Copy number variant analysis and expression profiling of the olfactory receptor-rich 11q11 region in obesity predisposition

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Abstract

Genome-wide copy number surveys associated chromosome 11q11 with obesity. As this is an olfactory receptor-rich region, we hypothesize that genetic variation in olfactory receptor genes might be implicated in the pathogenesis of obesity. Multiplex Amplicon Quantification analysis was applied to screen for copy number variants at chromosome 11q11 in 627 patients with obesity and 330 healthy-weight individuals. A ≥ 80 kb deletion with an internally 1.3 kb retained segment was identified, covering the three olfactory receptor genes OR4C11, OR4P4, and OR4S2. A significant increase in copy number loss(es) was perceived in our patient cohort (MAF = 27%; p = 0.02). Gene expression profiling in metabolic relevant tissues was performed to evaluate the functional impact of the obesity susceptible locus. All three 11q11 genes were present in visceral and subcutaneous adipose tissue while no expression was perceived in the liver. These results support the 'metabolic system' hypothesis and imply that gene disruption of OR4C11, OR4P4, and OR4S2 will negatively influence energy metabolism, ultimately leading to fat accumulation and obesity. Our study thus demonstrates a role for structural variation within olfactory receptor-rich regions in complex diseases and defines the 11q11 deletion as a risk factor for obesity.

1. Introduction

Obesity is a complex heterogeneous disorder in which body fat has accumulated as a result of the chronic imbalance in energy homeostasis. Although excessive food intake and lack of physical activity are perceived as the major contributors, heritability studies revealed that 40–70% of the interindividual variability in body mass index (BMI) is attributed to genetic factors [1–3]. Extensive search for genes involved in body weight regulation led to the recognition of the leptin-melanocortin signaling pathway as a key regulator of food intake and energy expenditure [4]. Mutations in a number of genes from this pathway have been proven to be responsible for early-onset monogenic obesity [5]. Nevertheless, obesity in most individuals has a complex etiology [6]. As these findings only partially explain the heritability estimates for BMI, it warrants the need to examine other forms of genetic variation.

Copy number variants (CNVs) have been predicted to play a significant role in the genetic susceptibility of human disease [7–9]. They are defined as DNA segments ranging in size from 1 kb to several Mb and present themselves as variable copy numbers across individuals. To explore the contribution of CNVs to obesity, genome-wide surveys have been performed in patient populations (Table 1). In 2009, Sha et al. [10] associated a 194 kb copy number variable region (CNVR) at chromosome 10q11.22 with BMI. The region spans four genes of which the neuropeptide Y4 receptor (NPY4R) is acknowledged as an important regulatory gene in food intake. Statistical analysis revealed that 1.6% of the estimated BMI variation could be explained by the CNVR. This was later supported by the study of Aerts et al. (2016), which showed an essential role for genetic and structural NPY4R variation in the pathogenesis of obesity [11]. Furthermore, a meta-analysis by the GIANT Consortium revealed a 10 kb and a 45 kb CNV upstream of neuronal...
growth regulator (NEGR1). Both deleted regions affect non-overlapping conserved elements present at the locus [12]. NEGR1 is expressed in the brain and hypothalamus, which are implicated in central nervous system processes of body weight regulation. Although its function in the pathogenesis of obesity still has to be determined, the 1p31.1 deletion has been established as a CNVR regulating energy balance [13,14]. In 2010, Bochukova et al. identified a few rare CNVs with a size range of 220 kb to 1.7 MB at chromosome 16p11.2. Different genes were reported for the deleted regions but always contained Src homology 2B adaptor protein 1 (SH2B1) [15]. The gene is part of the leptin-melanocortin pathway and involved in leptin and insulin signaling. Subjects carrying the 16p11.2 deletion exhibit hyperphagia and severe insulin resistance. In 2011, a genome-wide CNV analysis associated a 80 kb deletion at chromosome 11q11 with early-onset extreme obesity [16]. The CNVR represents a locus enclosing the three olfactory receptor genes olfactory receptor family 4 subfamily P member 4 (OR4P4), subfamily S member 2 (OR4S2) and subfamily C member 6 (OR4C6). While the phenotypic diversity in olfactory receptors (ORs) by copy number variability is well-known [17–19], Jarick et al. (2011) were the first to link the CNVR 11q11 with early-onset extreme obesity.

