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Large, rare chromosomal deletions associated with severe early-onset obesity

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Abstract

Obesity is a highly heritable and genetically heterogeneous disorder¹. Here we investigated the contribution of copy number variation to obesity in 300 Caucasian patients with severe early-onset obesity, 143 of whom also had developmental delay. Large (>500 kilobases), rare (<1%) deletions were significantly enriched in patients compared to 7,366 controls ($P < 0.001$). We identified several rare copy number variants that were recurrent in patients but absent or at much lower prevalence in controls. We identified five patients with overlapping deletions on chromosome 16p11.2 that were found in 2 out of 7,366 controls ($P < 5 \times 10^{-5}$). In three patients the deletion co-segregated with severe obesity. Two patients harboured a larger *de novo* 16p11.2 deletion, extending through a 593-kilobase region previously associated with autism²⁻⁴ and mental retardation⁵; both of these patients had mild developmental delay in addition to severe obesity. In an independent sample of 1,062 patients with severe obesity alone, the smaller 16p11.2 deletion was found in an additional two patients. All 16p11.2 deletions encompass several genes but include *SH2B1*, which is known to be involved in leptin and insulin signalling⁶. Deletion carriers exhibited hyperphagia and severe insulin resistance disproportionate for the degree of obesity. We show that copy number variation contributes significantly to the genetic architecture of human obesity.

Although the rise in obesity prevalence is driven by environmental factors, there is considerable evidence that weight is highly heritable^{1,7}. Genome-wide association studies (GWAS) have identified common single nucleotide polymorphisms (SNPs) associated with increased body mass index (BMI)⁸⁻¹¹; however, together these account for a small percentage of the inherited variation in BMI. Studies in patients with severe early-onset obesity have led to the detection of rare variants, many of which have an impact on the leptin–melanocortin system involved in energy homeostasis^{7,12,13}. To explore the contribution of copy number variants (CNVs) to obesity, we studied 300 Caucasians with

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severe early-onset obesity. Rare CNVs can cause developmental diseases¹⁴; as such we enriched for patients with developmental delay in addition to severe obesity ($n = 143$) in this discovery set (Supplementary Information). Genomic DNA was hybridized to the Affymetrix 6.0 array and the frequency of CNVs in patients was compared to 7,366 controls of European ancestry. Data were analysed using Affymetrix Power Tools and Birdsuite software¹⁵. We performed extensive post-calling quality control (QC) and filtering to arrive at a final set of CNVs (Supplementary Information). We detected 15,407 CNVs in the 284 patients passing QC and 403,098 CNVs in the 7,366 controls passing QC. The median number of CNV calls per individual was similar between patients and controls (53 and 55, respectively), as was the median size of CNVs (22 kilobases (kb) and 23.5 kb, respectively).

Following criteria set previously¹⁶, we defined large, rare deletions as present in $<1\%$ of individuals and >500 kb in size. We observed a twofold enrichment of large, rare deletions in patients compared to controls (Table 1). We assessed the significance of this observation using conditional permutation tests controlling for differences in total number of CNV calls or data quality between patients and controls, and found it to be highly significant ($P = 0.0005$, Supplementary Information). Patients with developmental delay in addition to severe early-onset obesity were more likely to harbour rare, large deletions (Table 1). The trend towards enrichment of large, rare deletions remains in patients with severe obesity alone, but is no longer statistically significant.

We identified several rare CNVs recurrent among patients and enriched relative to controls (Table 2 and Supplementary Fig. 1). We confirmed these by multiplex ligation-dependent probe amplification (MLPA) and investigated co-segregation in families. We identified four patients with the 16p11.2 (29.5–30.1 megabases (Mb)) deletion reported in autism and mental retardation^{2,4} (Table 2). These patients had mild developmental delay requiring special educational needs support; two had autistic spectrum behaviour. This frequency ($\sim 1\%$ of all obesity patients, $\sim 2\%$ of patients with obesity and developmental delay) is slightly higher than the frequency (0.5–1%) in published studies^{2,3,5}, possibly due to the inclusion of patients with developmental delay in addition to severe obesity in our study ($n = 143$). The literature¹⁷ suggests that 16p11.2 (29.5–30.1 Mb) deletions may be associated with being overweight⁵ and with severe obesity in one patient⁴. Two of three patients harbouring duplications in this region were reported as underweight¹⁷. However, our study design did not permit detailed investigation of the contribution of 16p11.2 (29.5–30.1 Mb) deletions to altered body weight.

