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## Copy Number Variants Are Enriched in Individuals With Early-Onset Obesity and Highlight Novel Pathogenic Pathways

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**Context:** Only a few genetic causes for childhood obesity have been identified to date. Copy number variants (CNVs) are known to contribute to obesity, both syndromic (15q11.2 deletions, Prader-Willi syndrome) and nonsyndromic (16p11.2 deletions) obesity.

**Objective:** To study the contribution of CNVs to early-onset obesity and evaluate the expression of candidate genes in subcutaneous adipose tissue.

**Design and Setting:** A case-control study in a tertiary academic center.

**Participants:** CNV analysis was performed on 90 subjects with early-onset obesity and 67 normal-weight controls. Subcutaneous adipose tissue from body mass index-discordant siblings was used for the gene expression analyses.

**Main Outcome Measures:** We used custom high-density array comparative genomic hybridization with exon resolution in 1989 genes, including all known obesity loci. The expression of candidate genes was assessed using microarray analysis of messenger RNA from subcutaneous adipose tissue.

**Results:** We identified rare CNVs in 17 subjects (19%) with obesity and 2 controls (3%). In three cases (3%), the identified variant involved a known syndromic lesion (22q11.21 duplication, 1q21.1 deletion, and 16p11.2 deletion, respectively), although the others were not known. Seven CNVs in 10 families were inherited and segregated with obesity. Expression analysis of 37 candidate genes showed discordant expression for 10 genes (*PCM1*, *EFEMP1*, *MAMLD1*, *ACP6*, *BAZ2B*, *SORBS1*, *KLF15*, *MACROD2*, *ATR*, and *MBD5*).

**Conclusions:** Rare CNVs contribute possibly pathogenic alleles to a substantial fraction of children with early-onset obesity. The involved genes might provide insights into pathogenic mechanisms and involved cellular pathways. These findings highlight the importance of CNV screening in children with early-onset obesity. (*J Clin Endocrinol Metab* 102: 3029–3039, 2017)

Obesity is a complex and common disease, affected by interacting genetic and nongenetic factors. Underlying genetic variants have been extensively studied, and mutations in single genes coding for enzymes and hormones regulating pathways of hunger control, satiety, and lipid metabolism have been identified as a plausible cause of early-onset obesity (1). Genome-wide association studies have shown >100 associations between genetic variants and the susceptibility for morbid obesity; however, together, these explain only a minor part of the variation in body mass index (BMI). Also, lifestyle factors have been shown to have a major effect on the development of morbid obesity (2–5). Several monogenic drivers of isolated early-onset obesity have been identified, emphasizing the importance of energy homeostasis (*LEP*, *LEPR*, *POMC*, *MC4R*) and cilia function (*CEP19*) (6–10). Furthermore, ciliopathy syndromes such as Bardet-Biedl syndrome [BBS; Mendelian Inheritance in Man (MIM) #209900] and Alström syndrome (MIM #203800) display obesity as a hallmark characteristic (11, 12). The underlying genetic defects in rare disorders with a predisposition to develop obesity have elucidated the cellular pathways involved in development of common obesity (13).

Several lines of evidence have indicated that copy number variants (CNVs) might also contribute to obesity. Recurrent deletions of 16p11.2 (MIM #611913) have early-onset obesity as a main characteristic, and 70% of cases of Prader-Willi syndrome (MIM #176270) is caused by a paternal deletion of 15q11-13 (14). Furthermore, deletions of 6q16, 1p36 (MIM #607872), 2q37 (MIM #600430), and 9q34 (MIM #610253) have also been linked to obesity (15–18). More recently, we have shown that the recurrent *NPHP1* deletion might be a primary driver of BBS and that exon-disrupting intragenic CNVs affecting known disease genes are important contributors to BBS (11, 19). Finally, both we, and others, have shown that a low copy number of the common polymorphic CNV affecting the *AMY1* locus, encoding salivary amylase, is associated with common obesity, early-onset female obesity, and an increased BMI in normal-weight prepubertal boys (20–22).

We performed a systematic analysis of both large CNVs and small single exon deletions and duplications in a cohort of 90 subjects with early-onset severe obesity (median BMI Z-score, +3.7) and 67 normal-weight controls (median BMI Z-score, +0.2). The findings showed an enrichment of rare CNVs in the subjects with obesity and segregation of the CNVs with obesity in several families. Second, we investigated whether genes affected by rare CNVs in our cohort have altered messenger RNA (mRNA) expression in subcutaneous adipose tissue from BMI-discordant siblings. Our analysis

indicated that rare CNVs could have a role in the development of early-onset obesity and the combined data highlight candidate loci for obesity.