ORs are generally known for their function in odor recognition. They interact with odorants in the nasal cavity to initiate a neural cascade resulting in the perception of smell [20]. In this way, odor signals can act as a sensor of the metabolic state with the intention of influencing appetite and satiety in humans [21]. The interindividual differences perceived in food intake behavior could be explained by genetic variation in OR genes [22,23]. These findings support the idea for a possible link between chemosensation and obesity. Nevertheless, deep sequencing revealed that these G-protein coupled receptors are also expressed in non-olfactory tissues where they exert diverse functions beyond chemosensation [24–27]. A recent study by Wu et al. (2017) determined the possible function of an ectopically expressed OR in cellular energy metabolism and obesity. They discovered that mouse olfactory receptor 544 (Olfr544 - human homologue OR52K1) is highly expressed in the two major metabolic tissues; activation of the receptor stimulates lipolysis in adipocytes and induces fatty acid oxidation and ketogenesis in the liver. Another study by Giusepponi et al. (2018) analyzed gene expression of olfactory receptor family 6 subfamily C member 3 (OR6C3) in human adipose tissue samples of various body weight. They observed significantly lower OR6C3 expression in subjects with obesity compared to normal-weight individuals. Both studies imply an antiobesogenic effect of OR genes where disruption of their function will result in body fat accumulation.

Although it is not known whether OR genes can have a direct influence on appetite control or energy homeostasis, the OR-rich 11q11 CNV has previously been recognized as an interesting region for obesity [16,30]. Further comprehensive investigation of this CNV might offer novel insights into the genetic architecture of obesity (missing heritability) and can help in revealing new disease mechanisms. In this respect, our objective was to examine the association of the 11q11 CNV candidate region in a Caucasian population of children and adults with obesity. Structural variation screening and expression profiling in metabolic relevant tissues were performed to determine susceptibility for disease.

2. Materials and methods

2.1. Structural variation screening of the olfactory receptor-rich 11q11 region

2.1.1. Study population

A total of 627 patients with obesity (324 children and 303 adults) and 330 normal-weight adults were included for structural variation screening in our study (Table 2). The pediatric patient cohort consists of unrelated children and adolescents (age ≥ 12 years) with obesity that were recruited at the Obesity Clinic for Children from the Antwerp University Hospital (Antwerp) and Jessa Hospital (Hasselt) in Belgium. The adult patient cohort consists of unrelated adults (age ≥ 18 years) with obesity that were recruited at the Obesity Clinic from the Antwerp University Hospital (Antwerp) in Belgium. Patients with mutations in the Melanocortin-4 receptor gene, the most common cause of monogenic obesity, have been excluded from the screening samples. The control population includes normal-weight adults of Caucasian origin recruited among employees from the Antwerp University Hospital and the University of Antwerp as well as among couples seeking prenatal counselling at the Centre of Medical Genetics (due to increased triple test or high maternal age). Couples seeking prenatal genetic counselling because of familial disease history were excluded. All subjects gave their written informed consent and parental permission was provided in case children participated. The study protocol was approved by the local ethics committee (Medical Ethics committee UAntwerp - registration number A04 21) and performed according to the Declaration of Helsinki.

2.1.2. Anthropometry

Weight was measured on a digital scale to the nearest 0.01–0.2 kg

| Locus | Position (Mb) | Size (kb) | Overlap Genes | Regulatory pathways |
|-------|---------------|-----------|----------------|---------------------|
| 1p31.1 | Chr1: 72,541,074-72,583,749 | 42.7 | NEGR1 | Feeding behavior |
| 10q11.22 | Chr10: 46,943,377-47,136,996 | 193.6 | SYT15, GPRIN2, NPY4R, LOC2872643 | Locomotory behavior, Neuron project development, Pancreatic polypeptide receptor signaling, GPCR signaling, Olfactory signaling pathway, GPCR signaling, Intracellular signal transduction |
| 11q1 | Chr11: 55,374,020-55,453,589 | 79.6 | OR4P4, OR4S2, OR4C6 | |
| 16p11.2 | Chr16: 28,823,927-29,043,875 | 220.0 | ATXN21, TUFM, SH2B1, ATP2A1, RABEP2, CD19, NFATC2IP, SPNS1, LAT | |

Genes responsible for association of CNV with obesity pathogenesis are underlined.