A large 15q11-q13 duplication is well-known to be associated with autism^{4,18}; we found smaller, distinct 15q13.2-q13.3 duplications in two patients, which have previously been observed in controls¹⁹, as well as patients with mental retardation, autism and other phenotypes²⁰ (Table 2). We also identified two patients with a 11q22-q23 deletion overlapping minimally with a CNV associated with mental retardation^{20,21} on a single brain-expressed gene *GUCY1A2* (Table 2). Some of the CNV carriers in these studies were obese²⁰. The finding of these rare recurrent CNVs in our cohort may point to a shared mechanistic basis for these neurodevelopmental diseases. Rare CNVs encompassing brain-expressed genes such as *TSNARE1* and *PDE2A* were unique to patients with severe early-onset obesity and require further investigation.

The commonest CNV enriched in patients with severe obesity was identified in five unrelated patients harbouring deletions on 16p11.2 (Table 2) with a minimal overlapping segment of 220 kb (Fig. 1a). This 220-kb deletion (28.73–28.95 Mb) was found in 2 out of 7,366 controls (Supplementary Information); BMI data were unavailable. The minimal deleted region contains genes involved in neurological diseases (*TUFM*, *ATP2A1*), immunity (*CD19*, *NFATC2IP*, *LAT*) and genes of unknown function (*ATXN2L*, *RABEP2*,

SPNS1), as well as *SH2B1*, which encodes an adaptor protein involved in leptin and insulin signalling. Disruption of *Sh2b1* in mice results in obesity and severe insulin resistance²². As CNVs can unmask a recessive allele, we sequenced *SH2B1* in these patients but did not find any coding/splice site mutations (Supplementary Information).

The 220-kb 16p11.2 deletion (28.73–28.95 Mb) was seen in three patients and co-segregated with severe early-onset obesity alone (Fig. 1b). A longer ~1.7-Mb deletion detected in two patients (Fig. 1c) encompassed the 220-kb deletion and extended through a 593-kb region (29.5–30.1 Mb) where deletions are associated with autism and mental retardation^{2,5,17} (Fig. 1c). These longer deletions occurred *de novo*; both these patients had mild developmental delay in addition to their severe obesity. These findings are consistent with a role for the *SH2B1*-containing 220-kb region (28.73–28.95 Mb) in severe obesity and the 29.5–30.1-Mb region in brain development. We used MLPA to screen for this rare *SH2B1*-containing (28.73–28.95 Mb) deletion in an independent set of 1,062 Caucasian patients with severe obesity alone from the same cohort, as no other comparable cohort of the same ancestry and size exists. We found two additional patients with this deletion. The deletion co-segregated with obesity in one family whose samples were available (Supplementary Information). The prevalence of the *SH2B1*-containing deletion was lower in the replication cohort (2 out of 1,062) compared to the discovery set with severe obesity alone (3 out of 157). Overestimation of case frequency in the primary screen is a recognized phenomenon especially with the investigation of rarer variants²³. Overall the prevalence of the *SH2B1*-containing deletion in patients with severe early-onset obesity alone (5 out of 1,219; 0.41%) is significantly greater than in controls (2 out of 7,366; 0.027%) ($P < 0.001$).

The breakpoints of both classes of deletion are embedded within complex, segmentally duplicated regions of 16p11.2 containing directly-oriented, highly-similar (>98% sequence similarity) duplicated sequences greater than 15 kb in length (Fig. 1a). This observation strongly supports the hypothesis that these deletions arise through non-allelic homologous recombination between duplicated sequences¹⁴.

We characterized the phenotype of patients with the *SH2B1*-containing deletion. Patients rapidly gained weight in the first years of life (Fig. 2a); their excess weight was predominantly fat mass (Fig. 2b). They exhibited hyperphagia with increased *ad libitum* food intake (Fig. 2c). Fasting plasma insulin levels were disproportionately elevated compared to age- and obesity-matched controls and patients with other obesity syndromes including MC4R deficiency, where we have reported disproportionate hyperinsulinaemia¹² (Fig. 2d). Fasting plasma glucose concentrations were in the normal range. Adult patients with the *SH2B1*-containing deletion had delayed, exaggerated insulin secretion in response to an oral glucose load compared to equally obese controls (Fig. 2e).

