Original Article

Molecular Syndromology

Mol Syndromol 2010;1:176–184
DOI: 10.1159/000322054

Accepted: September 21, 2010
by A. Rauch
Published online: November 25, 2010

Olfactory Receptor-Related Duplicons Mediate a Microdeletion at 11q13.2q13.4 Associated with a Syndromic Phenotype

A. Wischmeijer[a] P. Magini[a] R. Giorda[d] M. Gnoli[a] R. Ciccone[e] I. Cecconi[b] E. Franzoni[b] L. Mazzanti[c] G. Romeo[a] O. Zuffardi[e] M. Seri[a]

[a]U.O. Genetica Medica, [b]Neuropsichiatria Infantile e Disturbi del Comportamento Alimentare, e [c]U.O. Pediatria, Dipartimento di Scienze Ginecologiche, Ostetriche e Pediatriche, Università di Bologna, Bologna, [d]Biologia Molecolare, Istituto Scientifico Eugenio Medea, Bosisio Parini, e [e]Dipartimento di Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italia

Key Words
Array-CGH · Microdeletion · Olfactory receptor pseudogenes · Segmental duplications

Abstract
By array-CGH, we identified a cryptic deletion of about 3.4 Mb involving the chromosomal region 11q13.2q13.4 in a child with speech and developmental delay. Highly homologous segmental duplications related to the well-known olfactory receptor (OR)-containing clusters at 8p and 4p are located at the breakpoints of the imbalance and may be involved in its occurrence. Although these structural features are known to promote recurrent chromosomal rearrangements and previous studies had included the 11q13.2q13.4 deletion region among those considered potentially more unstable, neither deletions nor duplications of this region had been reported until now. Among the deleted genes, *SHANK2* might play a role in the phenotype of the patient since it encodes a postsynaptic scaffolding protein similar to *SHANK3*, whose haploinsufficiency is a well-known cause of severe speech delay and autistic-like behavior, and recently deletions and mutations of *SHANK2* have been described in patients with an autistic spectrum disorder or mental retardation.

The term 'genomic disorders' refers to the growing number of human diseases caused by recurrent and non-recurrent DNA rearrangements involving genomic regions containing dosage-sensitive genes. Non-allelic homologous recombination (NAHR) appears to be the most important mechanism underlying recurrent rearrangements with identical breakpoints falling within low-copy repeats (LCRs), also named segmental duplications (SD). NAHR may occur by classical homologous recombination-mediated double-strand break repair between the repeats or break-induced replication (BIR) [Hastings et al., 2009]. The development of high resolution techniques, such as array-CGH, allowed the definition of several new recurrent deletions and duplications mediated by different mechanisms [Zhang et al., 2009].

As for chromosome 11, most deletions involve the distal portion of the long arm with a frequently observed breakpoint in 11q23q24; many of them are terminal deletions associated with Jacobsen syndrome [Coldren et al., 2009; Mattina et al., 2009].

Here we report the identification of a cryptic interstitial deletion in chromosome 11q13.2q13.4 spanning from

A.W., P.M. and R.G. contributed equally to this work.

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Anita Wischmeijer
Medical Genetics Unit, S. Orsola-Malpighi Hospital
Via Massarenti 9
IT–40138 Bologna (Italy)
Tel. +39 051 208 8434, Fax +39 051 208 8416, E-Mail anita.wischmeijer@unibo.it

67.525330 to 70.964483 Mb (hg18) in a child with developmental delay, moderate-severe mental retardation, severe speech delay and some dysmorphic features. The imbalance was likely mediated by highly homologous inverted LCRs related to the olfactory receptor (OR) pseudogene-containing regions involved in the recurrent 4p and 8p rearrangements [Giglio et al., 2001, 2002]. Since no other cases have been described so far, this microdeletion may occur at low frequency or be non-recurrent.

Materials and Methods

Patient

The proband is an 8 years and 6 months old Italian girl, first and only child of healthy, unrelated parents. At the time of her birth her mother was 33 and her father 35 years old. Family history is negative for recurrent miscarriages, congenital anomalies and mental retardation.

At 6 months of gestation, intrauterine growth retardation was suspected but subsequent ultrasound examinations did not confirm this hypothesis. The mother reported poor fetal movements during the last weeks of the pregnancy. The proband was born by normal delivery after 40 weeks of gestation. At birth, weight was 2,680 g (5–10th centile), length was 49 cm (± 50th centile), and head circumference was 34 cm (25–50th centile). She reportedly did well at birth (but no APGAR score is available) but presented congenital hip dysplasia and some dysmorphisms (small simple low-set posteriorly rotated ears with overfolded helices and a preauricular tag at the left side, and downward slanting palpebral fissures). Since she presented poor sucking, she was bottle-fed with breast milk.

