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Ph.D in Medical Genetics

Multiple congenital anomalies and mental retardation: analysis by oligo array-CGH

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Thesis suitable for the title of "Doctor Europeus"

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Review of the thesis submitted for PhD and Doctor Europeus by Rossella Caselli
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''Multiple congenital anomalies and mental retardation: analysis by oligo array-CGH''

This research sets out to address a major clinical problem which concerns the difficulty in establishing an etiological diagnosis in a large proportion of children with multiple malformations and mental retardation. A new technology is utilised, that of array-based comparative genomic hybridisation, which detects alterations in dosage of DNA sequences from throughout the genome in the subject as compared to a control individual.

The background to the study is succinctly presented and justification given for the platforms used at various phases of the research. The size and details of the patient cohort are not described in the methods section but referred to in the discussion section. 32 selected patients were investigated, all having previously been screened for subtelomeric rearrangements which may account for the high rate of pathogenic rearrangements (9/32 – 28%). It is not stated in the text whether parental DNA samples were routinely collected and what proportion of the patient cohort had variants which were shown to be familial and not pathogenic, however in the published papers presented in the thesis all these details are given for the pathogenic results.

The strength of this research is in the published papers and in the cases described in detail in the Results section. These are logically divided into several groups and all four cases of novel deletions are published and have added significantly to the literature. Several represent distinct phenotypes. The papers are well written and strong scientifically. The other published papers from the 'Known deletion' and the 'Array-CGH as a tool for identification of MR genes' are also of high quality.

Finally the Discussion and Future Perspectives chapter demonstrates a comprehensive knowledge of the field and of the mechanisms leading to some of the imbalances detected including non allelic homologous recombination. Strategies for linking elements of the phenotype with specific genes in the deleted or duplicated segments are discussed as well as the importance of databases such as DECIPHER to which all the pathogenic cases have been submitted.

I would strongly support the award of PhD and Doctor Europeus for this research and thesis.

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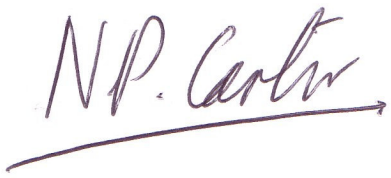
8th June 2007

Review of the thesis submitted for "Doctor Europeus" and PhD in Medical Genetics by Rossella Caselli entitled "Multiple congenital anomolies and mental retardation: analysis by oligo array-CGH".

The thesis comprises an introduction to the field of array-CGH for the analysis of patients with learning disability and congenital abnormalities, brief methods, results of the analysis of 18 cases and a discussion. Nine of the cases are presented in six published articles (Rossella being first authour in three of these) whilst the remaining nine patients are described in brief unpublished case notes.

The thesis is generally well written with only a small number of minor grammatical errors. The introduction is brief and only covers the area of array-CGH. While the introductions in the published articles provide the background relevant to those articles, it would have been nice to have seen a more detailed overview of the relevance of chromosomal abnormalities presented in the Introduction or Rationale sections. The published articles are well written and describe well the findings in the patients studied as well as providing good discussion of the results. Results from the unpublished cases are described more briefly. I would have liked to have seen more details and array data on these cases. Rosella has made good use of the DECIPHER database and the details of genes within the altered regions produced by this tool would have been good to have been included in these case reports. The results from these patients are analysed in the Discussion but this is done quite briefly. In particular, I would have liked to have seen a more detailed discussion of genotype-phenotype correlations in the three patients with overlapping X chromosome deletions. I would suggest that the unpublished cases would have been better presented in the form of mini-articles rather than case reports discussed in a later section.

The criticisms above are relatively minor. This thesis clearly represents a large body of work more than adequate for a Ph.D in Medical Genetics. In particular, the three first authour publications clearly demonstrate that Rossella is capable of academic research at the doctoral level.



Nigel P. Carter

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1. INTRODUCTION

1. INTRODUCTION

The array-based Comparative Genomic Hybridisation

Comparative Genomic Hybridization (CGH) was developed to measure alterations in dosage of DNA sequences throughout the entire genome in a single experiment [(1)]. CGH has been applied for the study of human diseases, given that gene dosage variations occur in many conditions from cancer to developmental abnormalities. Therefore, detection and mapping of copy number abnormalities provide an approach for associating aberrations with the phenotype and for localizing candidate genes.

Microarray formats for array-based Comparative Genomic Hybridisation technique (array-CGH) have been developed over the last 10 years [(2)]. In the array-CGH technique, metaphase chromosomes spreads are replaced by DNA immobilized on solid supports, representing a significant advantage in terms of quantity accuracy, resolution and repeatability with respect to traditional CGH. A microarray is an analytical device formed by an array of molecules (BAC or PAC clones, cDNAs, oligonucleotides, PCR products, polipeptides etc) or tissue sections immobilized at discrete locations on a solid porous or nonporous support. The distance between each immobilized target may vary from some millimetres to few micrometers depending on the microarray type [(3)]. In the context of microarrays, the molecule immobilized on the slide is indicated as “probe”, while the “target” is the molecule in solution exposed to the array [(3)]. The use of an array of mapped probes, instead of metaphase spreads, allows to overcome the main limitation of conventional CGH, that is the low resolution. Theoretically, the resolution of an array-CGH slide is limited only by the genomic distance between each DNA probe represented on the array (density of the probe) and by the size of the spotted sequences. However, it would be misleading to calculate the array resolution based on the mean of distances between probes. In fact, array elements may be not evenly distributed throughout the genome and some platforms may require multiple probes to detect an alteration [(4)]. A functional measure of resolution can be the size limit of detecting a segmental copy number alteration. Basic principles of array-CGH resembles

that of conventional CGH. Total genomic DNA obtained from a test sample (DNA test) and from control cells (DNA reference) are differentially labelled with green and red fluorochromes, then co-precipitated in presence of Cot-1 DNA to block repetitive sequences and co-hybridised onto an array. The slide is subsequently analysed with an array scanner and images are digitally quantified with dedicated softwares (Fig.1). The ratio of fluorescent intensities for each probe represented on the slide is normalized and plotted against the genome sequence position. Therefore, array-CGH allows to identify genomic copy number alterations. In addition, given that measurements can be referred directly to the positions on the genome it is possible to directly characterized the breakpoints of the rearrangement. The resolution of the experiment depends on the resolution of the array.

The main advantages in array-CGH technique application compared to conventional cytogenetic and other molecular cytogenetic approaches are: i) it is not required to culture cells; ii) the resolution is extremely high (virtually, it is possible to design arrays covering any target chromosomal region with any desired resolution); iii) the whole-genome may be analysed in a single experiment (with a screening potentiality equivalent to that of thousand FISH experiments); iv) sensitivity and specificity are very high [5 6]. Although array-CGH has proved to be an efficient and reproducible technique, the structural configuration of the abnormal chromosomes can not be characterized. The order and the orientation of the rearranged segments cannot be determined and also low levels of mosaicism may be difficult to detect (fig. 4). These limitations depend on the general principles of the methodology, while array-CGH performances may be strongly dependent also from the type of array-CGH platform employed [7].