Chromosomal locations shown in genome build GRCh37/hg19.

Table 1 Overview of the most important BMI-associated copy number variants.

| Locus | Position (Mb) | Size (kb) | Overlap Genes | Regulatory pathways |
|-------|---------------|-----------|----------------|---------------------|
| 16p11.2 Chr16: 28,823,927-29,043,875 | 220.0 | ATXN21, TUFM, SH2B1, ATP2A1, RABEP2, CD19, NFATC2IP, SPNS1, LAT | Intracellular signal transduction |

Table 2 Population characteristics.

|                     | Patients with obesity | Healthy-weight adults |
|---------------------|-----------------------|-----------------------|
|                    | Children | Adults | Children | Adults |
| N                   | 324      | 303     | 330      |        |
| Male (n)            | 140      | 81      | 136      |        |
| Female (n)          | 184      | 222     | 194      |        |
| Age (years)         | 12 ± 0.23 | 41 ± 0.74 | 34 ± 0.32 |        |
| Weight (kg)         | 72.3 ± 2.26 | 113.9 ± 1.13 | 65.8 ± 0.54 |        |
| Height (m)          | 1.49 ± 0.018 | 1.69 ± 0.005 | 1.73 ± 0.005 |        |
| BMI (kg/m²)         | 31.57 ± 0.40 | 29.87 ± 0.32 | 21.85 ± 0.10 |        |
| BMI Z-score         | 2.67 ± 0.03 | N.A.     | N.A.     |        |

Mean value ± standard error of the mean is shown for all parameters, except N and gender distribution (absolute numbers). N.A.: not applicable.
whereas height was measured to the nearest 0.1–0.5 cm, respectively for children and adults. The BMI was calculated for all individuals as weight (in kg) divided by height (in m) squared. For adults, BMI cut-off values were applied as defined by the World Health Organization [31]. Only patients with a BMI ≥ 30 were included while controls were excluded in case 18.5 < BMI ≥ 25. Children with obesity were identified by the use of the Flemish Growth Charts 2004 [32,33]. Per centile lines that cross a BMI of 30 kg/m² at 18 years of age on the Flemish age- and sex-specific BMI growth curves were used as cut-off values for the diagnosis of obesity. BMI Z-scores were calculated based on data depicted from the Flemish Growth Charts 2004.

2.1.3. CNV analysis by multiplex amplicon quantification

Copy number changes in the genomic region of interest were detected by the use of Multiplex Amplicon Quantification (MAQ), which has been recognized as a valuable diagnostic tool with an assay performance approaching 100% [34]. The technique involves the simultaneous amplification of several fluorescently labelled target and reference amplicons, followed by capillary electrophoresis and fragment analysis.

Genomic DNA was extracted from blood samples for all patient and control samples. The target region was set at chr11:55,300,000-55,700,000 (genome build GRCh37/hg19) and includes the previously reported OR-rich CNVR of Jarick et al. (2011) as well as ten additional OR genes. Primer pairs were designed with the MAQ primer design tool and are available upon request (Supplementary Fig. 1). MAQ-assays were performed with a total of 50 ng input DNA and following the manufactured protocol (Agilent Technologies, Antwerp, Belgium). Two negative control samples were included in each experiment for accurate normalization.

The resulting MAQ-PCR products were analyzed by capillary electrophoresis on an ABI Prism Genetic Analyzer 3130xl (Applied Biosystems Inc., Foster City, CA, USA). Generated raw data were sized relative to the GS500 ROX internal-lane size standard and target amplicons were scored using Genemarker software V2.6.4 (SoftGenetics LLC., Oakwood, PA, USA). This software computes and visualizes the dosage quotient (DQ) by comparing the intensities of the target and reference amplicons in the test individual with those in the experimental control. A DQ of 0.25–0.75 was considered indicative of a deletion while a DQ of 1.25–1.75 was indicative of a duplication.