Considering her psychomotor development, she achieved trunk control by 8 months; she did not crawl and made her first steps at the age of about 18–20 months. First teeth appeared at the age of about 6–7 months. At the age of 5 years and 11 months she came to our attention for the first time. She then pronounced less than 5 words and presented behavioral and relational problems as well as repetitive hand movements. Self-destructive behavior with wrist-biting had just been resolved. She attended kindergarten with supplementary education help; she liked to be with other children and tried to imitate them but did not really look for interaction. She was more confidential towards adults. She was followed by a speech therapist and attended psychomotor education and music therapy.

Physical examination revealed growth parameters within the normal range: height 113 cm (<50th centile), weight 17.9 kg (<25th centile), head circumference 50 cm (25–50th centile). In addition to the dysmorphic features described at birth, she presented deep-set eyes, ptosis of the left eyelid, long and fine lashes, broad nasal bridge, tubular somewhat beaked nose with round overhanging tip and hypoplastic nares, short philtrum, small and thin upper lip, preauricular tag and small low-set simple ears (fig. 1a–c). The central part of her lower lip appeared full and prominent. The teeth were small and the central incisors widely spaced. She had a short and wide neck and a clearly visible pattern of superficial veins on her chest, which appeared broad. Total hand length was 12 cm (3rd–25th centile) bilaterally; hands were narrow with long slender fingers, clinodactyly of both fifth fingers (fig. 1d), normal palmar creases and normal nails. Her feet presented metatarsus adductus and partial cutaneous syndactyly between the second and third toes (fig. 1e). Her skin presented cutis marmorata and there was a hyperchromic area on her right flank. The right nipple appeared slightly enlarged and elevated, and hypertrichosis was apparent on her back and lower limbs.

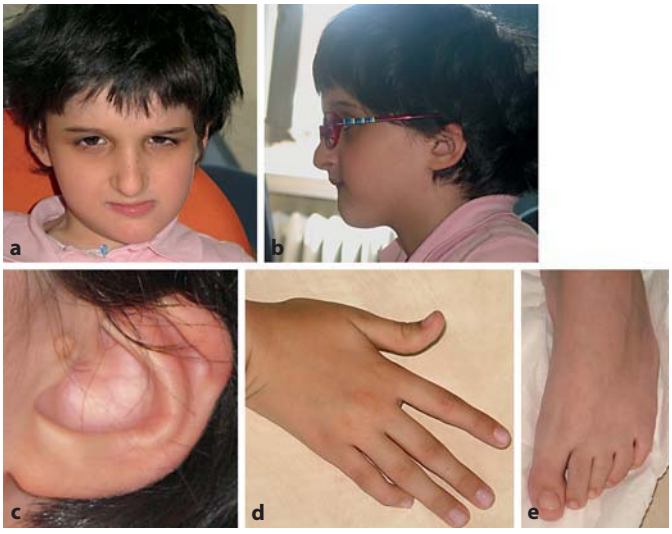


Fig. 1. Proband at the age of 7 years and 6 months. **a** Frontal view. **b** Profile. **c** Preauricular tag. **d** Long slender fingers. **e** Foot.

Magnetic resonance imaging and computed tomography of the brain did not reveal structural anomalies. Repeated EEGs did not show clear alterations. Abdominal ultrasound screening resulted normal. Ophthalmological evaluations revealed strabismus, ptosis of the left eyelid, and astigmatism. It was not possible to determine visus. The degree of mental retardation was difficult to test, but probably lies in the moderate to severe range.

We last saw the proband at the age of 7 years and 4 months. Her height was 121.8 cm (25–50th centile), her weight was 21.9 kg (25th centile) and head circumference measured 52.5 cm (50–75th centile). Verbal and non-verbal communication as well as comprehension seemed to be slightly improved. She pronounced about 10 words at that time. Her parents referred that initial chewing difficulties had disappeared by then, and that she had a varied diet. Intestinal function was regular. She was not completely toilet-trained yet. Her sleeping pattern had always been normal. There was no history of recurrent infections, apart from several episodes of conjunctivitis during the last years. She still followed the same therapeutic strategies.

Physical examination did not reveal new findings, apart from possible initial breast development. We also noted hyperhidrosis of the palmar and plantar regions of hands and feet, respectively.