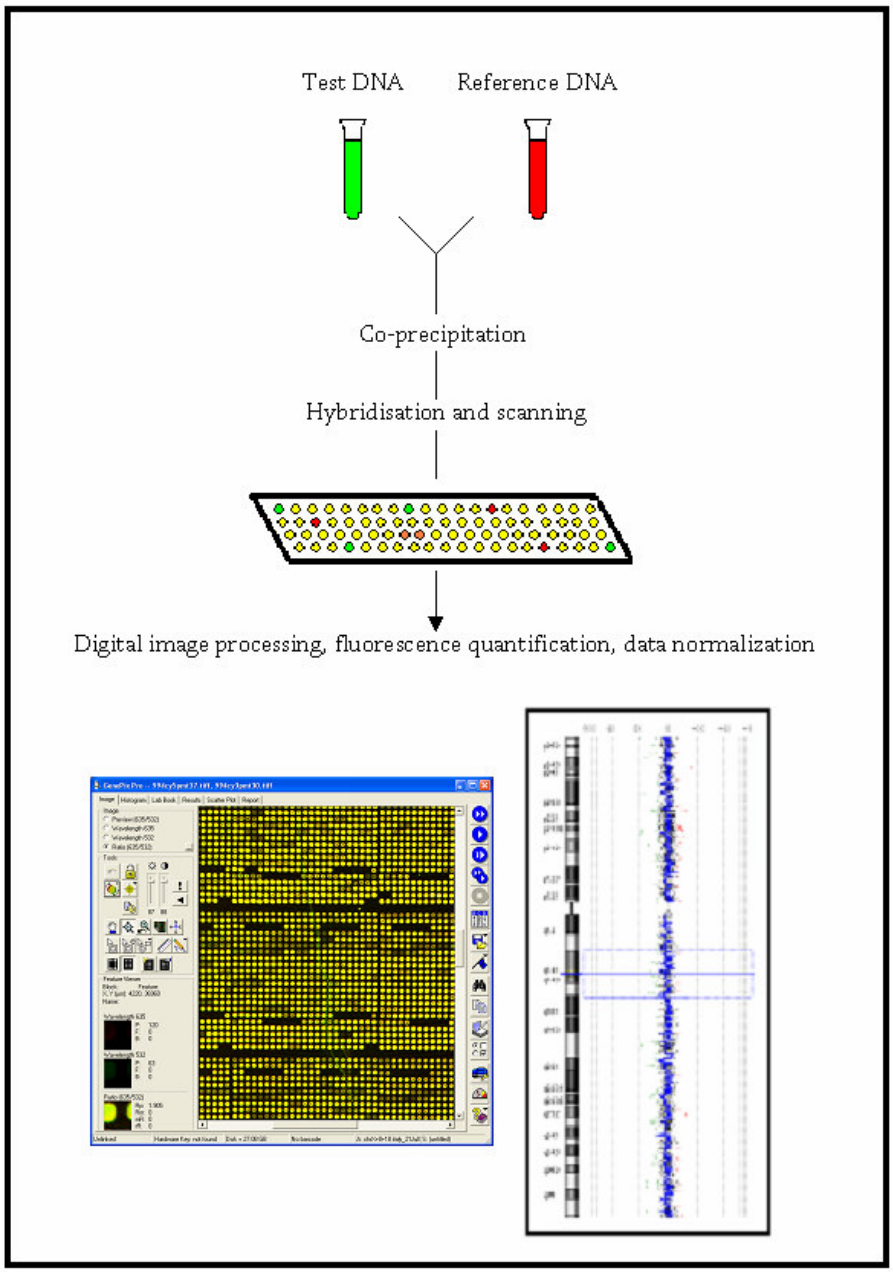

Fig.1. Schematic representation of an array-CGH experiment. Test and reference DNA are differentially labelled, co-precipitated and hybridised to an array. After wash procedures, the slides are analysed through a scanner and fluorescence intensities of each probe are determined. After imaging processing and data normalization, the log2 ratios of the probes are plotted as a function of chromosomal position. Probes with a value of zero represent equal fluorescence intensity ratio between sample and reference. Each dot represents a single probe spotted on the array. In this representation, copy number losses shift the ratio to the left and copy number gains shift the ratio to the right.

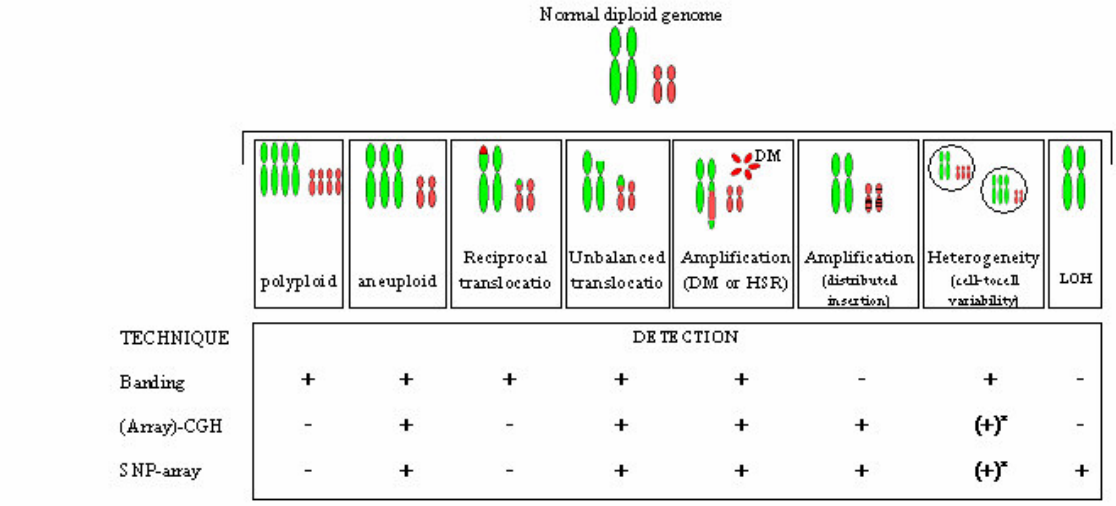

Fig.2. **Comparison of standard banding techniques and array-based approaches for identifying chromosomal abnormalities.** Various chromosomal aberrations that might be present in clinical samples are shown. The "+" symbol indicates that the technique is suited for identifying the chromosomal imbalance; the "-" symbol indicates that the aberration would be missed. (+)* indicates that a numerous of small subpopulations has to be analysed to detect the aberration. DM=double minutes; HSR= homogeneously stained region (modified from [8]).

Oligo Array-CGH

Oligonucleotide arrays were introduced to detect single-nucleotide polymorphisms (SNP) [9]. This array type contains 21-25 mer probes synthesized using a photolithographic method. Each SNP is represented on the array by several different probes that interrogate the site both on the sense and the antisense strand [9 10]. Bignell et al (2004) optimized a method to employ SNP-arrays for copy number variations analysis, using a different strategy for target preparation [9]. Sample DNA was prepared using whole-genome sample assay (WGSA), a PCR-based method performed to enrich sample DNA for small XbaI restriction sites. This method reduces sample complexity prior to hybridization and decreases the probability of cross-hybridization when small 25-mer probes are used. In SNP-arrays applications the test and the normal samples are hybridized to different arrays. The SNP platform allows the identification of deletions/duplications but shows greater variation in detection capability and a lower signal to noise ratio with respect to BAC arrays [4 9 11]. The advantage of this approach is the possibility to relate copy-number and allelic status at selected loci. Subsequently, oligonucleotides spotted arrays containing longer probes (60-70 mer) were developed. The use of typically 60-mer probes increases hybridisation specificity and improves the signal to background ratio to a level that is comparable to that of BAC arrays on a whole-genome scale. In addition, oligonucleotides-libraries are cheaper, easier to work with and faster than cDNAs or BAC/PAC clones, because no DNA isolation or PCR amplification steps are necessary [10 12 13]. This array-CGH platform can reproducibly detect genomic lesions, including single copy and homozygous deletions with an extremely high resolution [12 14-16]. Whole genome oligonucleotide arrays with a resolution of about 35 kb and 16 kb are currently commercially available.

Array-CGH and MCA-MR

Several studies have shown the potency of array CGH as a diagnostic tool. Up to now, thirteen studies performing array-CGH in several patients with idiophatic mental retardation and multiple congenital anomalies are reported [17]. In particular, Menten et al (2006) reported 1 Mb resolution array-CGH evaluation of 140 patients with mental retardation and multiple congenital anomalies [18]. They identified chromosomal imbalances in about 20% of patients. The 13.5% of the imbalances were causative of the phenotype and, excluding those aberrations that can also be detectable by subtelomeric screening, array-CGH analysis was able to identify the causative aberration in the 9% of patients. Up to now, considering all the analysed patients, de novo causative chromosomal imbalances were detected by array-CGH, in about the 9% of cases with a normal analysis of karyotype and subtelomeric regions [17].

DECIPHER database

The introduction of array-CGH allowed to detect submicroscopic chromosomal imbalances across the entire genome in patients with idiopathic MCA/MR. Its application led also to deal with emerging information about large copy number variations in normal human populations. Consequently, it was necessary to improve our knowledge about the type and the frequency of normal variations in the human genome. Several databases containing information on genomic variability in normal individuals are available on line, such as the database of Genomic Variants (http://projects.tcag.ca/variation/). Another important tool is the DECIPHER database, where users, beside the polymorphic variants, can also visualize pathogenic imbalances and the linked phenotype. DECIPHER is an acronym for **D**atabase of **C**hromosomal **I**mbalances and **P**henotype in **H**umans using **E**nsembl **R**esource. The DECIPHER database is a powerful molecular cytogenetic database for clinicians and researches linking genomic microarray data with phenotype using the Ensembl genome browser (http://www.sanger.ac.uk/PostGenomic/decipher/). This database is able to visualise the chromosomal location of the clones which are found to be deleted or duplicated in an

array-CGH analysis and eventually similar deletions or duplications that have been previously reported by any members of the Decipher consortium. The availability of a detailed description of the phenotype of each inserted patient allows to compare the phenotype of the patients with similar rearrangements. In particular, the phenotype terms are selected interactively from the London Neurogenetics and Dysmorphology database. The availability of this accurate description is of enormous value in determining the potential significance of copy number changes identified by array. As well as showing the existence of patients with the same or similar microdeletion/duplication, DECIPHER facilitates contacts with responsible clinicians in contributing centres, thereby accelerating the recognition of novel syndromes. The database lists all of the known and predicted genes that are implicated in an altered region, including OMIM genes. In particular, genes of established high significance can thus be identified immediately. This visualization of the known genes, putative genes and expressed-sequence tags (ESTs) within the deleted/duplicated region that can be see in the database is very useful to perform *in silico* analysis and to find the function of genes of unknown function. In addition, it is possible to visualise directly whether the imbalances characterized in a patient coincide with a recognized polymorphism. Recently, a tiling-path BAC array was used, together with an oligonucleotide array (Affimetrix 500K) that genotypes 500000 single nucleotide polymorphisms (SNP) to compare the genome of 270 individuals from different populations [(19)]. The analysis identified 1447 copy-number variations occurring across 12% (360Mb) of the human genome, including hundreds of genes and disease loci [(19)]. The CNVs visualized by the DECIPHER database can be used in order to align any identified imbalance and eventually to individuate those that are pathogenic, excluding the polymorphic ones.

The sharing content of DECIPHER database with the scientific community may allow progress in understanding the genetic bases of the phenotypes associated with copy number changes, opening opportunities for gene identification and for refining genotype-phenotype correlation.

2. RATIONALE, AIM and OUTLINE

2. RATIONALE, AIM and OUTLINE OF THE STUDY

Chromosomal abnormalities are a major cause of mental retardation and congenital malformations. It is known that a considerable fraction of patients with multiple congenital anomalies and mental retardation have submicroscopic chromosomal imbalances. The introduction of whole genome array-CGH allows to investigate the DNA for the presence of copy number alterations with high resolution. In patients with multiple congenital anomalies and mental retardation, 15-24% of segmental aneusomies were reported. Given these data, we have decided to set up the array-CGH technique in our laboratory in order to investigate for the presence of submicroscopic imbalances patients with mental retardation and multiple congenital anomalies, normal analysis of G-banded karyotype and subtelomeric regions.