2.1.4. Characterization of the olfactory receptor-rich 11q11 deletion

OR genes involved in the 11q11 deletion were confirmed by Sanger sequencing (Genbank accession nos. AB065774, AB065775, and BK004390). Gene-specific primers were designed using Primer3 software and are available upon request (genome build GRCh37/hg19). Polymerase chain reactions (PCR) were carried out under standard conditions followed by direct sequencing of the purified PCR product on an ABI Prism Genetic Analyser 3130xl (Applied Biosystems Inc., Foster City, CA, USA). Sequences were analyzed using CLC DNA workbench (CLC Bio, Aarhus, Denmark).

2.2. Olfactory receptor expression profiling of 11q11 genes in metabolic relevant tissues

Qualitative gene expression of relevant 11q11 ORs was investigated in adult human liver and adipose tissue from three biological replicates. Total RNA from cells and tissues of specimens affected by obesity was isolated using the Quick-RNA™ Microprep kit (Zymo Research, Irvine, CA, USA) for liver and RNeasy® Lipid Tissue kit (Qiagen, Hilden, Germany) for visceral and subcutaneous adipose tissue. cDNA was synthesized with 0.5–3 μg input RNA using SuperScript® III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA). Amplification was performed by a touchdown PCR protocol under standard conditions. The presence of OR expression in both tissues was examined by visualization after gel electrophoresis. The housekeeping gene actin beta (ACTB) was included as internal control. Gene-specific cDNA primers were designed using Primer3 software and are available upon request (genome build GRCh37/hg19).

Supplementary, expression in the olfactory epithelium and metabolic relevant tissues was assessed by browsing the Genotype-Tissue Expression (GTEx) Portal and available liver transcriptome data.

2.3. Statistics

The power of the current study was estimated using the genetic power calculator [35]. Assuming a prevalence of the disease of 14% and a disease allele frequency of 25%, the current study design with 627 cases and 330 controls holds 80% power to detect a disease allele with a genotype relative risk of 1.31 under an additive model, at a significance level of 0.05.

The genotypic probability of disease between patients with obesity and lean adults was statistically evaluated by the Cochran-Armitage trend test. The effect of genotype on BMI was assessed by simple linear regression in case BMI was treated as continuous variable while a Chi-square Test was applied when the different BMI categories (mild – moderate – extreme obesity) were used for analysis. Significance level was set at $p = 0.05$. All statistical analyses were performed using Rstudio.

3. Results

3.1. Structural variation screening of the olfactory receptor-rich 11q11 region

3.1.1. Common deletion at chromosome 11 associated with obesity

Nine hundred fifty-seven individuals were screened for copy number changes in the 11q11 region. A ± 80 kb deletion with an internally 1.3 kb retained segment was identified. We observed 42.57% heterozygotes and 5.28% homozygotes for the deletion in the adult patient cohort. For the children and adolescents with obesity, heterozygous and homozygous deletion carriers were observed in respectively 35.19% and 9.57%. Natural variation at CNVR 11q11 was considered in controls and identified 33.33% as carriers of heterozygous deletions and 5.76% as carriers of homozygous deletions (Fig. 1). No duplications in the OR-rich region were perceived among subjects with obesity nor in individuals with a healthy weight.

Significant differences in genotype frequency (Table 3) were perceived for age-matched patients with obesity versus controls ($p = 0.041$, odds ratio = 1.25 [0.97–1.62]) as well as for not age-matched patients with obesity versus controls ($p = 0.028$, odds ratio = 1.29 [1.00–1.59]). These consistent findings allowed us to take both patient groups together, which resulted in an even higher significance level and effect size ($p = 0.018$). The calculated odds ratio of 1.27 [1.02–1.59] indicates an increased prevalence of the deletion in our patient cohort (MAF = 26.87%) compared to our control population (MAF = 22.42%). No significant difference between both patient groups (children versus adults) could be assigned in respect to the 11q11 deletion frequency ($p = 0.41$, odds ratio = 1.03 [0.80–1.32]); neither a correlation between BMI and genotype could be recognized when BMI was treated as a continuous variable ($p = 0.19$), nor when the different BMI categories were assumed ($p = 0.07$).