Clinical investigations concerning the suspicion of precocious breast development have been performed. At a calendar age of 7 years and 6 months, her bone age corresponded to around 9 years. Pelvic ultrasound scanning showed small uterus and adnexes. LHRH test showed a pituitary response (FSH base level 1.7 mIU/ml, 8.4 after 30 min and 10.1 after 60 min; LH base level 0.1 mIU/ml, 4.4 after 30 min and 4.3 after 60 min). Repetition of pelvic ul-

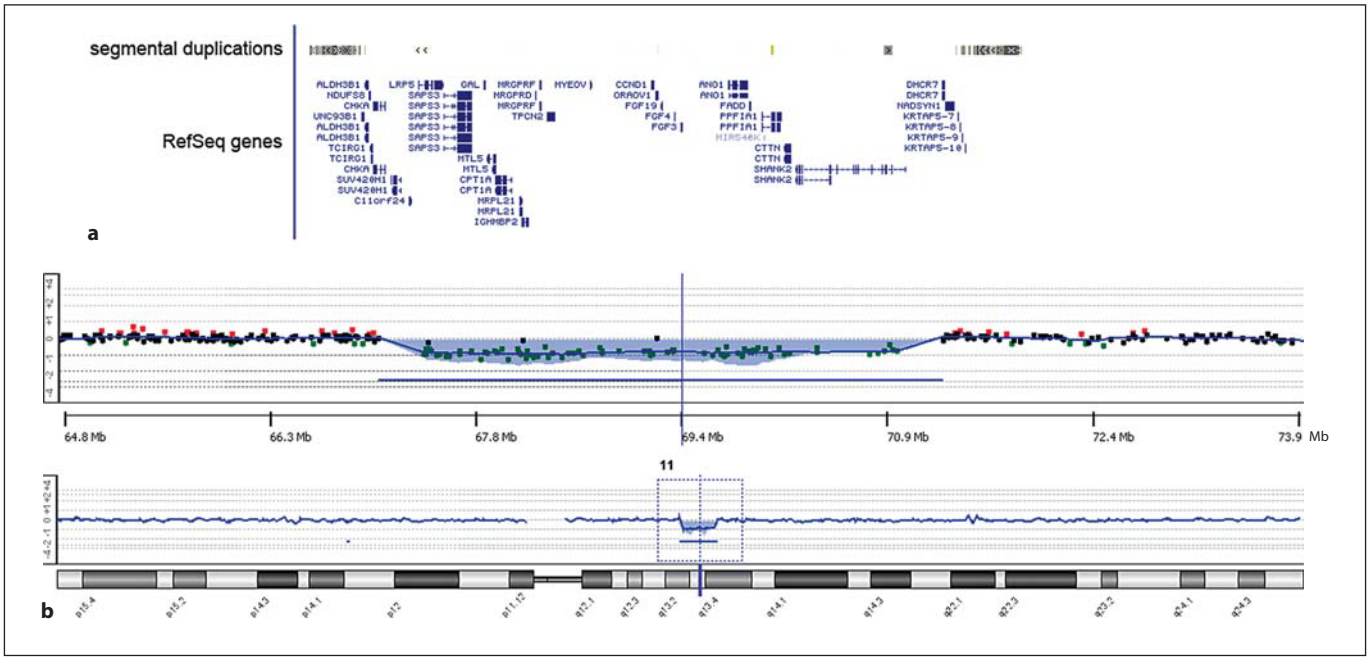


Fig. 2. ~3.4-Mb deletion in 11q13.2q13.4. **a** Deleted genes and the flanking blocks of segmental duplications (image modified from UCSC Genome Browser, http://genome.ucsc.edu/). **b** Array-CGH profile with indication of the chromosomal and the genomic positions of the deletion.

trasound and LHRH test at the age of 8 years and 6 months revealed similar results. No pharmacological therapy has been started so far.

Cytogenetic Examinations

Standard karyotyping on lymphocytes and search for subtelomeric rearrangements using FISH were performed following conventional protocols.

Array-CGH

Genomic DNA was extracted from peripheral blood and its quality was monitored using a NanoDrop spectrophotometer (Celbio). The entire array-CGH procedure has been done according to the Agilent protocol for the 44B platform.

Microsatellite and FISH Analyses

Polymorphic loci (D11S987, D11S1337 and D11S4136) were selected within the deleted region according to the UCSC Human Genome Assembly (Build 36, March 2006), amplified on the proband's and the parental DNAs with primers labeled with fluorescent probes (ABI 5-Fam and Hex) and genotyped on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

FISH analysis was performed following previously described protocols [Pramparo et al., 2003] on maternal chromosomes with BAC clones RP11-60O13 and RP11-892P16, whose map positions are 67.61–67.77 and 70.67–70.86 Mb, respectively (Genome Assembly March 2006, Build 36). They were labeled by nick-translation with biotin and digoxigenin, respectively, and visualized by FITC-avidin and TRITC-conjugated anti-digoxigenin antibodies on chromosomes stained with DAPI. Hybridizations were analyzed with an epifluorescence microscope and images captured with a PowerGene FISH System (PSI).