Firstly, we have worked on the set up of the array-CGH protocols for a commercially available oligonucleotide array with 44.000 probes and an average resolution of about 75 kb (44K, Agilent). By this method we have analyzed a cohort of 32 patients. In this cohort we have identified four novel *de novo* chromosomal deletions, four known deletions in atypical patients, one reciprocal duplication and two inherited imbalances (one deletion and one duplication). In particular, we have identified two 2q non overlapping interstitial deletions, one in a female patient with developmental delay, severe seizures and dysmorphic features and the other one in a male patient with severe mental retardation and behavioural problems (Result 4.1.1). Then, we have identified a 6q deletion of 2.6 Mb in a female patient with growth failure, atrial septal defect and moderate mental retardation. Finally, we have found a 7q36.2 deletion in a female case with mild mental retardation, renal hypoplasia and cardiac defects. (Result 4.1.1).

Subsequently, we have set up an higher resolution array consisting of 105,000 probes with a resolution of about 16 kb (105K, Agilent). By this method we have studied cases with already well known deletions to better define the deletion breakpoints. We performed an *in silico* analysis of the gene content of the deleted region to select genes potentially involved in generating the phenotype (Result 4.2). In particular, we have compared the deletion present in a familial case with X-linked ichthyosis (XLI) and MR in probands and isolated ichthyosis in maternal uncles to that present in a sporadic case with XLI and Kallmann syndrome (KAL) (Result 4.2.1**).** In addition, we have characterised two patients with 13q deletion syndrome and another

one with isolated retinoblastoma in order to narrow the critical region responsible for mental retardation and to identify the causative gene(s). We have also studied another case with retinoblastoma and developmental anomalies that shows only a maternally inherited 7q deletion (Result 4.2.2).

This study allowed the characterization of several chromosomal imbalances in patients with complex phenotype, confirming the power of the array-CGH method to clarify the molecular basis of these difficult cases. Through the employment of this innovative approach, several families finally received a definitive diagnosis and a correct recurrence risk.

All patients described in this thesis have been inserted in the DECIPHER database (http://www.sanger.ac.uk/PostGenomic/decipher/) (Fig.3).

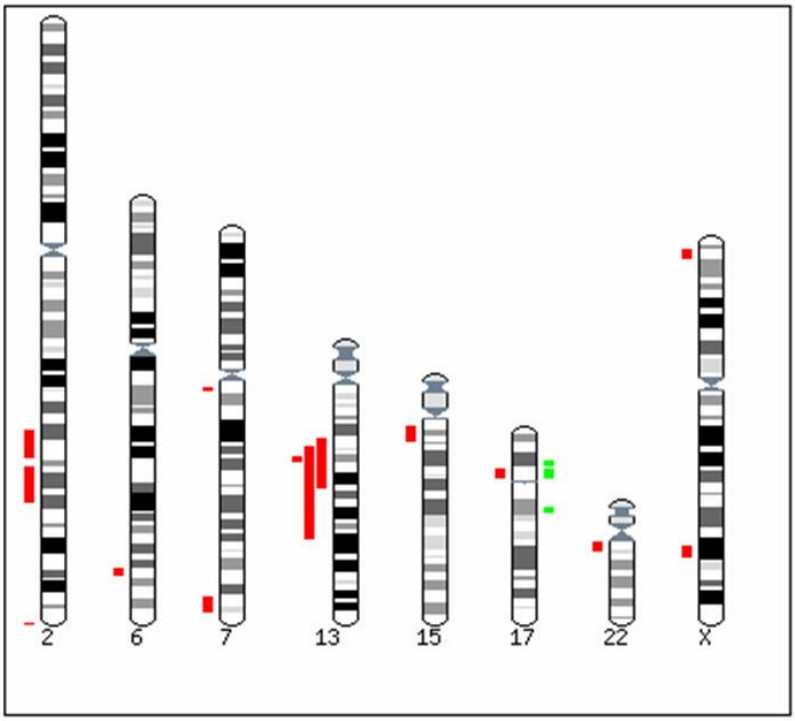

Fig.3. Ideogram of copy number changes of our patients that are inserted in the DECIPHER database. Red bars denote deletions; green bars denote duplications.

3. MATERIALS and METHODS

3. MATERIALS & METHODS

Patients collection

Patients with mental retardation and multiple congenital anomalies enrolled in this study have been selected among those attending the Medical Genetics Unit of the University of Siena. Patients were selected among those having mental disability without known etiology, in association with one or more major congenital anomalies, or dysmorphisms or both, and a normal karyotype on G banding analysis. A broad spectrum of clinical phenotyping measures, including a comprensive and standardized clinical genetic evaluation was applied. Physical examination, craniofacial anthropometry, standardized somatic morphological characterization and 2-D photogrammetry were performed. Comparison was made between the growth of different parts of the body, for example, to see whether head circumference, height and weight are at the same percentile or at different percentiles. Measurement to graphs in percentile instead of standard deviation was used. Specific neuropsychological measures were performed using Wechsler scale and Vineland test.

For each case enrolled in the study DNA samples of probands and both parents were collected.

Array-based CGH

Samples preparation

Genomic DNA of normal controls was obtained from Promega. Genomic DNA was extracted from peripheral blood using a QIAamp DNA Blood Maxi kit according to the manufacturer protocol (Qiagen, www.qiagen.com). The OD260/280 method on a photometer was employed to determine the appropriate DNA concentration [20]. Patient and control DNA samples were sonicated to produce a homogeneous smear DNA extending from approximately 600 bp to 2 kb. DNA samples were then purified using the DNA Clean and Concentrator kit (Zymo Research, Orange, CA). Ten micrograms of genomic DNA both from the patient and from the control were sonicated. Test and reference DNA samples were subsequently purify using dedicated columns (DNA

Clean and Concentrator, Zymo research, CA92867-4619, USA) and the appropriate DNA concentrations were determine by a DyNA Quant™ 200 Fluorometer (GE Healthcare).

Human oligonucleotides array

Array based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 43.000 60-mer probes with an estimated average resolution of 75 kb (Human Genome CGH Microarray 44B Kit, Agilent Technologies). DNA labelling was executed essentially according to the Agilent protocol (Oligonucleotide Array-Based CGH for Genomic DNA Analysis 2.0v) using the Bioprime DNA labelling system (Invitrogen). Genomic DNA (2 µg) was mixed with 20 µl of 2.5X Random primer solution (Invitrogen) and MilliQ water to a total volume of 41 µl. The mix was denatured at 95° C for 7 minutes and then incubated in ice/water for 5 minutes. Each sample was added with 5 µl of 10X dUTP nucleotide mix (1.2 mM dATP, dGTP, dCTP, 0.6 mM dTTP in 10 mM Tris pH 8 and 1 mM EDTA), 2.5 µl of Cy5-dUTP (test sample) or 2.5 µl of Cy3-dUTP (reference sample) and with 1.5 µl of Exo-Klenow (40 U/µl, Invitrogen). Labeled samples were subsequently purified using CyScribe GFX Purification kit (Amersham Biosciences) according to the manufacturer protocol. Test and reference DNA were pooled and mixed with 50 µg of Human Cot I DNA (Invitrogen), 50 µl of Blocking buffer (Agilent Technologies) and 250 µl of Hybridization buffer (Agilent Technologies). Before hybridization to the array the mix was denatured at 95° C for 7 minutes and then pre-associated at 37°C for 30 minutes. Probes was applied to the slide using an Agilent microarray hybridization station. Hybridization was carried out for 40 hrs at 65° in a rotating oven (20 rpm). The array was disassembled and washed according to the manufacturer protocol with wash buffers supplied with the Agilent 44B kit. The slides was dried and scanned using an Agilent G2565BA DNA microarray scanner. Image analysis was performed using the CGH Analytics software v. 3.1 with default settings. The software automatically determines the fluorescence intensities of the spots for both fluorochromes performing background subtraction and data normalization, and compiles the data into a spreadsheet that links the fluorescent signal of every oligo on the array to the oligo name, its position on the array and its position in the genome. The linear order of the oligos is reconstituted in the ratio plots consistent with an ideogram. The ratio plot is arbitrarily assigned such that

gains and losses in DNA copy number at a particular locus are observed as a deviation of the ratio plot from a modal value of 1.0.

For the Human Genome CGH Microarray 105K Kit, Agilent Technologies, the following changes have been applied: DNA labelling was executed essentially according to the Agilent protocol "Oligonucleotide Array-Based CGH for Genomic DNA Analysis 4.0v" using Agilent Genomic DNA Labeling Kit PLUS.

Array–CGH data were confirmed by an independent method: Real-time Quantitative PCR or Multiplex Ligation-dependent Probe Amplification.

Real-time quantitative PCR

To design adequate probes in different regions of the human genome, we used an TaqMan Gene Expression Assays by design which provides pre-designed primers-probe set for real-time PCR experiments (Applied Biosystems, https://products.appliedbiosystems.com). PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 µl. A total of 100 ng (10 µl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a prerun of 2 min at 50°C and 10 min at 95°C. Cycle conditions were 40 cycles at 95°C for 15 sec and 60°C for 1 min according to the TaqMan Universal PCR Protocol (ABI). The TaqMan Universal PCR Master Mix and Microamp reaction tubes were supplied by Applied Biosystems. The starting copy number of the unknown samples was determined using the comparative Ct method as previously described [21].

Multiplex Ligation-dependent Probe Amplification (MLPA)

DNA of the patients was isolated from an EDTA peripheral blood sample by using a QIAamp DNA Blood Kit according to the manufacturer protocol (Qiagen, www.qiagen.com). The Hoechest dye binding assay was used on a DyNA Quant™ 200 Fluorometer (GE Healthcare) to determine the appropriate DNA concentration.