3.1.2. Fine-mapping results in characterization of the involved OR genes

Our detailed MAQ design led to fine-mapping of the 11q11 CNV (Supplementary Fig. 1); absence of MAQ probes 5–9 and 12–13 was perceived while probes 1–4, 10–11 and 14–16 were present in CNV-carriers. This corresponded to a minimal size deletion of ± 80 kb spanning chr11:55,368,225-55,448,559 and a maximum size deletion of ± 148 kb spanning chr11:55,356,922-55,504,384. Internally, a
A retained segment was discovered with ± 1.3 kb as minimal size spanning chr11:55,432,491-55,433,765 and ± 16 kb as maximal size spanning chr11:55,419,331-55,435,229 (Table 4).

In-depth analysis showed that OR4C11, OR4P4, and OR4S2 were deleted in 11q11 carriers while OR4C6 was positioned in the internally retained segment. Genotype-specific Sanger sequencing of the four different genes confirmed CNV screening results. Our findings further indicated that the nine additional studied OR genes, lying on the long arm of chromosome 11, were not part of the deletion.

3.2. Olfactory receptor expression profiling of 11q11 genes in metabolic relevant tissues

The expression of genes positioned in the deletion was further examined in metabolic relevant tissues. Although qualitative gene expression and available GTEx transcriptome data did not allow the detection of transcripts coding for OR4C11 (NM_001004700), OR4S2 (NM_001004059) and OR4P4 (NM_001004124) in the liver, expression profiling in visceral and subcutaneous adipose tissue indicated the presence of these genes in body fat. Gene expression of 11q11 OR genes

Table 3
Characteristics of the 11q11 CNVR related to the different studied cohorts.

|                        | Patients with obesity | Healthy-weight adults | MAF obese     | MAF lean     | odds ratio | p-value  |
|------------------------|-----------------------|-----------------------|---------------|--------------|------------|----------|
| Age-matched cases versus controls | 303                   | 330                   | 26.57         | 22.42        | 1.25       | [0.98–1.62] | 0.041    |
| Not age-matched cases versus controls | 324                   | 330                   | 27.16         | 22.42        | 1.29       | [1.00–1.66] | 0.028    |
| All cases versus controls | 627                   | 330                   | 26.87         | 22.42        | 1.27       | [1.02–1.59] | 0.018    |

Table 4
Genomic features of 11q11 CNVR.

|                         | Minimal deleted region | Maximal deleted region | Minimum size (kb) | Maximum size (kb) |
|-------------------------|------------------------|------------------------|-------------------|-------------------|
| Chr11:55,368,225-55,448,559 | OR4C11                  | OR4C11                 | 80.3              | 147.5             |
| OR4P4                   |                         |                        |                   |                   |
| OR4S2                   |                         |                        |                   |                   |
| Chr11:55,432,491-55,433,765 | OR4C6                  |                         |                   |                   |
|                         | Minimal internal retained region | Maximal internal retained region | Minimum size (kb) | Maximum size (kb) |
| Chr11:55,432,491-55,433,765 |                         | Chr11:55,419,331-55,435,229 |                   |                   |
| OR4C6                   |                         | OR4C6                  | 1.3               | 15.9              |

Chromosomal locations shown in genome build GRCh37/hg19.
Fig. 2. Detected copy number variable region in current and initial 11q11 copy number variant loss study.