## Materials and Methods

### Study subjects

The overall study was designed to assess the involvement of genetic, metabolic, and skeletal characteristics in severe childhood-onset obesity. Two cohorts, ELLU (n = 64) and EPPE (n = 26), were investigated. In addition, 67 normal-weight controls (ELLU controls) were included. The cohorts were recruited and assessed at Children's Hospital, Helsinki University Hospital (ELLU, 2011–2013) and Seinäjoki Central Hospital, Seinäjoki (EPPE, 2014–2016), Finland. Both centers are located in western Finland, where the population is genetically similar and differs from the eastern and northern parts of Finland (23–25). Therefore, and because only genetic, and not epigenetic, changes were studied, the same controls were used for both patient groups, despite the discrepant age at recruitment. The research ethics committees of the Hospital District of Helsinki and Uusimaa and the ethical board of the Pirkanmaa Hospital district approved the present study. All the participants in the study, or parents of the subjects aged <18 years, gave informed written consent.

The subjects were identified through patient registries and invited to participate. The inclusion criteria for participation were severe early-onset obesity and referral because of severe obesity to the abovementioned hospitals during early childhood. All the patients fulfilled one of the following criteria for obesity before 10 years of age: (1) height-adjusted weight >60% (median age, 16 years; range, 4 to 23); (2) median BMI Z-score at the beginning of the study, +4.0; (3) BMI greater than the 97th percentile (median age, 20 years; range, 15 to 25); (4) median BMI Z-score, +2.9, according to the Finnish growth standards; (5) BMI Z-score >+2.0 (median age, 22 years; range, 16 to 24); and/or BMI Z-score +2.7 according to World Health Organization (26, 27). The controls were selected from the same area as the ELLU cohort using the national population register.

Altogether, the study involved 90 subjects (38 females and 52 males, median age, 17 years; range 4 to 25 years) with early-onset obesity and 67 controls (36 females and 31 males, median age, 20 years; range, 15 to 25 years) of normal weight (100% ethnic Finns). Controls were excluded from the study if they had developed obesity with a height-adjusted weight >40% during childhood. All subjects included in the final study had blood samples available for DNA and serum analyses. Also, any underlying endocrine disorders had been excluded. For subjects with rare CNVs found, the parents and siblings were also tested. All cohort characteristics are presented in Table 1.

### Clinical assessment

Background data on the sociodemographic factors, parental health and age, and BMI were collected using a questionnaire. Height, weight, and waist circumference were measured during the study visit, as described previously (Table 1) (27). The participants' height and weight at the time of DNA sampling were converted to sex- and age-specific BMI Z-scores using the World Health Organization reference values (26).

**Table 1. Baseline Characteristics of Subjects**

Characteristic	EPPE	ELLU Obese Subjects	ELLU Controls
Subjects, n	26	64	67
Male, n (%)	18 (69)	34 (53)	31 (46)
Age, y	11.5 (8.8–13.5)	18.8 (16.8–21.3)	19.5 (17.4–21.7)
Weight, kg	79.1 (58.8–102.0)	120.9 (102.5–140.2)	65.8 (57–73.8)
Height, cm	154.45 (140.4–167.3)	173.5 (167.0–182.7)	173 (165–182.2)
BMI z-score			
All	3.9 (3.4–4.5)	3.6 (2.7–4.8)	0.2 (–0.4 to 0.8)
Males	3.9 (3.4–4.6)	3.1 (2.5–4.3)	0.4 (–0.5 to 1.3)
Females	3.9 (2.9–4.4)	4.1 (3.4–4.9)	0.1 (–0.3 to 0.6)
Waist circumference, cm	NI	117 <sup>a</sup> (105–130)	73 (67–80)
Siblings, n	1 <sup>b</sup> (0–2)	1 (0–2)	1 (1–2)
Maternal age at delivery, y	30 (26–34)	32 <sup>a</sup> (28–34)	30 (28–33)
Maternal current BMI, kg/m <sup>2</sup>	32.3 (27.0–40.3)	30.8 (26.7–37.4)	24.3 <sup>c</sup> (22.6–27.3)
Paternal age at delivery, y	35 (28–38)	31 <sup>d</sup> (27–35)	31 (27–35)
Paternal current BMI, kg/m <sup>2</sup>	30.4 <sup>e</sup> (26.7–33.3)	27.8 (25.1–34.6)	27.8 <sup>f</sup> (25.1–34.6)

Data presented as median (interquartile range).

Abbreviation: NI, no information.

<sup>a</sup>n = 62.

<sup>b</sup>n = 20.

<sup>c</sup>n = 63.

<sup>d</sup>n = 57.

<sup>e</sup>n = 24.

<sup>f</sup>n = 61.