MLPA analysis was performed according to the provider's protocol with a specifically designed set of probes for testing DiGeorge (SALSA P023 kit; MRC-Holland, Amsterdam, Netherlands; http://www.mrc-holland.com) and Smith-Magenis (SALSA P064B MR1; MRC-Holland) critical regions. The ligation products were amplified by PCR using the common primer set with the 6-FAM label distributed by the supplier. Briefly, 100 ng of genomic DNA was diluted with TE buffer to 5 µl, denatured at 98°C for 5 minutes and hybridized with SALSA Probe-mix at 60°C overnight. Ligase-65 mix was then added and ligation was performed at 54°C for 15 minutes. The ligase was successively inactivated by heat (98°C for 5 minutes). PCR reaction was performed in a 50 µl volume. Primers, dNTP and polymerase were added and amplification was carried out for 35 cycles (30 seconds at 95°C, 30 seconds at 60°C and 60 seconds at 72 °C).

Amplification products were identified and quantified by capillary electrophoresis on an ABI 310 genetic analyzer, using GENESCAN software (version 3.7) all from Applied Biosystems (Foster City, CA, USA). The peak areas of the PCR products were determined by GENOTYPER software (Applied Biosystems). A spreadsheet was developed in Microsoft™ Excel in order to process the sample data efficiently. Data were normalized by dividing each probe's peak area by the average peak area of the sample. This normalized peak pattern was divided by the average normalized peak pattern of all the samples in the same experiment [22].

4. RESULTS

4.1 Analysis of a cohort of MCA/MR patients

4.1.1 Novel deletions

Case 1:

2q24-q31 deletion: report of a case and review of the literature.

Pescucci C, Caselli R, Grosso S, Mencarelli MA, Mari F, Farnetani MA, Piccini B, Artuso R, Bruttini M, Priolo M, Zuffardi O, Gimelli S, Balestri P, Renieri A.

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Original article

2q24–q31 Deletion: Report of a case and review of the literature

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Abstract

We report a patient with a de novo interstitial deletion of the long arm of chromosome 2 involving bands 2q24.3–q31.1. The patient shows postnatal growth retardation, microcephaly, ptosis, down-slanting palpebral fissures, long eyelashes and micrognathia. Halluces are long, broad and medially deviated, while the other toes are laterally deviated and remarkably short with hypoplastic phalanges. She also showed developmental delay, seizures, lack of eye contact, stereotypic and repetitive hand movements and sleep disturbances with breath holding. Prenatal and three independent postnatal karyotypes were normal. Array-CGH analysis allowed us to identify and characterize a "de novo" 2q interstitial deletion of about 10.4 Mb, involving segment between cytogenetic bands 2q24.3 and 2q31.1. The deletion was confirmed by quantitative PCR. About 30 children with 2q interstitial deletion have been reported. The deletion described here is overlapping with 15 of these cases. We have attempted to compare the clinical features of our patient with 15 overlapping cases. The emerging phenotypes include low birth weight, postnatal growth retardation, mental retardation and developmental delay, microcephaly, and peculiar facial dysmorphisms. Peculiar long and broad halluces with an increased distance between the first and the second toe are ("sandal gap" sign) present in most of the described patients. The gene content analysis of the deleted region revealed the presence of some genes that may be indicated as good candidates in generating

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both neurological and dysmorphic phenotype in the patient. In particular, a cluster of *SCNA* genes is located within the deleted region and it is known that loss of function mutations in *SCNA1* gene cause a severe form of epilepsy.

Keywords: Chromosome 2; Mental retardation; Digital anomalies; Array-CGH; 2q interstitial deletion

1. Introduction

To date, more than 100 cases with a deletion of the long arm of chromosome 2 have been identified through standard cytogenetic analysis. In particular, over 70 patients with a terminal and over 30 with an interstitial deletion have already been reported [5,7]. The most frequent interstitial deletion involves the cytogenetic bands 2q31–q33 and corresponds to a specific phenotype [25,30]. Deletions involving different segments of the long arm of chromosome 2 are characterized by variability in breakpoints location and are clinically heterogeneous. Only few cases show overlapping deletions, although of quite different extensions. Moreover, most of the reported cases with 2q interstitial deletion were analysed with standard cytogenetic techniques and shows a poor definition of breakpoints. Given these data, genotype–phenotype correlations in cases with a 2q deletion not involving 2q31–q33 bands is more difficult.

We here report clinical description and molecular data of a new patient with a de novo 2q24–q31 deletion and compare her features with the phenotypes of other patients with overlapping deletions. The location of the chromosomal breakpoints and the size of the deleted region were identified by array-based Comparative Genomic Hybridisation (array-CGH) method.

1.1. Case report

The patient, a 4 years and 2 months old girl, is the third child of healthy unrelated parents. At birth, mother and father were 40 and 42 years old, respectively. Two brothers, 19 and 14 years old, are healthy. Family history is unremarkable. No teratogen exposure during pregnancy was reported. The girl was born after 35 weeks of gestation. Apgar scores were 9 at 1 min and 9 at 5 min, birth weight was 1950 g (10th–25th percentile), length was 45 cm (25th–50th percentile) and head circumference was 31 cm (25th percentile). At birth, clinical examination showed cleft palate, corrected at 11 months. At 6 months gastroesophageal reflux was diagnosed. Partial seizures, with secondary generalization, occurred at 3 months. EEG investigations showed slow background with high amplitude delta waves mixed with spikes and sharp waves on the temporo-occipital areas. She had a severe neuromotor developmental delay: she gained head control at 7 months, and began to sit alone at 3 years of age. Presently, at the age of 4 years and 2 months, she is still unable to walk and speech is absent. Daily partial seizures occurred during the first years of life in spite of several antiepileptic drugs used in different therapeutic combinations. She was first admitted to our medical unit at the age of 3.9 years. Her weight was 10 kg (−2.6 DS), head circumference 43.5 cm (−2.7 DS), and length 78 cm (−3 DS). At our physical examination she had hypotelorism, down-slanting palpebral fissures, long eye lashes, ptosis, high nasal bridge with large nose, thick helices and ear lobule, mild micrognathia, cupid

bow mouth, tapering fingers with clinodactyly of the fifth finger (Fig. 1). Medially deviated, broad and long halluces were present. The other toes were deviated laterally, resulting in an increased distance between the first and the second toe. Other toes were remarkably short with hypoplastic phalanges (Fig. 2). She also presented lack of eye contact, severely impaired visual-motor coordination, stereotypic and repetitive movements of the hands, bruxism and sialorrhea. The patient showed sleep disturbances characterized by breath holding and episodes of screaming, with opisthotonus and eye deviation towards the right side, followed by secondary generalization with tonic or tonic–clonic seizures. Moreover, flushing and hypertonia at the upper limbs with flexion of the right arm and extension of the contralateral arm were observed. Oral automatisms appeared during the crisis, sometimes. Sporadic myoclonic jerks were also present. In the clinical course, seizures became more polymorphic with both focal and generalized seizures. EEGs recorded slow background, sharp waves of high amplitude in the frontal region, spikes and spikes–waves in the fronto-central areas, bilaterally. The review of the EEG investigations, represented by intercritical recordings, was consistent with the clinical manifestations. In fact, temporo-occipital paroxysmal activity was persistently recorded during the first year of life. Subsequently, epileptiform activity shifted towards the anterior areas (fronto-central regions) with evident secondary generalization. Seizures were resistant to several antiepileptic drugs in different combinations. Recently, the association of fenobarbital and levetiracetam was able to considerably reduce seizure frequency (Fig. 1).

At the time of our second evaluation, at the age of 4 years and 2 months, growth parameters were unchanged. Development was still severely delayed and seizures remained uncontrolled by drug therapy.

Screening for neurometabolic disorders, organic acids, and amino acids levels were normal or negative as were serum and CSF lactate and pyruvate levels. MRI showed enlarged lateral ventricles and diffuses cortical atrophy associated with incomplete white matter myelination.

Four independent karyotypes were performed, one prenatal and three postnatal executed in three different centers (major resolution been 550 bands). Sequence analysis of exons 2, 3 and 4 of the *MECP2* gene showed no mutation.

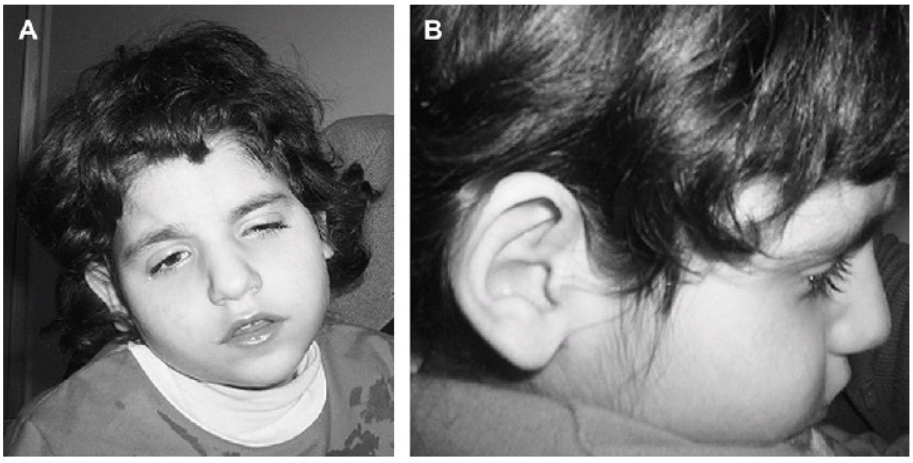


Fig. 1. Photographs of the patient at the age of 4 years and 2 months. (A) Frontal view showing hypotelorism, downslanting palpebral fissures, ptosis, high nasal bridge with large nose, cupid bow mouth. (B) Side view showing long eye lashes, thick helices and ear lobule.