Computational Methods

The chromosomal location of the repetitive extragenic palindromic (REP) sequences was identified in the self chain track of the UCSC Genome Browser (Human Genome assembly, March 2006); REP structure and identity was analyzed using PipMaker (http://pipmaker.bx.psu.edu/cgi-bin/pipmaker) [Schwartz et al., 2000] and MAVID (http://baboon.math.berkeley.edu/mavid/) [Bray and Pachter, 2004]; the phylogenetic tree calculated by MAVID was drawn with Phylodendron (Phylodendron: by D.G. Gilbert; software@bio.indiana.edu); Chimpanzee *(Pan troglodytes)* REPs were searched on the UCSC Browser (Chimp Genome assembly, November 2003; http://genome.ucsc.edu/cgi-bin/hgGateway).

Results

Array-CGH with a resolution of about 100 kb detected in the proband a cryptic deletion (fig. 2) of 3.4 Mb involving the chromosomal region 11q13.2q13.4, from 67,525,330 to 70,964,483 bp (table 1); normal flanking probes were located at 67,194,884 and 71,305,248 bp (hg18).

The deletion was flanked by blocks of highly homologous segmental duplications. Computational analysis

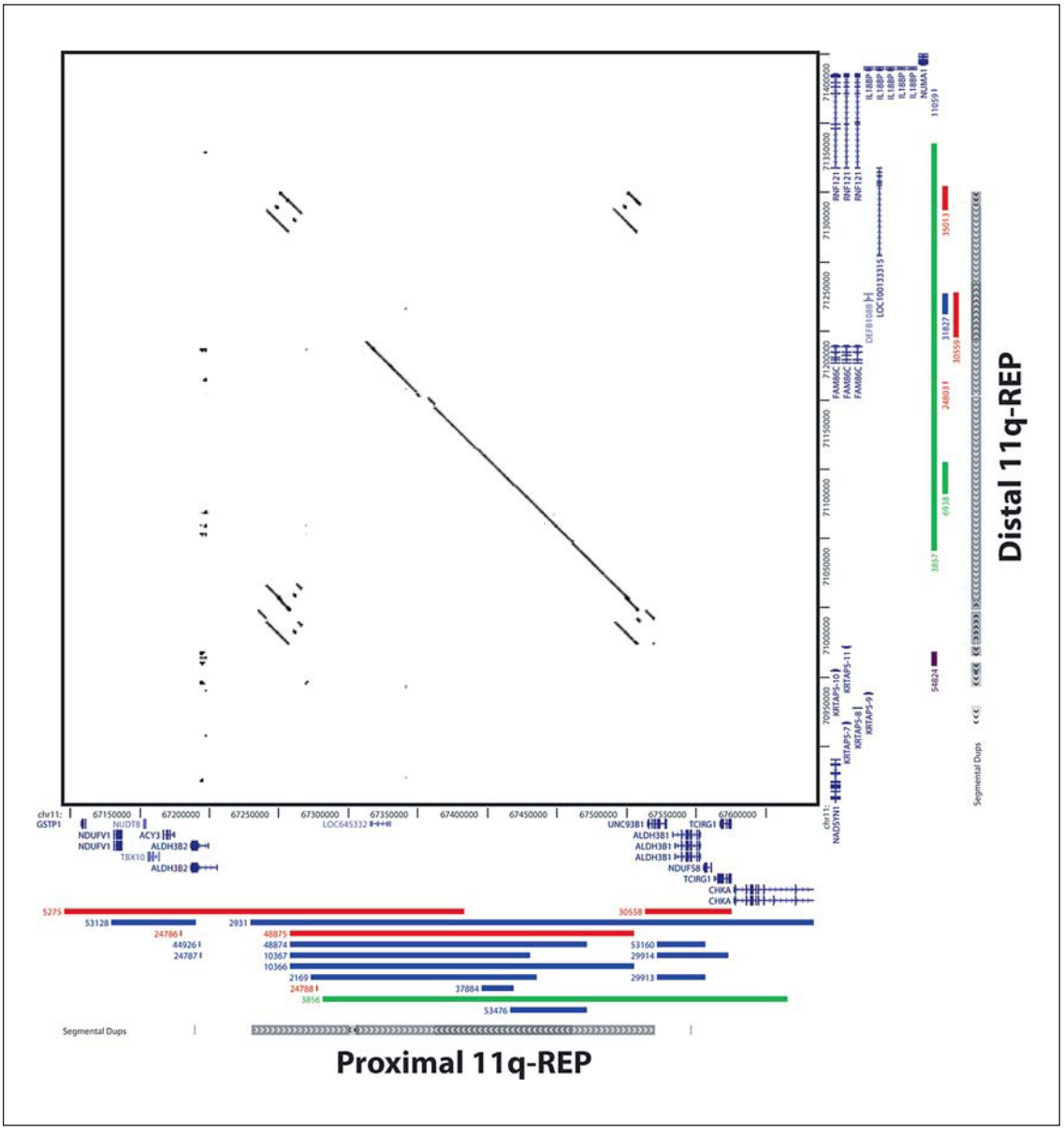
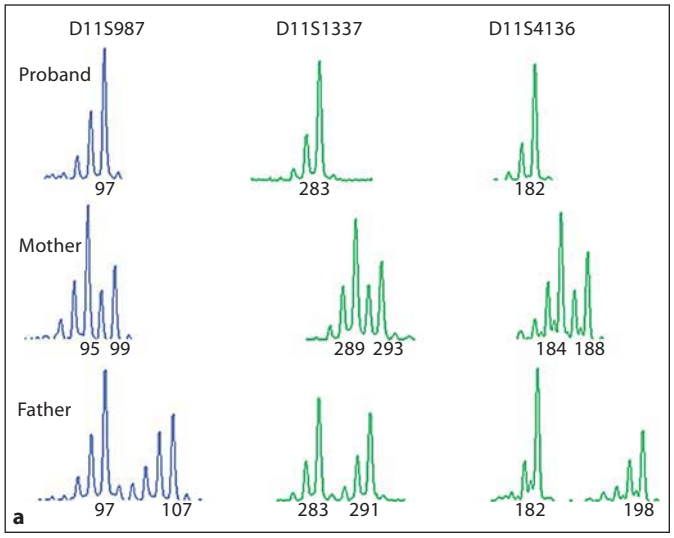