### Array comparative genomic hybridization analyses

Using eArray, an online web tool for array design (Agilent Technologies, Palo Alto, CA), we created a custom array comparative genomic hybridization (aCGH) design. We used the Agilent 2×400K high-definition comparative genomic hybridization microarray (Agilent Technologies) with a total of 400,000 oligonucleotide probes, 180,000 probes evenly spaced across the genome, and the remaining probes targeting 1989 genes from the cilia proteome and/or genes involved in obesity, obesity syndromes, lipid metabolism, neuropsychiatric diseases, and intellectual disability, with 1 probe per 100 bp in coding sequences and 1 probe per 500 bp in intragenic noncoding sequences. Array slides were ordered from Oxford Gene Technology (Oxfordshire, UK). The controls used for the aCGH experiment consisted of pooled healthy individuals matched for sex (Promega, Madison, WI). In brief, 1.2 μg of samples and controls were digested using restriction enzymes *AluI* and *RsaI* (Promega), followed by labeling using Cy3 (subjects) and Cy5 (controls) using the CGH Labeling Kit for Oligo Arrays (Enzo Life Sciences, Farmingdale, NY). Hybridization solution was prepared using blocking agent (Agilent Technologies), hybridization buffer (Agilent Technologies), and Cot1 DNA (Invitrogen, Carlsbad, CA), and samples were hybridized with controls for 48 hours at 65°C. After hybridization, array slides were washed in Wash buffer 1 and 2 (Agilent Technologies) and acetonitrile (Sigma-Aldrich, St. Louis, MO). The slides were scanned using a DNA Microarray Scanner (Agilent Technologies). The scanned TIFF file was processed using Agilent Feature Extraction Software (Agilent Technologies) and analyzed using Cytosure Interpret Software, version 4.6 (Oxford Gene Technology, Oxfordshire, UK). All aberrations noted by the software were manually inspected and classified into five categories: benign, likely benign, possibly pathogenic, pathogenic, or known syndrome. Variants were classified as benign if they were found in the controls or databases of known variants, likely benign

if they were found multiple times in both controls and patients, possibly pathogenic if the variant was rare and affecting a cellular pathway of interest in obesity, pathogenic if the variant was in a known obesity predisposing gene, and known syndrome if the variant had been described in an obesity syndrome previously.

### Expression analysis in subcutaneous adipose tissue from BMI-discordant sibling pairs

To further elucidate the potential role of the genes detected in the aCGH analysis, we studied their expression in subcutaneous adipose tissue of BMI-discordant sibling pairs. Data were obtained from the Swedish Obese Subjects (SOS) Sib Pair study, consisting of 154 nuclear families with BMI-discordant sibling pairs (BMI difference ≥10 kg/m<sup>2</sup>). The entire study population consists of 732 subjects (28). The subjects were extensively phenotyped, and subcutaneous adipose tissue needle biopsies were obtained for all participants (28). Total RNA had previously been isolated from the subcutaneous adipose tissue using RNeasy Lipid Tissue Mini Kit (Qiagen, Chatsworth, CA) or the phenol-chloroform method. Gene expression was analyzed using Human Genome U133 Plus, version 2.0, arrays (Affymetrix, Santa Clara, CA). All arrays were analyzed according to the manufacturers' instructions, and expression data were analyzed using the robust multi-array algorithm (Affymetrix). Probe sets for each candidate gene are presented in Table 2 and Supplemental Table 1.

### Statistical analysis

The frequencies of rare CNVs were compared between two groups (obese and controls) using Fisher's exact test. A comparison of continuous variables between obese and controls was performed using an independent samples *t* test or, in case of a non-normal distribution, the Mann-Whitney *U* test. Differences in gene expression between siblings (obese vs normal-weight)

**Table 2. Discordant Gene Expression in 10 Candidate Genes**

Gene	U133Plus 2.0 Probe	Sib Pair Expression <i>P</i> Value (Obese vs Normal-Weight)	Obese/Normal-Weight Ratio
<i>PCM1</i>	202174_s_at	$6.26 \times 10^{-29a}$	0.78
	209996_x_at	$1.67 \times 10^{+4}$	0.89
	209997_x_at	$7.45 \times 10^{+0}$	0.88
	214118_x_at	$4.63 \times 10^{-13a}$	0.83
	214937_x_at	$8.48 \times 10^{-25a}$	0.79
	228905_at	$8.75 \times 10^{+3}$	0.96
<i>EFEMP1</i>	201842_s_at	$4.01 \times 10^{-10a}$	1.24
<i>MAMLD1</i>	201843_s_at	$3.03 \times 10^{-8a}$	1.23
	205088_at	$2.35 \times 10^{-5a}$	0.81
<i>ACP6</i>	218795_at	$2.12 \times 10^{-10a}$	0.72
<i>ATR</i>	209902_at	$3.48 \times 10^{-19a}$	1.51
	209903_s_at	$8.90 \times 10^{-17a}$	1.34
	233288_at	$1.12 \times 10^{+4}$	0.9
<i>BAZZB</i>	203080_s_at	$2.73 \times 10^{-7a}$	0.85
	217392_at	$3.18 \times 10^{+4}$	1.1
<i>SORBS1</i>	211705_s_at	$2.89 \times 10^{+3}$	0.8
	211819_s_at	$3.65 \times 10^{+3}$	0.91
	218087_s_at	$1.46 \times 10^{+4}$	0.98
	222513_s_at	$7.20 \times 10^{-5a}$	0.85
<i>KLF15</i>	221302_at	$2.27 \times 10^{+4}$	0.94
	231015_at	$1.48 \times 10^{-18a}$	0.7
<i>MACROD2</i>	1553564_at	$5.25 \times 10^{+4}$	1
	1561677_at	$1.13 \times 10^{+4}$	0.9
	1563209_a_at	$7.45 \times 10^{-6a}$	0.67
	235278_at	$2.74 \times 10^{-8a}$	0.75
<i>MBD5</i>	220195_at	$2.98 \times 10^{-4a}$	0.83
	227839_at	$1.24 \times 10^{+3}$	0.93
	244662_at	$5.04 \times 10^{+4}$	1.01