2. Materials and methods

2.1. Genomic DNA isolation, labelling and hybridization

Genomic DNA of a normal female control was obtained from Promega. Genomic DNA of the patient was isolated from an EDTA peripheral blood sample by using a QIAamp DNA Blood Kit according to the manufacturer protocol (Qiagen, www.qiagen.com). We used the OD260/280 method on a photometer to determine the appropriate DNA concentration [26].

Array-based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 43.000 60-mer probes with an estimated average resolution of about 75 kb (Human Genome CGH Microarray 44B Kit, Agilent Technologies). DNAs (7 μg) of the patient and controls of the same sex (Promega) were double-digested with RSAI and AluI for 2 h at 37°. After columns purification, the appropriate DNA concentrations were determined by a DyNA Quant™ 200 Fluorometer (GE Healthcare, www.gehealthcare.com). DNA labelling was executed essentially according to the Agilent protocol (Oligonucleotide Array-Based CGH for Genomic DNA Analysis 2.0v) using the Bioprime DNA labelling system (Invitrogen). Genomic DNA (2 μg) was mixed with 20 μl of 2.5X Random primer solution (Invitrogen) and MilliQ water to a total volume of 41 μl. The mix was denaturated at 95 °C for 7 min and then incubated in ice/water for 5 min. Each sample was added with 5 μl of 10X dUTP nucleotide mix (1.2 mM dATP, dGTP, dCTP, 0.6 mM dTTP in 10 mM Tris, pH 8, and 1 mM EDTA), 2.5 μl of Cy5-dUTP (test sample) or 2.5 μl of Cy3-dUTP (reference sample) and with 1.5 μl of Exo-Klenow (40 U/μl, Invitrogen). Labeled samples were subsequently purified using CyScribe GFX Purification kit (Amersham Biosciences) according to the manufacturer protocol. Test and reference DNA were pooled and mixed with 50 μg of Human Cot I DNA (Invitrogen), 50 μl of Blocking buffer (Agilent Technologies) and 250 μl of hybridization buffer (Agilent Technologies). Before hybridization to the array, the mix was denatured at 95 °C for 7 min and then pre-associated at 37 °C for 30 min. Probes were applied to the slide using an Agilent microarray hybridization station. Hybridization was carried out for 40 h at 65° in a rotating oven (20 rpm). The array was disassembled and washed according to the manufacturer protocol with wash buffers supplied with the Agilent 44B kit. The slides were dried and scanned using an Agilent G2565BA DNA microarray scanner.

2.2. Image and data analysis

Array was analysed through the Agilent scanner and the Feature Extraction software (v8.1). The software automatically determines the fluorescence intensities of the spots for both fluorochromes performing background subtraction and data normalization, and compiles the data into a spreadsheet that links the fluorescent signal of every probe on the array to the oligonucleotide name, its position on the array and its position in the genome. Graphical overview was obtained using the CGH analytics software (v3.2.32) with default settings (aberration algorithm: z-score, moving average with window of 0.5 Mb, cut-off 1.25). The linear order of the oligonucleotides is reconstituted in the ratio plots consistent with an ideogram. The ratio plot is arbitrarily assigned such that gains and losses in DNA copy number at a particular locus are observed as a deviation of the ratio plot from a modal value of 1.0.

2.3. Real-time quantitative PCR

Real-time quantitative PCR was performed to confirm array-CGH data. We used a TaqMan Gene Expression Assay by design (Applied Biosystems, http://products.appliedbiosystems.com) which provides pre-designed primers-probe set for real-time PCR experiments. We designed the probe in the exon 2 of the Nostrin gene related to the 2q24.3 locus. Nostrin exon 2 forward primer: 5′-GCC AGA AAG TGG TGA TGC AAA-3′; Nostrin exon 2 reverse primer: 5′-CAA AGC TTG GAC TAA GTT C-3′; Nostrin exon 2 TaqMan probe: 5′-GCA GGC AGT GCA GAA CAG AT-3′. PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 μl. A total of 100 ng (10 μl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a prerun of 2 min at 50 °C and 10 min at 95 °C. Cycle conditions were 40 cycles at 95 °C for 15 s and 60 °C for 1 min according to the TaqMan Universal PCR Protocol (Applied Biosystems). The TaqMan Universal PCR Master Mix and Microamp reaction tubes were supplied by Applied Biosystems.

The starting copy number of the unknown samples was determined using the comparative Ct method as previously described [2].

3. Results

The association of developmental delay, epilepsy and dysmorphic features prompted us to search for genomic rearrangements. Seventy-five kilobase resolution array-CGH experiments were performed. Despite the normal result of karyotyping, the analysis revealed a chromosome 2 interstitial deletion of 10.29–10.58 Mb (46,XX,del(2)(q24.3q31.1)) (Fig. 3A). The proximal breakpoint is mapped in 2q24.3 (last oligonucleotide present located in 165.47 Mb, first deleted in 165.58 Mb position); while the distal breakpoint is located between 175.87 and 176.05 Mb in 2q31.1 (last oligonucleotide deleted and first present, respectively). To confirm array data, real-time quantitative PCR experiments was performed in the patient and in her parents. The deletion was confirmed in the patient, while the parents showed a normal result (Fig. 3B). Analysis of the gene content of the deleted region showed the presence of 58 known genes (Fig. 3C).

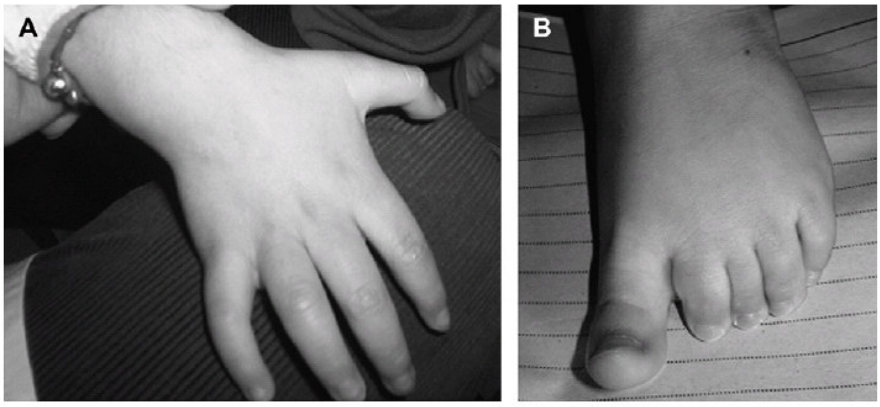
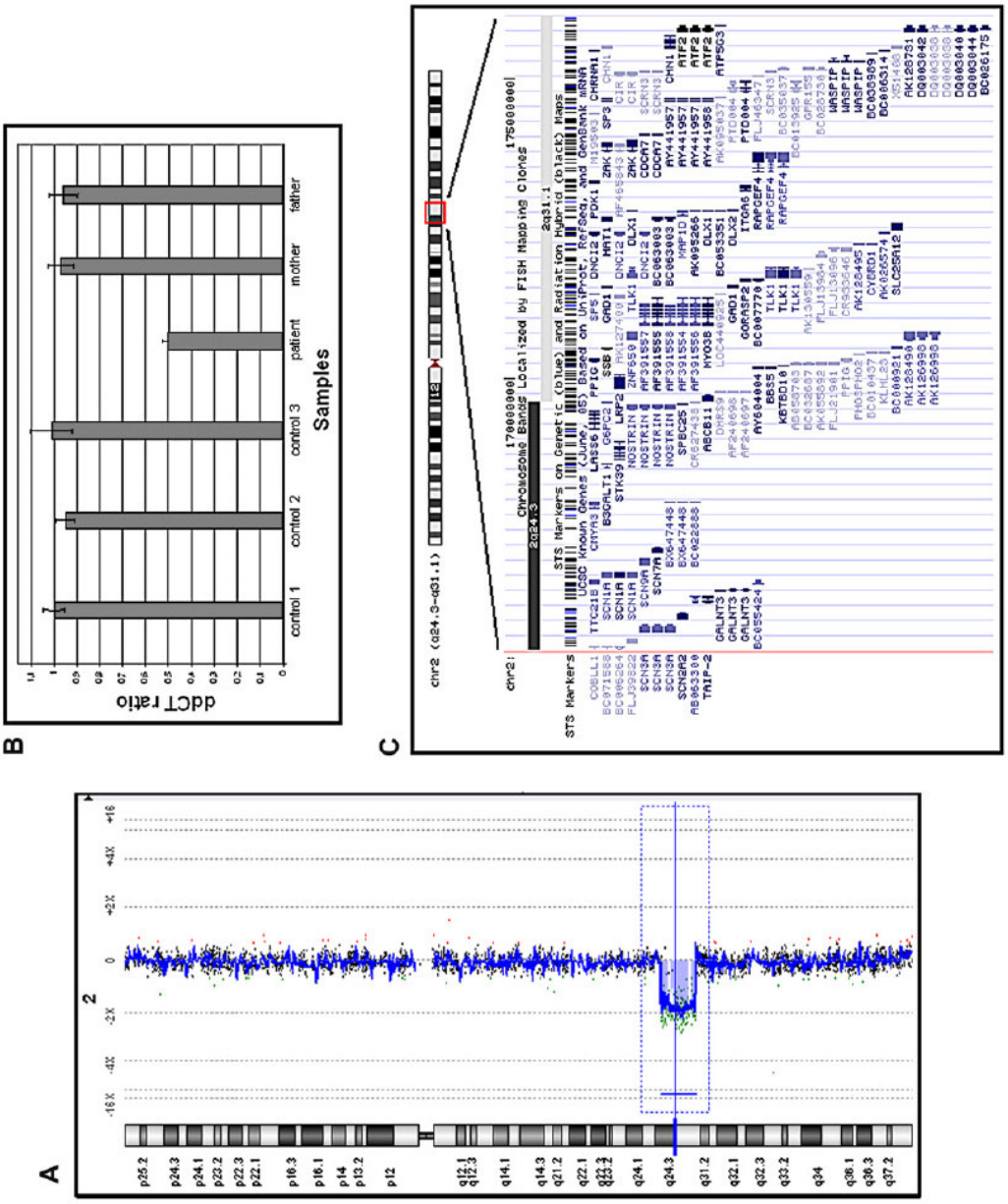


Fig. 2. Hands and feet anomalies in the proband. (A) View of the right hand showing the tapering fingers. (B) View of the right foot showing the broad and long halluces and the increased distance between the first and the second toe. Clinodactyly of the fifth finger is also present.