Fig. 3. Dot-plot alignment of distal and proximal breakpoint regions of the 11q deletion. The identity between the 2 sequences is indicated by a diagonal line. The position of genes, genomic variants according to the DGV structural variations track, and inverted segmental duplications as shown in the UCSC map are indicated.

Table 1. Array-CGH findings in the proband. Chromosomal bands, breakpoint positions and deletion size are reported. Map positions refer to Genome Assembly 2006 (Build 36). The presence of segmental duplications is also indicated.

Array-CGH findings	Proximal breakpoint, Mb		Distal breakpoint, Mb		Deletion size, Mb	Flanking LCRs
	Last not deleted probe	First deleted probe	Last deleted probe	First not deleted probe		
del(11)(q13.2q13.4)	67.19	67.52	70.96	71.30	3.4	Several



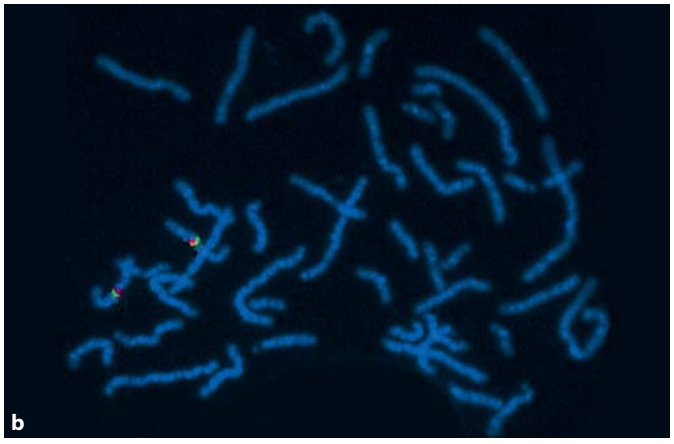


Fig. 4. Microsatellite- and FISH analysis findings. **a** Microsatellite peaks and their respective sizes for the proband and both parents. The markers, chosen according to the Genome Assembly 2006, were labeled with different fluorochromes, FAM (blue) for D11S987, and HEX (green) for D11S1337 and D11S4136. **b** FISH results on maternal chromosomes. BAC clones RP11-60O13 and RP11-892P16, mapping at the 2 ends of the studied region, were revealed by FITC-avidin (green) and TRITC-conjugated anti-digoxigenin antibodies (red), respectively. The fluorescent signals indicate the position of the 2 probes and their relative orientation in relation to the centromere on both chromosomes 11.

demonstrated that proximal and distal breakpoints fell inside highly identical (96.9%) 150-kb inverted repeats (11q-REPs) (fig. 3). These paralogous regions, initially defined as OR-containing repeats on the basis of the analysis of chromosome 4 and 8 LCRs [Giglio et al., 2001, 2002], actually consist of conserved regions of variable size with copies on chromosome 3 (75, 127 and 131 Mb), 4 (4 and 9 Mb), 7 (6 Mb), 8 (REPD: 8 Mb and REPP: 12 Mb), 11p (3 Mb), 11q (67 and 71 Mb), 12 (8 Mb) and 16 (5 Mb). Identity between all copies of the repeat is over 94%.

The microsatellite analysis, performed with markers D11S987, D11S1337 and D11S4136, confirmed the array-CGH finding and showed that the deletion originated de novo on the maternally-derived chromosome 11 (fig. 4a).

FISH analysis performed on metaphase chromosomes of the proband's mother demonstrated that no inversion polymorphisms underlie the imbalance; on both chromosomes 11, the 2 probes mapped in the same relative orientation with respect to the centromere and the long arm telomere (fig. 4b).