<sup>a</sup>Statistically significant.

were analyzed using a paired *t* test. Because the Human Genome U133 Plus 2.0 arrays simultaneously measures the expression of 54,675 transcripts, we applied a Bonferroni correction to the resulting *P* values to adjust for multiple testing.

## Results

### Cohort characteristics

The median BMI z-score at the time of DNA sampling for the EPPE cohort was +3.9 [interquartile range (IQR) +3.4 to +4.5] and for the ELLU cohort was +3.6 (IQR +2.7 to +4.8). The corresponding median BMI z-score for the control cohort was +0.2 (IQR -0.4 to +0.8). We also observed an indication for obesity segregation within families. At the time of inclusion, the median maternal and paternal BMI for EPPE cohort was 32.2 kg/m<sup>2</sup> and 30.4 kg/m<sup>2</sup> and for the ELLU cohort was 30.8 kg/m<sup>2</sup> and 27.8 kg/m<sup>2</sup>, respectively. The corresponding values in the control cohort were 24.3 kg/m<sup>2</sup> and 27.8 kg/m<sup>2</sup>. The cohorts' characteristics are presented in Tables 1 and 3.

### Identification of rare CNVs in subjects with obesity and normal-weight controls

The data from 90 subjects with early-onset obesity and 67 normal-weight controls were analyzed for rare CNVs

using the custom aCGH. First, we analyzed 64 subjects with obesity from the ELLU cohort and identified 12 who harbored rare CNVs (19%). This was an enrichment compared with the control subjects (*n* = 67), in whom we identified two rare CNVs (3%; *P* < 0.01, Fisher's exact test). To confirm these results, we used a second smaller cohort of obese children (EPPE). The data from 26 EPPE subjects were analyzed, and five rare CNVs were identified (19%). This was substantial compared with the results from the ELLU controls (3%) analyzed previously (*P* = 0.02, Fisher's exact test). The combined results from the ELLU and EPPE cohorts showed 10 duplications and 9 deletions in 17 subjects with obesity and 2 deletions in 2 controls (19% vs 3% in subjects with obesity and normal-weight controls, respectively; *P* < 0.01, Fisher's exact test; Supplemental Fig. 1; Tables 3 and 4). Finally, one individual in the control cohort had trisomy X, which was not considered a rare CNV. Two subjects with obesity harbored two CNVs (ELLU106 and EPPE14; Table 4). The size of the CNVs ranged from 2 kb to 2.7 Mb, with six CNVs (32%) >500 kb (Table 4). CNVs were similarly present in both sexes (*P* = 0.7, Fisher's exact test). In obese males, we identified 11 rare CNVs (19%) but none in the control males (*P* < 0.001, Fisher's exact test). In the obese and control females, we found six

**Table 3. Characteristics of Study Subjects With CNVs**

Participant	Sample ID	Sex	Age, y	BMI Z-Score	Additional Phenotype/Comments
Subject	ELLU018	Male	21	3.3	
Subject	ELLU021	Female	18	6	
Subject	ELLU023	Male	19	5.8	Dysphasia
Subject	ELLU036	Female	16	4.8	Learning disability
Subject	ELLU040	Female	19	3.5	
Subject	ELLU059	Female	20	5.9	Hydrocephalus
Subject	ELLU101	Male	16	3	
Subject	ELLU106	Female	22	4.9	
Subject	ELLU107	Male	16	5.3	
Subject	ELLU111	Male	17	3.6	
Subject	ELLU206	Female	16	2.9	
Subject	ELLU210	Male	24	4.3	Learning disability
Subject	EPPE9	Male	13	2.8	
Subject	EPPE14	Male	13	3.3	Legg-Calve-Perthes disease
Subject	EPPE21	Male	16	4.6	
Subject	EPPE26	Male	11	2.8	Type 1 diabetes
Subject	EPPE28	Male	9	3.7	Asthma
Control	ELLU072	Female	20	-0.3	
Control	ELLU120	Female	18	0.8	

Abbreviation: ID, identification.