The similarities with the human leptin-receptor-deficient phenotype are striking¹³ and consistent with a role for central disruption of SH2B1 (ref. ⁶). Deletion of peripheral *Sh2b1* in rodents impairs insulin signalling in muscle, liver and adipose tissue²⁴, which might explain the severe and disproportionate insulin resistance in these patients. As heterozygous deletion of *Sh2b1* in mice leads to obesity on a high-fat diet⁶, haploinsufficiency may be a plausible mechanism underlying the phenotype seen in humans (Supplementary Information).

Common SNPs near the *SH2B1* locus have been associated with BMI in GWAS^{10,11}. We investigated CNVs upstream or downstream of known obesity genes or other BMI-associated loci. We identified a 153-kb deletion 60 kb downstream from *MC4R* (chromosome 18: 55,975,710–56,128,721) in a single patient (Supplementary Fig. 2a), inherited from an obese father (Supplementary Fig. 3). This deletion removes a BMI

association interval²⁵ (Supplementary Fig. 2a) and its distal end partially overlaps with a CNV found in a single control (chromosome 18: 55,804,160–56,063,854). Further studies will be required to ascertain the significance of this finding. A 100-kb duplication encompassing part of *LEPR* was found in a single patient but also in 16 controls (Supplementary Fig. 2b). Although we did not find any rare CNVs close to *FTO*^{8,9}, we detected the common 45-kb deletion upstream of *NEGR1*^{10,11} at comparable prevalence in patients and controls (Supplementary Fig. 2c).

We report here the first rare CNVs associated with severe early-onset obesity. We show that deletion of 16p11.2 is associated with highly penetrant familial severe early-onset obesity and severe insulin resistance. Although the contribution of other genes or non-coding genetic material cannot be excluded, the phenotype is consistent with a role for *SH2B1* in human energy homeostasis and glucose metabolism. We did not identify any coding/splice site mutations in *SH2B1* in 500 Genetics of Obesity Study (GOOS) patients (Supplementary Information). Segmental duplications on 16p11.2 and concomitant increase in deletion rate through non-allelic homologous recombination may explain why deletions are a relatively more frequent source of loss of function in *SH2B1* than at other monogenic obesity loci. Novel CNVs unique to patients with severe early-onset obesity and the increased CNV burden (which is not fully accounted for by *SH2B1* deletions) suggest a role for additional genes in the aetiology of severe obesity. Our findings indicate the presence at the same loci of both common variants influencing susceptibility to common obesity and more highly penetrant rare variants, including CNVs, associated with severe early-onset forms of the disease. Strategies aimed at looking for rare variants near common susceptibility loci may well prove to be fruitful in other common complex diseases.

METHODS SUMMARY

A discovery set of 300 UK Caucasian patients from the Genetics of Obesity Study (GOOS) cohort were randomly selected for this study (143 had developmental delay). All patients had severe obesity defined as a BMI standard deviation score (s.d.s.) >3 and onset of obesity before 10 years of age (Supplementary Information). Mutations in *LEPR*, *POMC* and *MC4R* were excluded by direct nucleotide sequencing and a karyotype performed. DNA samples were run on Affymetrix Genome-Wide Human SNP Array 6.0 by Aros, Inc., and compared to control data on the same platform obtained on over 7,000 controls recruited from the Wellcome Trust Case Control Consortium 2 (WTCCC2) and the Genetic Association Information Network (GAIN). The WTCCC2 data set contains 6,000 individuals, 3,000 from the 1958 British Birth Cohort and 3,000 from the UK Blood Service Collection, used as common controls in genome-wide association studies of 13 disease conditions undertaken by Wellcome Trust Case Control Consortium 2 (<http://www.wtccc.org.uk/cc2>). The GAIN data set contains 1,442 individuals of European ancestry used as part of the control cohorts in genome-wide association studies of schizophrenia and bipolar disorders. The data set was obtained from the database of Genotype and Phenotype (dbGaP) found at <http://www.ncbi.nih.gov/gap> through dbGaP accession numbers phs000021.v1.p1, phs000021.v2.p1 and phs000017.v1.p1. Further methods for CNV calling, analysis and MLPA are included in the Supplementary Information. All clinical studies were conducted in accordance with the principles of the Declaration of Helsinki using methods that have previously been reported¹³.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Discovery of 16p11.2 CNV associated with severe early-onset obesity