A
B
C
ddCT ratio
Samples
control 1
control 2
control 3
patient
mother
father
chr2
chr2 (q24.3-q31.1)
Chromosome Bands Localized by FISH Mapping Clones
STS Markers on Genetic (blue) and Radiation Hybrid (black) Maps

4. Discussion

Even though the extension of the chromosome 2 interstitial deletion present in the patient (about 10 Mb) is significantly higher than the resolution limit of standard cytogenetic techniques, three independent chromosomal analyses failed to identify it. Prenatal diagnosis performed on the fetus with standard cytogenetic techniques (Q banding with an estimate resolution of about 320 bands) showed a normal female karyotype. Retrospectively, the deleted region is located in bright Q banded 2q region, hardly to evaluate on a standard resolution level, as usually performed in prenatal diagnosis (Fig. 3).

After birth, given that the phenotype of the proband was strongly suggestive of a chromosomal imbalance, the cytogenetic analysis was performed on three distinct peripheral blood samples. In all cases the karyotype was reported as female normal. The deletion was subsequently revealed by the use of array-based Comparative Genomic Hybridization, a molecular cytogenetic technique with an extremely high resolution (about 75 kb). These data confirm the importance of a deeper investigation in patients in which the complex phenotype is strongly suggestive for the presence of a chromosomal aberration and point at array-CGH as a powerful technique for the identification of the rearrangements where conventional cytogenetic failed.

The patient shows a de novo interstitial deletion of the long arm of chromosome 2, involving a segment of band 2q24.3 and a segment of band 2q31.1. About 30 children with 2q interstitial deletion have been reported [4–6,8,10,11,18,19,21,22,25,27,28,31]. We have attempted to compare the deletion reported here with other previously described cases. Unfortunately, most of them are only cytogenetically studied with poorly defined breakpoints. In spite of these limits, the deletion described here is overlapping with 15 cases (Table 1). Considering these 15 patients, the emerging phenotype include low birth weight, postnatal growth retardation, mental retardation and developmental delay, microcephaly, down-slanting palpebral fissures, long eye lashes, micrognathia and low-set ears. Moreover, the presence of peculiar long and broad halluces with an increased distance between the first and the second toe is observed in 5 out of 15 patients and additional digital anomalies of hands and feet are present in all the reported cases (Table 1). These anomalies include camptodactyly and clinodactyly of fifth finger, syndactyly of the fingertoes, duplicated halluces and hypoplastic phalanges of the third, fourth and fifth finger [6]. These features were first associated with the 2q24–q31 deletion by Moller et al. [21]. In the reported case, the presence of medial deviation of the hallux and lateral deviation of the toes, brachysyndactyly, and clinodactyly of fifth finger confirm the association of digital anomalies with the 2q24–2q31 region. On the other hand, the vast majority of 2q deletion not overlapping with this region usually does not show significant digital anomalies, bearing to the conclusion that the common limb phenotype in deletions involving the region 2q24–q31 might be cause by genes in this area involved in distal limb morphogenesis, according to Boles et al. [6].

Fig. 3. Molecular data of the patient. (A) Array-CGH ratio profile. On the left, the chromosome 2 ideogram. On the right, the log2 ratio of chromosome 2 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about −2X). (B) Real-time quantitative PCR validation experiment. Nostrin ddCT ratios and standard deviations of three different controls and of the patient and her parents. The patient shows a ddCT ratio of about 0.5 that indicates the presence of a single copy of Nostrin (deletion), while parents as controls show ddCT ratios of about 1.0, that indicate a double copy of the gene. (C) Gene content of the deleted region (UCSC Genome Browser; http://genome.ucsc.edu).

Table 1
Clinical findings in 2q24–q34 deletions

Clinical findings	McConnell, 1980	Shabtai, 1982	Moller, 1984			Bernar, 1985	Wamsler, 1991	Boles, 1995	Chinen, 1996	Nixon, 1997	McMillin, 1998	Slavotinek, 1999		Maas, 2000	Bijlsma, 2005	Present, case
			Case A	Case B	Case C						Case 4	Case 1	Case 2			
Karyotype	q22–q31	q23–q34	q24.2–q31.05			q24.3	q24–q31	q24.2–q31.1	q24.3	q24.3–q32.1	q24.2–q31	q31.1–q31.3	q24.3–q31.3	q23–q24.3	q24.2–q32.2	q24.3–q31.1
Sex	F	F	F	F	M	M	F	M	F	M	M	F	M	M	F	F
Age	Died at birth	20 years	4 years and 11 months	3 years and 2 months	6 years	4 months	18 months	4 months		9 years	7 years	9 years	8 years	1 year and 6 months	Fetus	3 years 9 months
Birth weight < 5th centile	+	−				+	−	−	+	+	−	+	−	+		+
Growth failure		+	+	+	+	+	+	+	+	+		+	+	+		+
Developmental delay/mental retardation		+	+	+	+	+	+	+	+	+	+	+	+	+		+
Seizures			−	−	−	+	+	+			+	+	+	+		+
Microcephaly	−	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Short palpebral fissures	+		−	−	−		+	+		+	+	+	+			+
Down-slanting palpebral fissures		+	+	+	+	+	+	−	+	+		+	+	−		+
Ocular abnormalities		+, Cataracts	−	−	−	−	−	−		+, Coloboma iris, retina, optic nerve		+	+, Left convergent squint			
Micrognathia	+	+	+	+	+	+	+	+	+	+		+	+	+	−	+
Clef palate	+	+	−	−	−	−	High arched	+	+	High arched		+, Cleft uvula		High arched		+
Ear anomalies	+	+	+	+	+	+	+	+	+	+		+	+	+	+	
Brachysyndactyly			+	+	+		+	+		+		+	+		+	+
Increased distance between 1st and 2nd toes	+	+	+	+	+	−	+	+		+	+	+	+	−	+	+
Wide halluces			+	+	+		−	+				+	−			+
Long halluces			+	+	+		−	−				+	+		+	+
Clinodactyly of 5th finger			+	+	+		+	−		+		+	+			+
Cardiac anomalies	+	+	−	−	−	*	+	+	+	+			−	−	+	
Other anomalies	a	b			c			d	e	f	g	h	i		l	m

*. Heart murmur.

a. Scaphocephaly, occipital meningomyelocele, internal hydrocephalus.

b. Cataract, microphthalmia.

c. Turricephaly. Hand: postaxial polydactyly type A, absence of proximal phalanges of the first toes.

d. Hands: ulnar deviation of both hands. Feet: equinovarus, absent second digits and hypoplastic third, fourth and fifth digits, wide gap.

e. Occipital encephalocele, ventricular enlargement, lacunar skull defect.

f. Craniosynostosis, proptosis.

g. Sagittal synostosis marked ridging of the sagittal suture, dysplastic nails of the halluces.

h. Sex reversal: female external genitalia with hypoplasia of clitoris and labia minora.

i. Prominent metopic suture with bifrontal narrowing. Persistently open anterior fontanelle at age of 3 years, facial hirsutism and a small sacral dimple. Bilateral single palmar creases.

l. Contractures of both the elbows and the wrists, with webbing of the elbows. Right hand: only one digit presumed the fifth; left hand: only the fourth and fifth digits. Bilateral split foot. Right foot: absence of the second and third toes and soft tissue syndactyly between the fourth and the fifth toes. Left foot: absence of the third toe and soft tissue syndactyly between the hallux and the second toe and between the fourth and the fifth toes.

m. Feet: broad and long halluces with a wide gap between first and second toes. Third, fourth and fifth digits remarkably short with hypoplastic phalanges.

The gene content analysis of the deleted region shows the presence of 58 known genes. The cluster of genes *HOXD3–HOXD13* is reported as good candidate for limb anomalies in patients with a 2q24–q31 deletion [11]. In our patient, this cluster is excluded from the deleted region; however, she shows tapering fingers with clinodactyly of the fifth finger, broad and long halluces with a wide gap between first and second toes, and shortness of the other toes with hypoplastic phalanges. A hypothesis might be that the deleted region may contain regulatory elements for the *HOXD* genes that are located at an estimated distance of 0.8 Mb from the distal breakpoint [13]. Most reported cases described as 2q31–q33 deletions does not show major limb involvement as in our patient or in patients by Del Campo et al. [11]. One explanation might reside in an erroneous definition of breakpoints and in particular of the proximal one. Alternatively, these deletions might not involve *HOXD* cluster being located immediately telomeric to it. To confirm this hypothesis a patient with an overlapping deletion to ours (2q22–q31) shows the peculiar first toes phenotype with cleft between first and second toes [9]. The absence of such regulatory elements could explain the milder digital anomalies observed in the patient, with respect to other patients with a deletion including the *HOXD* cluster [11].