(16%) and two (6%), respectively ( $P = 0.03$ , Fisher's exact test). Maternal and paternal age and BMI did not differ between those with and without CNVs ( $P > 0.4$ , Mann-Whitney  $U$  test). In addition, subjects with CNVs had a higher BMI z-score compared with the whole cohort (+3.6 vs +2.0;  $P < 0.001$ , Mann-Whitney  $U$  test). However, the difference became nonsignificant when the control subjects were excluded (+4.1 vs +3.7;  $P = 0.2$ , Mann-Whitney  $U$  test).

Of the total 19 rare CNVs detected among the subjects with obesity, 3 were considered pathogenic and clinically important: 1q21.1 deletion (MIM #612474; ELLU106), 22q11.21 duplication (MIM #608263; ELLU018), and 16p11.2 deletion (MIM #613444; ELLU023). The remaining 16 CNVs involved 37 genes. Five had been previously linked to obesity or related disorders or pathways (*EFEMP1*, *PCM1*, *MCTP2*, *SORCS1*, and *ACE*) and were classified as possibly pathogenic (29–33). The remaining loci had not previously been linked to obesity or related disorders or pathways and were classified as being of unknown significance (Table 4).

### Segregation of rare CNVs in families

Segregation analysis of 12 CNVs in 10 unrelated families revealed that 8 CNVs in seven cases were inherited and segregated with obesity (Fig. 1; Table 4). Of the remaining two pedigrees, ELLU106 harbored two CNVs that had arisen *de novo*, and for ELLU111, no paternal sample was available for analysis and the CNV had not been inherited from the nonobese mother. DNA was not available for segregation analysis for seven families.

### Candidate obesity genes show different expression levels in BMI-discordant siblings

All protein coding candidate genes affected by deletions and duplications in our cohort (37 genes) were expressed in subcutaneous adipose tissue (Table 2 and Supplemental Table 1). Ten genes showed discordant expression in sibling pairs from the SOS Sib Pair study. Nine (*PCM1*, *EFEMP1*, *MAMLD1*, *ACP6*, *BAZ2B*, *SORBS1*, *KLF15*, *MACROD2* and *MBD5*) had lower expression in subcutaneous adipose tissue from the siblings with obesity, and one (*ATR*) had higher expression compared with the expression in subcutaneous adipose tissue from normal-weight siblings (Table 2), highlighting several interesting pathways, such as gene transcription, gene expression, and ciliogenesis.

### Discussion

We performed a systematic screening for genomic deletions and duplications in subjects with severe childhood-onset obesity and compared them with the results from normal-weight controls. Rare CNVs were significantly more prevalent in the subjects with obesity (17 of 90; 19%) than in the controls (2 of 67; 3%). In a subset of the cohort, the identified CNVs were segregated to those overweight or obese in the family.

In three subjects with obesity, the identified CNVs were classified as pathogenic. Two of these were known syndromes previously described in the published data. ELLU023, who had presented with early-onset obesity and dysphasia, harbored a deletion on chromosome 16p11.2. The 16p11.2 region is a known BMI