Comparison of the 11q11 deletion discovered in (A) our study by multiplex amplicon quantification and (B) the study of Jarick et al. (2011) by the Affymetrix Genome-Wide Human CNV Array 6.0. Red coloring represents the minimal deleted region while yellow coloring signifies the maximal deleted region. Green coloring indicates genes that are not positioned in the deletion. Differences in size and chromosomal location are observed. In the current study, $\pm$ 80 kb spanning chr11:55,368,225-55,448,559 was identified as minimal region and a $\pm$ 147 kb deletion spanning chr11:55,356,922-55,504,384 as maximal region. An internally retained segment was present with $\pm$ 1.3 kb as minimal size spanning chr11:55,432,491-55,433,765 and $\pm$ 16 kb as maximal size spanning chr11:55,419,331-55,435,229. In contrast, Jarick et al. (2011) identified a minimum size deletion of $\pm$ 80 kb spanning chr11:55,374,020-55,453,589 and a maximum size deletion of $\pm$ 97 kb spanning chr11:55,363,328-55,460,696. Both copy number variable regions cover the olfactory receptor genes $OR4P4$ and $OR4S2$; inconsistencies are perceived for genes $OR4C11$ and $OR4C6$.

HgLiftOver was applied to convert the deleted region of the initial 11q11 CNV loss study from genome build GRCh36/hg18 to GRCh37/hg19.
in the olfactory epithelium could not be demonstrated based on expression databases and literature.

4. Discussion

The present study explored whether structural variation within the olfactory receptor-rich 11q11 candidate region is implicated in the pathogenesis of obesity. An increased risk for childhood and adult obesity was present in case an individual carries at least one copy of the deleted allele (p = 0.018). Additionally, a high copy number loss frequency (MAF = 26.87%) and low odds ratio (1.27; 95% CI = 1.02–1.59) were observed, assuming that the 11q11 CNV will have a rather small effect size. Although this is true for monogenic disease forms, common CNVs have already been associated with susceptibility for complex diseases [36–38]. We therefore conclude that the 11q11 deletion can be recognized as a risk factor for complex obesity.

Fine-mapping of the 11q11 CNV revealed a minimal size deletion of ± 80 kb enclosing the three olfactory receptor genes OR4C11, OR4P4 and OR4S2. An internally retained ± 1.3 kb segment was discovered that encompasses OR4C6. A difference in size and chromosomal location was perceived with the initial 11q11 CNV study by Jarick et al. (2011). However, we believe that our results identified the same CNVR (Fig. 2). The observed dissimilarity can be ascribed to the used technique for CNV calling. The initial 11q11 CNV loss study applied a GWAS approach using the Affymetrix Genome-Wide Human CNV Array 6.0 while we designed a MAQ assay specifically for the 11q11 region. High-density SNP array experiments (1.6 million probes) provide a cost-effective way for CNV discovery as they can easily detect the total number of copies in a genomic region. Nonetheless, precise breakpoint identification as well as predicting CNV length is rather restricted by the coverage of probes on the microarray [39]. A wide variation in probe distribution along the genome is noticed [40]; some regions are densely located (e.g. known copy number polymorphic sites) while others lack probes (e.g. centromeres). Another drawback is the genomic architecture of the target region. Genomic regions such as segmental duplications, tandem repeats, and complex CNV areas are hard to call by SNP arrays due to limited sensitivity [41]. Both reasons can give an explanation for the perceived differences in the deleted region as it is located around the centromere of chromosome 11 encompassing multiple genes of the hypervariable OR family. Consequently, Jarick et al. (2011) detected a CNV containing OR4P4, OR4S2 and OR4C6. The use of a more targeted CNV screening approach by our research group leads to the discovery that OR4C11 is also involved in the 11q11 deletion while OR4C6 is still present in deletion carriers. These findings result from a well-thought-out probe design. The strength of using a MAQ assay for CNV screening is that you can specifically design probes in the region of interest. In this way, we were able to find primer pairs within three genes of the OR-rich 11q11 region that were not covered by SNP array probes (Supplementary Fig. 1 & Supplementary Fig. 2). Probes directly positioned up- and downstream of the gene (gene boundaries) were chosen in case no primer pairs were found within the gene itself. This made it possible to discover the involvement of OR4C11 in the 11q11 deletion, a gene that was not identified in the initial CNV loss study by Jarick et al. (2011). However, this gene is positioned in the deletion when considering the maximal deleted region identified by Jarick et al. (2011). Fine-mapping of the 11q11 deletion by our research group makes it possible to hypothesize that genetic variation in OR4C11, OR4P4 and OR4S2 increases the risk for obesity while OR4C6 does not contribute to the observed disease susceptibility resulting from the deletion.