DLX1 and *DLX2* genes are located in the deleted region. The highly conserved *Dlx1*, *2*, *5* and *6* homeobox transcription factors are involved in the regulation of basal ganglia and cortical local circuit neurons development [1]. Mice lacking *Dlx1* show epilepsy due to the apoptotic death of cortical neurons and murine *Dlx* genes have a central role in controlling the development and function of forebrain GABAergic neurons [1,9,29]. The *DLX* genes are clustered on chromosome 2q (*DLX1*/*DLX2*) and on chromosome 7q (*DLX5*/*DLX6*) [14]. Since these genes play a major role in control craniofacial patterning and differentiation and survival of forebrain inhibitory neurons [12,23], *DLX1*/*DLX2* haploinsufficiency might indeed be responsible for both neurological and dysmorphic phenotype in the patient.

A cluster of *SCN* genes including *SCN3A*, *SCN2A2*, *SCN1A*, *SCN9A*, and *SCN7A* is located within the deleted region. These genes codify for distinct alfa subunits of voltage-gated sodium channels and are involved in different types of epilepsy [3,24,16,17]. Missense mutations in *SCN1A* are associated with GEFS+ (generalized epilepsy with febrile seizures plus; OMIM#604233) a mild, dominantly inherited epilepsy. Non-sense and frameshift *SCNA1* mutations cause SMEI (Severe Myoclonic Epilepsy of Infancy; OMIM#182389) that is often associated with mental deterioration [15]. The patient described here shows a worsening of the seizures during the clinical course, with both focal and generalized seizures. The monosomy for *SCN* genes cluster may be responsible for the severe form of epilepsy observed in the patient, given that loss of function *SCNA1* heterozygotic mutations are responsible for severe epilepsy phenotypes [20]. To establish whether an electroclinical pattern distinctive of patients with a 2q24–q31 deletion exists, clinical and EEG studies are necessary in other patients with overlapping deletions.

In the light of literature data and of the clinical and molecular features of our proband, we suggest to accurately evaluate the 2q24–q31 region in that cases presenting a syndromic condition with mental retardation, developmental delay, severe epilepsy and digital anomalies.

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References

[1] S.A. Anderson, D.D. Eisenstat, L. Shi, J.L. Rubenstein, Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes, Science 278 (1997) 474–476.

[2] F. Ariani, F. Mari, C. Pescucci, I. Longo, M. Bruttini, I. Meloni, et al., Real-time quantitative PCR as a routine method for screening large rearrangements in Rett syndrome: report of one case of *MECP2* deletion and one case of *MECP2* duplication, Hum. Mutat. 24 (2004) 172–177.

[3] S. Baulac, I. Gourfinkel-An, F. Picard, M. Rosenberg-Bourgin, J. Prud'homme, F. Baulac, et al., A second locus for familial generalized epilepsy with febrile seizures plus maps to chromosome 2q21–q33, Am. J. Hum. Genet. 65 (1999) 1078–1085.

[4] J.R. Bernar, S. Sparkes, S. Allensworth, Interstitial deletion 2q24.3: case report with high resolution banding, J. Med. Genet. 22 (1985) 226–228.

[5] E.K. Bijlsma, A.C. Knegt, C.M. Bilardo, F.R. Goodman, Increased nuchal translucency and split-hand/foot malformation in a fetus with an interstitial deletion of chromosome 2q that removes the SHFM5 locus, Prenat. Diagn. 25 (2005) 39–44.

[6] R.G. Boles, B.R. Pober, L.H. Gibson, C.R. Willis, J. McGrath, D.J. Roberts, et al., Deletion of chromosome 2q24–q31 causes characteristic digital anomalies: case report and review, Am. J. Med. Genet. 55 (1995) 155–160.

[7] M. Chaabouni, M. Le Merrer, O. Raoul, M. Prieur, M.C. de Blois, A. Philippe, et al., Molecular cytogenetic analysis of five 2q37 deletions: refining the brachydactyly candidate region, Eur. J. Med. Genet. 49 (2006) 255–263.

[8] Y. Chinen, T. Tohma, Y. Izumikawa, T. Iha, Y. Goya, K. Naritomi, Small interstitial deletion of the long arm of chromosome 2 (2q24.3): further delineation of 2q medial monosomy syndrome, Jpn. J. Hum. Genet. 41 (1996) 323–328.

[9] I. Cobos, M.E. Calcagnotto, A.J. Vilaythong, M.T. Thwin, J.L. Noebels, S.C. Baraban, J.L.R. Rubenstein, Mice lacking *Dlx1* show subtype-specific loss of interneurons, reduced inhibition and epilepsy, Nat. Neurosci. 8 (2005) 1059–1068.

[10] W. Courtens, F. Speleman, L. Messiaen, J. Bormans, N. Van Roy, E. Vamos, et al., Interstitial deletion 2q33.3–q34 in a boy with a phenotype resembling the Seckel syndrome, Am. J. Med. Genet. 71 (1997) 479–485.

[11] M. Del Campo, M.C. Jones, A.N. Veraksa, C.J. Curry, K.L. Jones, et al., Monodactylous limbs and abnormal genitalia are associated with hemizygosity for the human 2q31 region that includes the *HOXD* cluster, Am. J. Hum. Genet. 65 (1999) 104–110.

[12] M.J. Depew, C.A. Simpson, M. Morasso, J.L. Rubenstein, Reassessing the *Dlx* code: the genetic regulation of branchial arch skeletal pattern and development, J. Anat. 207 (2005) 501–561.

[13] F.R. Goodman, F. Majewski, A.L. Collins, P.J. Scambler, A 117-kb microdeletion removing *HOXD9–HOXD13* and *EVX2* causes synpolydactyly, Am. J. Hum. Genet. 70 (2002) 547–555.

[14] S.P. Hamilton, J.M. Woo, E.J. Carlson, N. Ghanem, M. Ekker, J.L. Rubenstein, Analysis of four *DLX* homeobox genes in autistic probands, BMC Genet. 6 (2005) 52.

[15] K. Kanai, S. Hirose, H. Oguni, G. Fukuma, Y. Shirasaka, T. Miyajima, et al., Effect of localization of missense mutations in *SCN1A* on epilepsy phenotype severity, Neurology 63 (2004) 329–334.

[16] J.A. Kearney, A.K. Wiste, U. Stephani, M.M. Trudeau, A. Siegel, R. Ramachandran Nair, et al., Recurrent de novo mutations of *SCN1A* in severe myoclonic epilepsy of infancy, Pediatr. Neurol. 34 (2006) 116–120.

[17] J.A. Kearney, Y. Yang, B. Beyer, S.K. Bergren, L. Claes, P. Dejonghe, W.N. Frankel, Severe epilepsy resulting from genetic interaction between *Scn2a* and *Kcnq2*, Hum. Mol. Genet. 15 (2006) 1043–1048.

[18] S.M. Maas, J.M. Hoovers, M.E. van Seggelen, D.M. Menzel, R.C.M. Hennekam, Interstitial deletion of the long arm of chromosome 2: a clinically recognizable microdeletion syndrome? Clin. Dysmorphol. 9 (2000) 47–53.

[19] T.S. McConnell, M. Kornfeld, G. McClellan, J. Aase, Partial deletion of chromosome 2 mimicking a phenotype of trisomy 18: case report with autopsy, Hum. Pathol. 11 (1980) 202–205.

[20] M.H. Meisler, J.A. Kearney, Sodium channel mutations in epilepsy and other neurological disorders, J. Clin. Invest. 115 (2005) 2010–2017.

[21] M.D. Moller, D. Garcia-Cruz, H. Rivera, J. Sanchez-Corona, J.M. Cantù, Pure monosomy and trisomy 2q24.2–q3105 due to an inv ins(7;2)(q21.2;q3105q24.2) segregating in four generations, Hum. Genet. 68 (1984) 77–86.

[22] J. Nixon, M. Oldridge, A.O.M. Wilkie, K. Smith, Interstitial deletion of 2q associated with craniosynostosis, ocular coloboma, and limb abnormalities: cytogenetic and molecular investigation, Am. J. Med. Genet. 70 (1997) 324–327.

[23] G. Panganiban, J.L. Rubenstein, Developmental functions of the Distal-less/*Dlx* homeobox genes, Development 129 (2002) 4371–4386.
[24] S. Pereira, J.P. Vieira, F. Barroca, P. Roll, R. Carvalhas, P. Cau, et al., Severe epilepsy, retardation, and dysmorphic features with a 2q deletion including *SCN1A* and *SCN2A*, Neurology 63 (2004) 191–192.
[25] J.C. Ramer, R.L. Ladda, C.A. Frankel, A. Beckford, A review of phenotype–karyotype correlations in individuals with interstitial deletions of the long arm of chromosome 2, Am. J. Med. Genet. 32 (1989) 359–363.
[26] J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[27] F. Shabtai, D. Klar, I. Halbrecht, Partial monosomy of chromosome 2. Delineable syndrome of deletion 2 (q23–q31), Ann. Genet. 25 (1982) 156–158.
[28] A.C. Slavotinek, C. Schwarz, J.F. Getty, O. Stecko, F. Goodman, H. Kingstone, Two cases with interstitial deletions of chromosome 2 and sex reversal in one, Am. J. Med. Genet. 86 (1999) 75–81.
[29] T. Stuhmer, L. Puelles, M. Ekker, J.L.R. Rubenstein, Expression from a *Dlx* gene enhancer marks adult mouse cortical GABAergic neurons, Cereb. Cortex 12 (2002) 75–85.
[30] G. Van Buggenhout, C. Van Ravenswaaij-Arts, N.M.C. Maas, R. Thoelen, A. Vogels, D. Smeets, et al., The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients, Eur. J. Med. Genet. 48 (2005) 276–289.
[31] C. Wamsler, B. Muller, G. Freyberg, M. Schmid, Interstitial deletion del(2)(q24q31) with a phenotype similar to del(2)(q31q33), Am. J. Med. Genet. 39 (1991) 204–206.

Case 2:

Clinical and molecular characterization of a patient with a 2q31.2-32.3 deletion identified by array-CGH.