**Table 4. Rare CNVs Identified in 17 Obese Individuals and 2 Normal-Weight Controls**

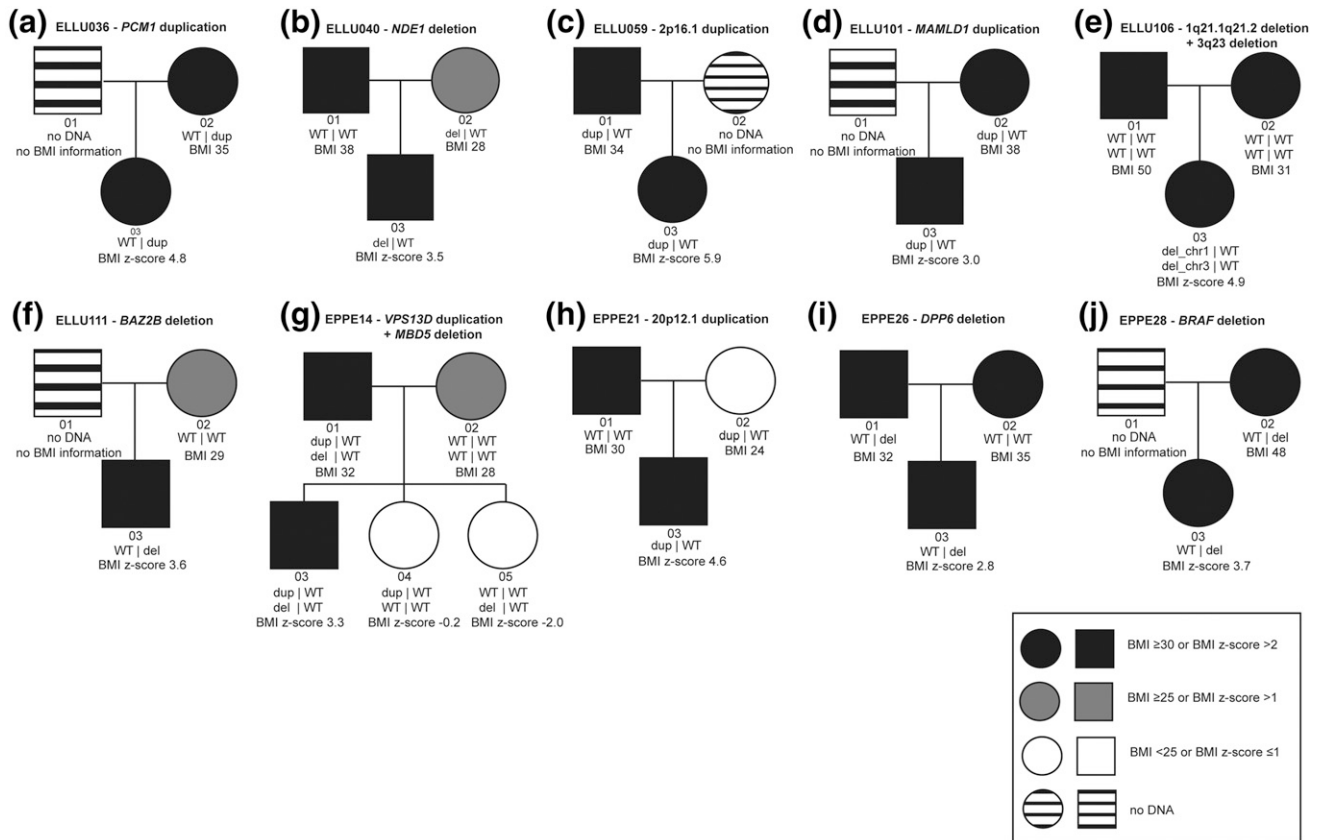
Sample ID	Array Result	Inheritance	Hg19 CNV Coordinates	Size (bp)	Affected Genes
ELLU018	Duplication	NI	22q11.21 (18,787,913-21,505,425)	2717512	<i>DGCR6, PRODH, DGCR5, DGCR2, DGCR14, TSSK2, GSC2, SLC25A1, CLTCL1, HIRA, C22orf39, MRPL40, UFD1L, CDC45, CLDN5, SEPT5, GP1BB, TBX1, GNB1L, C22orf29, TXNRD2, COMT, MIR4761, ARVCF, TANGO2, MIR185, DGCR8, MIR3618, MIR1306, TRMT2A, RANBP1, ZDHH8, RTN4R, MIR1286, DGCR6L, GGTLC3, RIMBP3, FAM230A, USP41, ZNF74, SCARF2, KLHL22, MED15, SLC9A3P2, BCRP5, TMEM191A, PI4KA, SERPIND1, SNAP29, CRKL, AIFM3, LZTR1, THAP7, SLC7A4, MIR649</i>
ELLU021	Duplication	NI	10q25.1 (107,170,743-108,506,663)	1335920	<i>SORCS1</i> (exon 6-27)
ELLU023	Heterozygous deletion	NI	16p11.2 (29,656,657-30,097,178)	440521	<i>SPN, QPRT, C16orf54, ZG16, KIF22, MAZ, PRRT2, PAGR1, MVP, CDIPT, SEZ6L2, ASPHD1, KCTD13, TMEM219, TAOK2, HIRIP3, INO80E, DOC2A, C16orf92, FAM57B, ALDOA, PPP4C, TBX6</i>
ELLU036	Triplication	Maternal (duplication)	8p22 (17,884,140-17,886,229)	2089	<i>PCM1</i> (exon 39)
ELLU040	Heterozygous deletion	Maternal	16p13.11 (15,764,120-15,777,066)	12946	<i>NDE1</i> (exon 5)
ELLU059	Duplication	Paternal	2p16.1 (56,088,362-56,426,697)	338335	<i>EFEMP1, MIR217, MIR216A, MIR216B, CCDC85A</i> (exon 1-2)
ELLU101	Duplication	Maternal	Xq28 (149,386,489-149,687,251)	300762	<i>MAMLD1</i>
ELLU106	Heterozygous deletion	<i>De novo</i>	1q21.1q21.2 (146,518,335-147,278,575)	760240	<i>PRKAB2, PDIA3P, FMO5, CHD1L, BCL9, ACP6, GJA5</i>
	Heterozygous deletion	<i>De novo</i>	3q23 (142,083,562-142,403,283)	320141	<i>XRN1</i> (exon 1-15), <i>ATR, PLS1</i>
ELLU107	Duplication	NI	17q23.3 (61,560,001-61,580,397)	20396	<i>ACE</i> (exon 8-25)
ELLU111	Heterozygous deletion	Not maternal	2q24.2 (160,457,207-160,556,234)	99027	<i>BAZ2B</i> (exon 1)
ELLU206	Duplication	NI	13q14.3 (52,344,911-52,439,400)	94489	<i>DHRS12</i> (exon 1-8), <i>CCDC70</i> (exon 1)
ELLU210	Duplication	NI	15q26.1q26.2 (93,800,851-95,943,789)	2142938	<i>MCTP2</i>
EPPE9	Heterozygous deletion	NI	3q21.3 (126,023,706-126,159,938)	136232	<i>KLF15, CCDC37, ZXDC</i> (exon 9-10)
EPPE14	Duplication	Paternal	1p36.22p36.21 (12,336,117-12,839,474)	503357	<i>VPS13D</i> (exon 19-70)
	Heterozygous deletion	Paternal	2q23.1 (149,079,137-149,159,043)	79906	<i>MBD5</i> (exon 5)
EPPE21	Duplication	Maternal	20p12.1 (13,243,375-15,453,152)	2209777	<i>ISM1</i> (exon 2-6), <i>TASP1, ESF1, C20orf7, SEL1L2, FLRT3, MACROD2</i> (exon 1-7)
EPPE26	Heterozygous deletion	Paternal	7q36.2 (154,423,461-154,585,594)	162133	<i>DPP6</i> (exon 6-10)
EPPE28	Heterozygous deletion	Maternal	7q34 (140,471,334-140,534,535)	63201	<i>BRAF</i> (exon 4-13)
ELLU072 (control)	Heterozygous deletion	NI	16p13.13 (12,281,891-12,344,218)	62327	<i>SNX29</i> (exon 14)
ELLU120 (control)	Heterozygous deletion	NI	10q24.1 (97,145,024-97,155,627)	10603	<i>SORBS1</i> (exon 12-3)