The same probes were used to search for possible inversion polymorphisms of this region in 50 healthy individuals with negative results (data not shown).

Discussion

Over the last years, rearrangements below the detection level of conventional cytogenetics have been proven to significantly contribute to mental retardation and allowed to define several new malformation syndromes [Zhang et al., 2009].

Several both apparently benign and causative deletions/duplications are caused by NAHR occurring between 2 blocks of homologous segmental duplications.

Sharp et al. [2006] predicted a series of chromosome regions as potential sites of recurrent chromosomal aberrations. They included chromosome region 11q13.2q13.4 from 67.253 to 71.242 Mb in their list, but neither deletions nor duplications of this region were detected in their patients' screening or by other investigators. By array-CGH, we identified a cryptic deletion of this 11q region, spanning from 67.525 to 70.964 Mb, in a patient with developmental and speech delay. The segmental duplications flanking this deletion are highly homologous to OR gene-rich regions on chromosomes 8p and 4p [Giglio et al., 2001, 2002]; they contain a conserved sequence with copies on several chromosomes. Some copies of the LCR have acquired additional homologous sequences also present on chromosome 21 (32 Mb). A single OR pseudogene is present on all copies of the REP, while additional OR sequences are present in the chromosome 8 REPs. A common core sequence, highly conserved in all copies of the LCR, corresponding to the chromosome 16 region between 5,069,047 and 5,089,700 bp, contains full-length copies of a hypothetical gene *(FAM86A)* and partial copies of another gene *(ALG1)* whose functional copies are both lo-

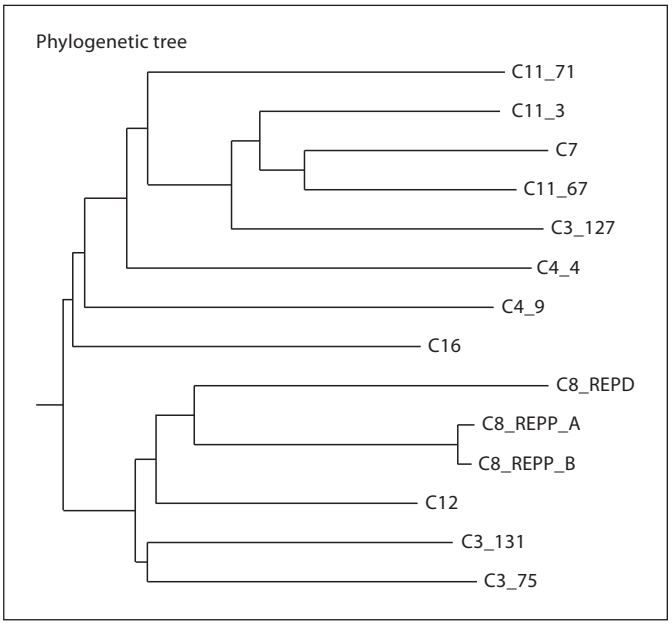


Fig. 5. Phylogenetic tree of the relationship between copies of the OR-related duplicons in the human genome.

Table 2. Summary of the clinical features of the proband

	Clinical features
Mental development	moderate-severe mental retardation
Speech	severe language delay
Hair	thick hair
Forehead	prominent forehead
Eyes	ptosis
	strabismus
	astigmatism
Ears	preauricular tag
	low set ears
	posteriorly rotated ears
	small simple ears with overfolded helices
Nose	round overhanging tip and hypoplastic alae nasi
	broad nasal bridge
Mouth	small, thin upper lip
Teeth	small and central incisors widely spaced
Hand	long slender fingers
Foot	metatarsus adductus
	partial cutaneous syndactyly of toes II–III

cated on chromosome 16. It is not known whether the additional copies of *FAM86A* are functional, while some exons from the *ALG1* copies seem to be incorporated in a number of transcripts (not shown). Several transcripts have been mapped to single REPs, most of them of retroviral origin.

A tree representation of the phylogenetic distance between REPs is shown in figure 5. A portion of each REP corresponding to the conserved core region of the LCR (chr16: 5069047–5089700) has been used for the comparison. Comparing the whole LCR sequences produced a tree with slightly different branching (not shown). REPs on the same chromosome usually have higher identity. REPs on chromosomes 4, 7 and 11 and, on the other hand, REPs on chromosome 3, 8 and 12 appear more closely related. On 4 chromosomes (3, 4, 8 and 11) REP pairs are found at a distance of approximately 4 Mb; in all instances the paired REPs are in inverted orientation.