a, Affymetrix 6.0 array data for five patients with deletions at 16p11.2 is shown. Log₂ ratios of the five samples are highlighted in dark red, with other samples in the same genotyping plate shown in grey. The structure of extensive segmental duplication that extends to the flanking regions is shown. Annotation of the segmental duplications was taken from UCSC Genome Browser and the darkness of colour coding represents sequence similarity between the duplicated pairs. Protein-coding genes are represented by dark-blue lines; *SH2B1* is highlighted in red and by blue vertical shading. The light-pink vertical shading indicates the range of a previous BMI-association signal found in two genome-wide association studies^{10, 11}; the light-grey vertical shading indicates the reported autism-associated CNV region^{2, 4, 17}. **b, c**, Pedigrees are shown for the five patients with 16p11.2 deletions. Families are numbered as in Table 2. Filled symbols represent patients with severe early-onset obesity; arrows indicate probands. Age, BMI (body mass index) and BMI s.d.s. (standard deviation score) for children are included where available. Presence (del) or absence (no del) of the 16p11.2 *SH2B1*-containing CNV is shown where known. Representative MLPA data are shown. MLPA probes for genes in the region of interest are shown. The MLPA target regions labelled as C are control probes located either on chromosome 16 but outside the deleted region or on other chromosomes (Supplementary Information). Patient MLPA traces are in red, overlaid upon the normal control MLPA traces in black. Arrows point to the deleted probes. **d**, Three probands in whom the 16p11.2 *SH2B1*-containing deletion co-segregates with severe early-onset obesity alone. **e**, Two probands harbouring larger *de novo*

16p11.2 deletions that also encompass a known autism-associated locus and are associated with developmental delay and severe early-onset obesity.

Figure 2. Metabolic phenotype of carriers of the 16p11.2 deletion

a, Weights for three boys with the 16p11.2 deletion are plotted on growth charts based on UK reference data. **b**, **c**, Percentage body fat measured by dual energy X-ray absorptiometry (DEXA) (**b**) and *ad libitum* energy intake at a test meal (adjusted for fat free mass (FFM) in kilograms) (**c**) is shown for 16p11.2 deletion carriers ($n = 5$), leptin-receptor-deficient subjects ($n = 10$) and normal weight controls ($n = 35$). Means $\pm$ s.e.m. (error bars) are indicated. **d**, Fasting plasma insulin levels for 16p11.2 deletion carriers ($n = 6$), leptin-receptor-deficient subjects ($n = 10$), MC4R-deficient subjects ($n = 35$), and age and BMI s.d.s. matched controls from the GOOS cohort ($n = 535$) are shown. Plasma insulin values were analysed after $\log_{10}$ -transformation and are presented as geometric mean (1 s.e. range) after back-transformation. **e**, Plasma insulin levels in response to a 75 g oral glucose load are shown in adult 16p11.2 deletion carriers ($n = 3$) and severely obese adult controls matched for fasting plasma insulin levels ($n = 10$) (Supplementary Information). Plasma insulin values were analysed after $\log_{10}$ -transformation and are presented as geometric mean (1 s.e. range) after back-transformation. Results were compared using unpaired *t*-tests. All *P* values were two-sided. $P < 0.05$ was considered statistically significant. * $P < 0.01$; *** $P < 0.001$.

Table 1

Global CNV burden analysis in patients with severe early-onset obesity

Samples	Type	Case rate	Case/control ratio	P value *	P value †
All patients	Losses and gains	0.2500	1.2996	0.0201	0.0433
	Losses	0.1127	2.0906	0.0005	0.0007
	Gains	0.1373	0.9917	0.4776	0.5800
Severe early-onset obesity only	Losses and gains	0.2089	1.0857	0.3150	0.3905
	Losses	0.0696	1.2917	0.2389	0.2884
	Gains	0.1392	1.0055	0.4790	0.5332
Severe early-onset obesity and developmental delay	Losses and gains	0.2937	1.5417	0.0098	0.0195
	Losses	0.1667	3.1318	0.0003	0.0001
	Gains	0.1270	0.9252	0.5701	0.6801

* Single-tailed P values derived from permutation conditioned on total number of calls per sample. Alternative hypothesis: ratio > 1.