Abbreviation: NI, no information.

quantitative trait locus and a neuropsychiatric disorder susceptibility locus (34). ELLU018 harbored a 2.6-Mb duplication at chromosome 22q11. One of the rare reported phenotypes in 22q11 duplication syndrome is increased bodyweight, although because of the extreme variability in phenotypes, the true pathogenicity remains unclear (35). Finally, in ELLU106, who had isolated severe early-onset obesity (BMI 48 kg/m<sup>2</sup>), the CNV analysis detected two *de novo* deletions affecting a total of seven genes on 1q21.1 and three genes on 3q23. Individuals with larger deletions of 1q21.1 often show intellectual disability, cardiac abnormalities, developmental delay, and, rarely, obesity (36). Further

studies are necessary to pinpoint the specific genes driving obesity in this case, but two strong candidate genes were discordantly expressed in the subcutaneous adipose tissue from BMI-discordant siblings, with lower *ACP6* and higher *ATR* expression in the subjects with obesity.

For the remaining 14 cases, both the pathogenicity of the CNV and the specific genes within the CNV possibly driving the development of early-onset severe obesity are still unclear. However, five of the identified genes (*EFEMP1, PCM1, SORCS1, ACE, and MCTP2*) have previously been associated with obesity or cilia pathways in humans or animal models (29–33).



**Figure 1.** (a–j) Segregation analysis of 12 CNVs in 10 families. DNA samples from 15 parents and 2 siblings from 10 families were available for segregation analysis. The analysis revealed nine CNVs in eight subjects were inherited and eight CNVs in seven subjects segregated with the obesity phenotype. Two CNVs in one subject (ELLU106) were *de novo*. The CNV in ELLU111 only had one parental sample available, and the inheritance could not be established. The BMI z-score is presented for the index subjects and siblings. The current BMI at the inclusion of the severely obese child is shown for the parents.

First, *EFEMP1*, which was duplicated in case ELLU059 and the father with obesity (BMI 34 kg/m<sup>2</sup>) showed significantly lowered expression in the subcutaneous adipose tissue from obese individuals in the SOS Sib Pair gene expression analysis. Normal variants in *EFEMP1* have been associated with waist circumference and overall body size in East Asians, making it a strong candidate gene for further study (33).

Second, individual ELLU036 harbored a triplication of exon 39 of *PCM1*. The mother with obesity (BMI 35 kg/m<sup>2</sup>), carried a duplication with the same coordinates. *PCM1* is known to be involved in cilia function and is required for recruitment of BBS proteins to the cilium (32). BBS is one of several ciliopathies presenting with obesity, supporting the idea that *PCM1* is a possible candidate gene in isolated obesity. Finally, in the expression data we have presented, *PCM1* showed significantly lower levels in subcutaneous adipose tissue from individuals with obesity compared with the expression in their normal-weight siblings.