The same CNVR on chromosome 11 was identified by a study extensively investigating copy number changes in OR-rich regions [42]. They noticed that the CNV was only present as a deletion across geographically diverse populations (MAF = 36%). Carriers of the deletion showed absence of OR genes OR4C11, OR4P2 and OR4S2 while OR4C6 was present in all individuals. Additionally, Young et al. (2008) examined the formation mechanism of the identified 11q11 CNV. The mutational process underlying this copy number change was inferred by the presence of alternative structural alleles around the rearrangement breakpoints. Different formation mechanisms were considered including non-allelic homologous recombination (NAHR), non-homologous end-joining (NHEJ), shrinking or expansion of variable number of tandem repeats (VNTRs) and mobile element insertions (MEI). The most convenient mechanism for OR-containing regions is NAHR since ORs typically occur as highly homologous tandem repeat sequences in the genome. However, Young et al. (2008) characterized the region as a complex CNV area resulting from a combination of multiple deletion and inversion events. They proposed that the presence of highly similar L1 repeats, flanking the 11q11 region, initiate improper pairing of the repeats with the corresponding region. The outcome is the creation of a loop structure wherein the different deletions and inversions could have occurred. Final analysis of the deletion/inversion breakpoints implicated NHEJ as the formation mechanism of this complex CNV area. This is in line with a paper by Mills et al. (2011) who associated specific formation mechanisms with structural variation chromosomal positioning, size and type. They observed (i) high abundance of VNTR near centromeres while NAHR was clustered near telomeres, (ii) occurrence of small structural variants as a result of VNTR or MEI (in presence of Alu or L1 repeats) while NHEJ- and NAHR-based mechanisms were perceived across a wide size range, and (iii) NHEJ as dominating deletion mechanism while MEI was seen as main insertion process [43]. Specific for the identified rearranged area, we infer the presence of NHEJ, VNTR and MEI as the 11q11 region is a complex CNV consisting of multiple small and large deletion and insertion events near the centromere.

Assessment of the functional impact of gene disrupting CNVs by Mills et al. (2011) noticed a significant enrichment for genes involved in cell defense and sensory perception. Accordingly, genetic variation affecting sensory acuity and perception could explain phenotypic differences observed in the sense of smell [22]. Recent evidence showed that orexigenic agouti-related protein-expressing neurons are regulated by energy status and sensory perception. Copy number loss of ORs could thus result in partial or total insensitivity of the corresponding odorant, for which might be compensated by an increase in food intake [21]. This ‘olfactory system’ hypothesis proposes that disruption of OR genes could influence eating behavior, ultimately leading to hyperphagia and obesity [23,44–47]. An alternative hypothesis has been suggested since the discovery of ectopic OR expression in metabolic relevant tissues. Research has demonstrated that ORs could have a protective role against fat accumulation in the liver and adipose tissue [28,29]. This ‘metabolic system’ hypothesis implies that gene disruption of ORs will negatively influence energy metabolism with obesity as a consequence. Although we were not able to confirm nor reject the ‘olfactory system’ hypothesis with our study design, we could exclude the presence of 11q11 OR gene expression in the liver. In addition, gene expression of OR4C11, OR4P4 and OR4S2 was detected in adipose tissue. This is the first time that expression of these genes has been studied in relation to obesity and gives us the possibility of postulating about the underlying mechanism. Based on our results, the ‘metabolic system’ hypothesis is most likely the responsible mechanism for the increased disease susceptibility perceived in 11q11 deletion carriers. This implies that the responsible OR genes regulate energy metabolism in visceral and subcutaneous adipose tissue. The 11q11 region could thus be recognized as an obesity susceptible locus in which copy number loss(es) will result in a disturbed energy balance with fat accumulation as outcome. Future research will be necessary to elucidate the involvement of the three OR genes in body weight regulation. On one hand, the possible association of obesity with olfactory dysfunction (“olfactory system” hypothesis) will need to be excluded. However, this is not easy as the primary tissue of interest for expression profiling is the olfactory epithelium. On the other hand, the results of the present study need to be confirmed in vitro.
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