BLAT analysis on the chimpanzee genome using the human *ALG1*/*FAM86A* core region shows that multiple copies of the REPs are present also in chimpanzee, on chromosomes 2 (two closely spaced copies), 3 (two closely spaced copies), 7 (two closely spaced copies), 9 (two closely spaced copies), 10 and 18; their position and relative orientation is the same in humans and chimp. The presence of multiple copies of the chromosome 8 REPs in chimpanzee, orangutan, gorilla and old world monkeys has been experimentally verified [Sugawara et al., 2003].

Inverted LCRs can mediate by NAHR the creation of inversion polymorphisms of the fragment they flank [Lupski, 1998], although these structural variants are not observed for all LCR pairs. Inversion polymorphisms of the region between paired REPs, involved in the genesis of a number of pathogenic rearrangements including deletions, duplications and complex duplications/deletions, have been demonstrated for chromosomes 4 and 8 [Giglio et al., 2001, 2002]. We have not been able to find any inversion, neither in the proband's mother, at whose meiosis the deletion occurred, nor in a sample of 50 Italian subjects. Unlike other regions flanked by inverted LCRs [Antonacci et al., 2009], the 11q-REPs do not seem to facilitate submicroscopic inversions. Alternatively, the presence of large regions of genome instability due to the LCRs may facilitate lower frequency/non-recurrent DNA breaks repaired by non-homologous end-joining (NHEJ) [Roth et al., 1985] or fork stalling and template switching (FoSTeS) [Lee et al., 2007], explaining the rarity of rearrangements mediated by the 11q-REPs. The extreme complexity and large number of homologs of the 11q-REP LCRs hindered us from cloning the breakpoints and elucidating the mechanism of the deletion.

Our patient presents a complex phenotype (fig. 1a–e) mainly characterized by preauricular tag, small low-set

simple ears, ptosis of the upper eyelid, tubular somewhat beaked nose with round overhanging tip and hypoplastic nares, short philtrum, moderate-severe developmental delay and severe language delay (table 2). She also presented congenital hip dysplasia as well as signs of precocious puberty. Remarkably, in spite of her moderate-to-severe mental retardation and almost absent speech, brain MRI and CT scans did not reveal any morphologic alterations.

Among the additional features, our proband presented poor sucking in early infancy and chewing difficulties during the first years of life, downward slanting palpebral fissures, thin upper lip, small teeth and widely spaced upper central incisors, small narrow hands with long slender fingers, clinodactyly of both fifth fingers, and bilateral metatarsus adductus.

The deleted region includes nearly 40 genes. Among them, *SHANK2* might have some relevance with respect to the patient's phenotype. Shank proteins (Shank1–3) are scaffolding molecules of the postsynaptic density (PSD) of excitatory synapses, which are crucial for PSD assembly and the formation of dendritic spines. Shank1 mRNAs are distinguished by their translational regulation [Falley et al., 2009] and Shank1b, together with specific binding partners, acts as a key contributor to the regulation of dendritic spine morphogenesis and brain function [Kim et al., 2009]. *SHANK3* codes for a scaffolding protein of the PSD that binds to neuroligins and regulates the size and shape of dendritic spines [Roussignol et al., 2005]. Its haploinsufficiency is mainly responsible for the distal 22q13 chromosome deletion syndrome that is characterized by the severe delay of language development also present in our patient [Bonaglia et al., 2006]. *SHANK3* is part of the *NLGN-NRXN* pathway and is probably required during the stabilization phase of the synapse in response to neuronal activity. Mutations of these genes are associated with autism spectrum disorder [Bourgeron, 2009; Gauthier et al., 2009]. Similarly to *SHANK3, SHANK2* also codes for a postsynaptic scaffolding protein directed towards the PSD of excitatory synapses via its C-terminal end that includes a sterile alpha motif (SAM) domain. Recent experiments on synaptogenesis of hippocampal neurons in primary cell culture from rat embryos demonstrated that Shank2 protein is amongst the first molecules of the PSD [Grabrucker et al., 2009].