† Single-tailed P values derived from permutation conditioned on median absolute deviation (MAD). Alternative hypothesis: ratio > 1.

Table 2

Rare recurrent CNVs found in patients with severe early-onset obesity

Locus	CNV type	Frequency		P value	Subject	Genomic coordinates	BMI s.d.s./additional features	Inheritance
		Cases	Controls					
3p11.2	Gain	3	0	5.07×10 ⁻⁵	1	89,245,197–89,343,751	3.8/–	n/a
					2	89,250,592–89,343,751	3.7/mild DD	n/a
					3	89,250,592–89,319,536	5.5/behavioural abnormalities	From obese parent
6p12.1	Loss	2	0	1.37×10 ⁻³	4	52,875,284–52,892,054	4.2/DD, dysmorphic features	n/a
					5	52,875,284–52,892,054	4.0/2	n/a
8q24.3	Gain	2	0	1.37×10 ⁻³	6	143,067,903–143,688,840	4.4/–	n/a
					7	143,268,033–143,634,461	6.0/disproportionate hyperinsulinaemia	n/a
10p15.3	Gain	2	1	4.02×10 ⁻³	8	432,207–876,909	7.1/–	n/a
					9	541,873–818,440	6.6/DD, seizures	n/a
11q22.2 ^{†‡}	Loss	2	0	3.71×10 ⁻²	10	103,489,260–106,419,349	5.9/DD	n/a
				3.71×10 ⁻²	11	105,716,030–108,818,442	5.3/DD, dysmorphic features	From obese parent
11q13.4	Gain	2	0	1.37×10 ⁻³	12	71,980,493–72,106,059	4.4/–	n/a
					13	72,013,333–72,089,312	5.2/–	From obese parent
15q13.2-q13.3 [†]	Gain	2	2	7.84×10 ⁻³	14	28,675,248–30,231,488	4.0/impaired speech, dyspraxia	n/a
					15	28,700,879–30,577,010	3.8/dyspraxia, memory impairment, DD	Found in obese sibling
16p11.2	Loss	3	2	1.37×10 ⁻⁶	16	28,731,428–28,951,376	3.8/–	From obese parent
					17	28,655,035–28,951,376	3.9/–	From obese parent
					18	28,731,428–28,951,376	6.0/–	From obese parent
16p11.2	Loss	2	0	1.37×10 ⁻³	19	28,291,978–30,085,309	4.1/DD	<i>De novo</i>
					20	28,443,702–30,099,397	3.9/DD	<i>De novo</i>
16p11.2 [†]	Loss	4	4	4.60×10 ⁻⁷	9	29,448,112–30,227,810	6.6/DD, seizures	n/a
					21	29,448,112–30,134,433	4.9/DD, speech delay	From normal weight parent
					22	29,487,535–30,134,444	3.0/DD, autistic	From obese parent

Locus	CNV type	Frequency		P value *	Subject	Genomic coordinates	BMI s.d.s./additional features	Inheritance
		Cases	Controls					
17p13.3	Gain	2	2	7.84×10 ^{−3}	23	29,487,535–30,235,818	4.0/DD, autistic	n/a
					12	2,218,774–2,259,124	4.4/−	n/a
					24	2,224,814–2,256,880	3.3/DD, syndactyly, dysmorphic features	From obese parent
22q13	Gain	2	0	1.37×10 ^{−3}	25	49,246,176–49,313,898	3.7/mild DD	n/a
					12	49,246,176–49,349,366	4.4/−	n/a

Rare recurrent and MLPA validated CNVs found in patients with severe early-onset obesity and controls are shown. Details about the CNV locus (CNV type, chromosomal location, size, inheritance pattern) are listed; genes contained within each CNV are listed in the Supplementary Information. Phenotypic information on the subjects carrying the CNV is included where available. Severity of obesity is reported as BMI standard deviation score (s.d.s.). A minus (–) indicates no additional phenotype reported. All those with developmental delay were in the subgroup of patients with ‘obesity and developmental delay’ in Table 1. DD, developmental delay; n/a, not available.

* P value derived from two-sided Fisher’s exact test.

[†] The two CNVs at this locus overlap at <50% of the sequence.

[‡] CNVs identified in previous studies (prevalence in cases and controls in these studies is provided in the Supplementary Information for comparison).