Three more candidate genes were affected by duplications in our cohort, *SORCS1* (ELLU021), *ACE* (ELLU107), and *MCTP2* (ELLU210). *SORCS1* has been identified in mice to be highly involved in obesity-related diabetes

mellitus type 2. *Sorcs1* underlies the diabetes mellitus type 2 locus (*T2dm2*) and seems strongly to regulate the development of diabetes mellitus type 2 in obese mice. However, no human studies have been performed (30). *ACE* was previously associated with a high BMI and waist circumference in obese and normal-weight Egyptian females, and *MCTP2* has previously been linked to adiposity in a group of 707 subjects with obesity from the Quebec Family Study (29, 31). Although all three genes are expressed in the subcutaneous adipose tissue, they were not expressed differently in the BMI-discordant siblings, indicating that tissues than other adipose tissue might be involved.

Finally, 20 genes with no previous connection to obesity were affected by rare CNVs in the subjects with obesity. Of these, *KLF15*, *MAMLD1*, *MACROD2*, and *MBD5* showed significantly lower expression in the subcutaneous adipose tissue from the obese subjects compared with their normal-weight siblings. All four genes are involved in different cellular functions: transcription regulation (*KLF15*), transcriptional coactivation (*MAMLD1*), deacetylation (*MACROD2*), and chromatin binding (*MBD5*). The pathogenic factors underlying childhood obesity might involve several different cellular

pathways, and our results could not prove or exclude any of the genes. Because all four genes that showed discordant expression between the lean and obese siblings have important roles in the regulation of gene expression and transcription, gene expression studies in the CNV carriers could be highly informative. With RNA sequencing of adipose tissue from these individuals, we could learn more about the genes and cellular pathways that are affected and possibly involved in the obesity-generating mechanisms.

Finally, we observed a deletion of seven exons in *MACROD2* in individual EPPE21. Vuillaume *et al.* (37) previously found the same deletion in 2 of 100 investigated obese children. Both in our subject (EPPE21) and in the previous study, the CNVs were inherited from a normal-weight mother. This could be interpreted that this particular gene does not contribute to the obesity phenotype or that the CNV is interacting with an undetected genetic variant in cis or trans with the deletion. Considering the gene expression results from the present study, sequence variants in *MACROD2* could be of interest in further studies.

The rare CNVs identified in our study affected many different genes, and only two genes belonged to the same pathway (vacuolar protein sorting family; *SORCS1* and *VPS13D*). Genes from the vacuolar protein sorting family are highly interesting and have been associated previously with the obese phenotype. Individuals with Cohen syndrome (MIM #216550), caused by mutations in the *VPS13B* gene, have, in addition to congenital neutropenia, retinopathy, and intellectual deficiency, an increased risk of truncal obesity and insulin resistance (38). Another cellular system of high interest is the primary cilia, and impaired cilia function has been connected to both syndromic and nonsyndromic obesity in both human patients and rodents (9, 10, 39). In the present study, four affected genes were suggested or known to be involved in ciliogenesis (*PCM1*, *ATR*, *NDE1*, and *EFEMP1*).

The parental origin and family segregation of the identified CNVs was possible in 10 families (in 1 family, only the maternal sample was available). Eight CNVs in seven subjects segregated with obesity or overweight and two CNVs in ELLU106 were *de novo*. However, obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) or overweight (BMI  $\geq 25$  kg/m<sup>2</sup>) was present in both parents, complicating the interpretation of the segregation analysis.

In the control group, we identified two rare CNVs and one case of trisomy X (ELLU008). The vast majority of females with trisomy X are never diagnosed because of the very light to no phenotype of the syndrome, hence explaining the accidental inclusion of this female in the control group (40).

The present study was limited by the small sample size and lack of an independent replication study. However,

hypothesizing that genetic early-onset obesity is heterogeneous, possibly involving several rare genetic components, traditional power calculations were not applicable. We sought to identify high-impact rare variants in carefully characterized individuals and then perform segregation analyses and expression analysis. Our aim was to highlight candidate genes for further study in larger cohorts, and, despite the small cohorts, our study was successful. However, replication studies are needed with larger cohorts and of other populations to confirm our findings. Another limitation of the study was the lack of an independent control cohort for the EPPE subjects. However, owing to the known genetic structure of the Finnish population and genetic homogeneity of Western Finland, where the EPPE, ELLU, and control cohorts were recruited, the lack of independent EPPE controls was less likely to affect the results of the present study.

## Conclusions

The amount of rare CNVs in the cohort with early-onset severe obesity was significantly greater than that in the control cohort. This supports our hypothesis that CNVs could be important contributors to isolated obesity. Our findings also suggest that genome-wide CNV screening, in addition to single gene analysis, would increase the diagnostic rate in children with early-onset obesity. Further studies are needed to explore the role of the identified candidate genes in the development of obesity, in particular, the genes involved in vacuolar protein sorting and ciliogenesis.

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