deletion carriers.

TBX6, which maps within the interval, is a candidate gene for
vertebral malformations since mice homozygous for a Ts6x
mutation showed rib and vertebral body anomalies.\(^46\)
Additionally, TBX6 polymorphisms were associated with
congenital scoliosis in the Han population.\(^47\)

In conclusion, our study demonstrates in two independent
datasets that the 16p11.2 BP4-BPS 600 kb deletion consistently
and quantitatively impacts cognitive functioning, HC, BMI,
and growth. A range of behavioural disorders affects the vast
majority of carriers. Probands referred for neurodevelopmental
disorders or morbid obesity and carriers who were not med-
ically ascertained (relatives of a proband) show similar values for
FSIQ and BMI, suggesting that ‘asymptomatic’ carriers are
uncommon. Five carriers within our cohorts presented an
FSIQ >100 and may therefore represent the higher end tail of
the FSIQ distribution of deletion carriers. All five presented language disorders, autism, disruptive behaviour or obesity. The deleterious impact of the deletion is further highlighted by its low fitness reflected by the rarity of multigenerational carrier families. In contrast to BMI, the variance of global cognitive functioning (FSIQ) is the same among carriers and control population, suggesting that the factors determining its variability are identical to those at play in the general population and unrelated to the 16p11.2 locus.

This comprehensive study of the 16p11.2 BP4-BFS phenotype helps to guide clinical monitoring and counselling of patients and families and to potentially overcome the genetic counseling challenge posed by its variability. It illustrates that the study of rare variants causing common diseases lacking pathogenic nemor features requires the assembly and detailed clinical characterisations of large cohorts, recruited using multiple ascertainment criteria.

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Contributors

All authors contributed to the concept, design, acquisition of data or analysis and interpretation of data. All authors contributed to drafting or revising the content and approved the final version.

Competing interests

Competing interests are disclosed in the ICMJE conflict of interest forms.

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This study was approved by the IRB of the University of Lausanne, the IRB of the Simons Foundation as well as the IRB of each site conducting the study.

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Data sharing statement

Data on the Simons VIP subjects are available through SFARI Base at https://sfari.org/resources/sfari-base.

REFERENCES


Copy-number variation


Miscellaneous

Corrections


Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012;49:578–90. In the above article updated figures were not included in the proof. These will be uploaded with the article as a data supplement.