Moreover, Berkel et al. [2010] identified intragenic deletions and nucleotide variants in patients affected with ASD or mental retardation, suggesting a potential role of this gene in the pathogenesis of these conditions. Altogether some phenotypic characteristics of our patient, essentially mental retardation and severe language delay, might well be related to *SHANK2* haploinsufficiency, although other genes' imbalance could have contributed to her malformation/mental retardation picture. Looking at the genes within the deletion, we observed that some of them are responsible for autosomal recessive diseases that have been excluded on clinical grounds. Others are associated with pathologies transmitted in an autosomal dominant way (online supplementary table 1, for all supplementary tables, see www.karger.com/doi/10.1159/000322054). Defects in *FGF3* are a cause of autosomal recessive congenital deafness with inner ear agenesis, microtia and microdontia [MIM: 610706]. Recently, Gregory-Evans et al. [2007] showed that *FGF3* haploinsufficiency could be the cause of otodental syndrome [MIM:166750], a rare but severe autosomal dominant craniofacial anomaly, characterized by grossly enlarged canine and molar teeth (globodontia) associated with sensorineural hearing deficit with variable onset, from early childhood to middle age. Our patient does not present dental anomalies. Since she is still young, she could not have expressed the hearing deficit yet, but this could also be due to incomplete penetrance or the effect of modifier genes. *LRP5* encodes a low-density lipoprotein receptor-related protein, involved in the Wnt/beta catenin signaling pathway and responsible for several autosomal dominant pathologies (familial exudative vitreoretinopathy [MIM:133780, 601813]; high bone mass trait [MIM:601884]; endosteal hyperostosis [MIM:144750]; osteosclerosis [MIM:144750]; osteopetrosis type I [MIM:607634]; van Buchem disease type 2 [MIM:607636]) that affect, in particular, retinal vessels and bone density, although they are sometimes asymptomatic. Among these diseases, only exudative vitreoretinopathy can be due to mutations that lead to frameshift and a premature stop codon, causing the disorder through a protein level reduction. Therefore, careful ophthalmological follow-up in the proband seems reasonable.

Other genes that could have played a role in the pathogenesis of the clinical picture are: *FGF19*, expressed in fetal but not in adult brain [Nishimura et al., 1999]; *PPFIA1,* coding for a member of the LAR protein-tyrosine phosphatase-interacting protein (liprin) family, known to be important for neuronal growth, axon guidance and synapses formation [Ackley et al., 2005; Yang et al., 2005].

Finally, *GAL* haploinsufficiency might explain the precocious breast development (with an LHRH test showing pituitary response at the age of 7 years and 6 months)

and advanced bone age present in our patient. In fact, the protein encoded by this gene seems to be involved in a series of processes among which are regulation of growth hormone and insulin release, and modulation of adrenal secretion, LH secretion and LHRH-induced LH release [López et al., 1991].

In conclusion, to our knowledge, this is the first report of a cryptic deletion of chromosome 11q13.2q13.4 (spanning from 67.525 to 70.964 Mb) mediated by inverted homologous segmental duplications in a patient with dysmorphic features and language and developmental delay. Sharp et al. [2006] predicted that this same chromosomal region could be a potential rearrangement hot spot, being flanked by LCRs. The absence of an inversion polymorphism, that would facilitate the occurrence of deletions/duplications, in the region between the two 11q-REPs, may well explain the rarity of this rearrangement. Alternatively, the presence of large regions of genome instability due to the LCRs may facilitate lower frequency non-recurrent DNA breaks repaired by NHEJ [Roth et al., 1985] or FoSTes [Lee et al., 2007].

The DatabasE of Chromosomal Imbalances and PHenotypes using Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk/application/) reports 3 patients with overlapping 11q deletions involving different breakpoints compared to our proband. Patient 2389 carries a deletion spanning from 68.072 to 70.307 Mb, completely encompassed by the one we presented here, and suffers from congenital deafness. We have no additional information hence we cannot precisely compare the phenotype of this patient with the clinical picture described above, but we could suppose an involvement of *FGF3* in the pathogenesis of congenital deafness (see above), presuming an alteration of the second allele.

Patient 251808 is characterized by a much smaller deletion between 67.644 and 68.044 Mb and shows a phenotype similar to our patient (low-set ears, down-slanting palpebral fissures, speech delay and mental retardation), but the poor clinical description and the presence of another chromosomal aberration (dup15q13.1) prevent us from defining a precise phenotype-genotype correlation and suitably comparing these to patients from both a clinical and molecular point of view.

Patient 251970 has a larger completely overlapping deletion spanning from 66.704 to 71.769 Mb and shares some clinical features with our patient, including low birth weight, feeding problems, prominent forehead, deep-set eyes, clinodactyly and mental retardation. Further pathological signs are short stature with prenatal onset, hypertelorism, micrognathia, cleft palate, patent ductus arteriosus, hypotonia and anarthria and they could be due to the haploinsufficiency of the nearly 40 additional genes disrupted by this larger deletion (in online suppl. table 2 genes with known functions are listed). However, none of these genes seems to have functions directly related to the condition reported for patient 251970, although *INPPL1* may contribute to mental retardation, being involved in the neurite outgrowth process by regulating actin dynamics [Muraille et al., 2001; Prasad and Decker, 2005], and *TBX10* might be responsible for the cleft palate through an unknown mechanism, different from the gain of function described by Bush et al. [2004].

Nevertheless, the description of more patients with microdeletion 11q13.2q13.4 or with partially overlapping deletions would be useful to further delineate the main clinical features of this possible new syndrome and to understand the function of the genes contained in the deleted chromosomal interval.

Acknowledgements

We would like to thank the family of our proband for the kind collaboration to this article. This work was supported by project CHERISH grant agreement No. 223692 to M.S. from the European Commission's FP7 funding.

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