Mencarelli MA, Caselli R, Pescucci C, Hayek G, Zappella M, Renieri A, Mari F.

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Clinical Report

Clinical and Molecular Characterization of a Patient With a 2q31.2-32.3 Deletion Identified by Array-CGH

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We report on a patient with a de novo interstitial deletion of the long arm of chromosome 2 involving bands 2q31.2-2q32.3. The patient shows severe mental retardation, absence of speech, sleep disturbances, behavioral problems, and some dysmorphic features. In particular, he presents with macrocephaly, high forehead, thick and coarse hair, thick eyebrows, synophrys, increased inner and outer canthal distance, bifid nasal tip, high palate, micrognathia, dysmorphic right ear, and long and tapering fingers. Array-CGH analysis allowed us to identify and characterize a 2q interstitial deletion of about 13 Mb, involving the segment between cytogenetic bands 2q31.2 and 2q32.3. The deletion was confirmed by quantitative PCR. We compare the phenotype of our patient with those already reported in literature. In particular, we discuss the similarities shared with two recently reported patients, studied by array-CGH, who show an overlapping deletion. The common clinical features are: long face, high forehead, abnormal teeth and ears, midface hypoplasia, high palate, micrognathia, transparent and thin skin, high frequency of inguinal hernia, severe development impairment, and behavioral problems. Some genes located in the deleted region may be good candidates for the neurological phenotype such as *ZNF533* and *MYO1B*, which are both involved in neuronal function. Furthermore, the *GLS* gene could be a good candidate in generating the behavioral phenotype in the patient. In fact, it encodes for the major enzyme yielding glutamate from glutamine and it can be implicated in behavioral disturbances in which glutamate acts as a neurotransmitter.

Key words: chromosome 2; mental retardation; array-CGH; 2q interstitial deletion; behavioral phenotype; wrinkly skin syndrome (WSS); Ehlers–Danlos syndrome (EDS)

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INTRODUCTION

Patients with a deletion of chromosome 2 involving 2q31 and 2q32 bands have been already reported. Most of them has been identified through standard or high-resolution cytogenetic analysis without clear definition of breakpoints [Ramer et al., 1989, 1990]. Ramer reported a total of six patients with a del(2)(q31q33) and compared the phenotype of these patients with other six previously described cases with an apparently identical deletion and with other patients with either overlapping or non-overlapping 2q deletions [Ramer et al., 1989, 1990]. Common manifestations in cases with del(2)(q31q33) include: small size at birth, growth and developmental retardation, microcephaly and distinctive craniofacial anomalies such as hypoplastic midface, small nose, long philtrum, short palpebral fissures, thin upper lip, cleft palate, unusual ear shape, and micrognathia [Ramer et al., 1989].

Recently four unrelated cases with a 2q32-q33 deletion identified by high-resolution cytogenetic analysis and characterized by BAC array-based comparative genomic hybridization analysis (array-CGH) have been reported [Van Buggenhout et al., 2005]. The common clinical features include pre- and

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postnatal growth retardation, severe mental retardation, distinct facial dysmorphism, thin and sparse hair and fair built, micrognathia, cleft or high palate, relative macroglossia, dacrocystitis, persisting feeding difficulties, inguinal hernia and broad-based gait [Van Buggenhout et al., 2005].

We report on the clinical description and molecular characterization of an additional patient with a de novo 2q31.2-32.3 deletion and compare his features with the phenotype of other patients with partially overlapping deletions. The chromosomal breakpoints and the size of the deleted region were characterized by oligonucleotide array-CGH method. We have analyzed the gene content of the deleted region in order to propose the possible involvement of specific genes in the clinical phenotype of the reported patient. Since a familial case with wrinkly skin syndrome (WSS, OMIM 278250) and a 2q32 deletion has been reported, we have compared the phenotype of our patient with the features of the WSS. Given that the deleted region contains the *COL3A1* and *COL5A2* genes associated with Ehlers–Danlos syndrome (EDS), we discuss the possibility of EDS diagnosis in our patient as well.

CLINICAL REPORT

The patient is a male, first child of unrelated parents (Fig. 1). At the time of delivery the mother was 29 years old and the father was 28 years old. The father reports a unilateral facial nerve palsy in childhood and unilateral hearing loss. The mother is healthy. The second child of the couple is a healthy girl. During the pregnancy, eclampsia occurred and the patient was born preterm at 29 weeks gestational age. Birth weight was 1,560 g (75th–90th centile), while length and OFC are not available. After birth the proband was hospitalized for about 10 weeks. He showed developmental delay: he developed head control at 8 months; he began to sit alone at 14 months and to walk independently at 22 months. Speech and language are still absent. From the age of 7 years he had nocturnal apnea and sleep disturbances. He underwent surgery for unilateral cryptorchidism and for unilateral inguinal hernia repair. The ophthalmological examination, performed at the age of 3, revealed astigmatism, hypermetropia, and intermittent exotropia. ABR index at 4 years was normal. Brain MRI at 8 years revealed a mild enlargement of lateral ventricles. The EEG investigation at 7 years showed non-specific epileptiform abnormalities in the central region. A follow-up EEG performed 2 years later showed only disorganization of the background activity. A standard G-banded karyotype on a peripheral blood sample was performed eleven years ago and reported a male normal karyotype. The resolution of the karyotype was not specified in the report. However, given the image attached to the report, the resolution was probably below 300 bands. Clinical examination at the age of 13 years and 8 months showed height of 152 cm (10th–25th centile), weight of 54 kg (50th–75th centile; the mother refers a marked increase after the introduction of the pharmacological therapy) and OFC of 57 cm (97th centile). OFC of the father is 61.5 cm (97th centile). The propositus shows high forehead with short neck, thick and coarse hair, thick eyebrows, synophrys, inner canthal distance of 3.8 cm (> +2 SD), outer canthal distance of 11.3 cm (+2 SD), bifid nasal tip, high palate, micrognathia (Fig. 1A). Teeth are broad and overcrowded with abnormal shape. The right medial incisor was removed for a fracture. The right ear is dysmorphic with prominent anti-helix and extra folds occupying the concha (Fig. 1B). He shows kyphoscoliosis and bilateral genu valgum. Hyperextensible skin and joint-hyperlaxity are not present (Beighton's Criteria for Joint Hypermobility score 0) [Beighton, 1988]. The venous pattern is evident over the anterior thorax and the arms (Fig. 1C,D). Palm length is 9.5 cm (25th centile), middle finger length is 7.6 cm (50th centile), fingers are tapering with clubbing of the distal part (Fig. 1E). Foot length is 23.5 cm (3rd–25th centile) with bilateral sandal gap (Fig. 1F). He is

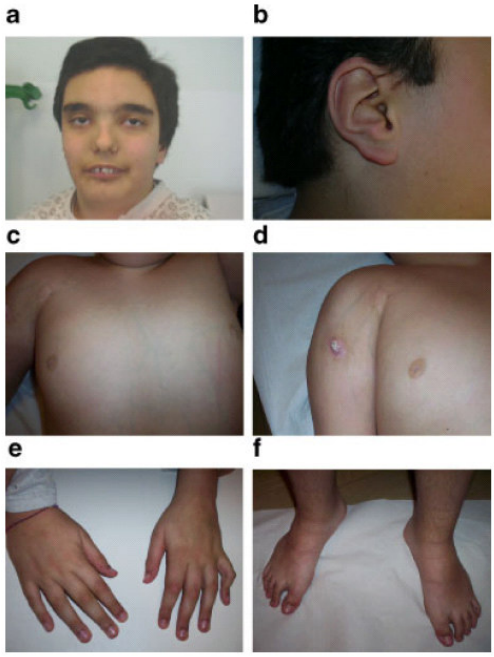

Fig. 1. Photographs of the patient at the age of 13 years and 8 months. (**a**) Frontal view; (**b**) particular of the right ear; (**c**) thorax showing the extremely visible veins pattern; (**d**) particular of the right shoulder showing a scar produced by the patients for continuous pinching; (**e**) hands; (**f**) feet. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

able to walk independently even if with short steps. He presents rigidity in the upper limbs, which are flexed and adduced and he has poor manual abilities. Severe mental retardation and behavioral problems such as hyperactivity, screaming and periods of anxiety, aggressiveness, and self mutilation occur (Fig. 1D). At the second examination at the age of 14 years and 9 months, height is 160 cm (10th–25th centile), weight 62 kg (75th centile), OFC 58 cm (>97th centile). The clinical and behavioral features are unchanged. Actually he is treated with carbamazepine, haloperidol, and niaprazin.

MATERIALS AND METHODS

Array-CGH Analysis

Array-based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 43.000 60-mer probes (Human Genome CGH Microarray 44B Kit, Agilent Technologies, Santa Clara, Ca) as previously reported [Pescucci et al., 2007]. The average resolution is about 75–100 Kb.

Real-Time Quantitative PCR

Real-time quantitative PCR was performed to confirm array-CGH data. We used a Custom TaqMan Gene Expression Assays by design (Applied Biosystems, Foster City, CA; http://products.appliedbiosystems.com) which provides predesigned primers-probe set for real-time PCR experiments. We designed the probe in the exon 9 of the *CALCRL* gene related to the 2q32.1 locus. *CALCRL* exon 9 forward primer: 5′-TCT GTT GTA ACA ATC ATT CAC CTC ACT-3′; *CALCRL* exon 9 reverse primer: 5′-AGG GCA CAA TCT TGG TTT ACT TAC AG-3′; *CALCRL* exon 9 TaqMan probe: 5′-CAG TGG CCA ACA ACC AG-3′. PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 µl. A total of 100 ng (10 µl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a prerun of 2 min at 50°C and 10 min at 95°C. Cycle conditions were 40 cycles at 95°C for 15 sec and 60°C for 1 min according to the TaqMan Universal PCR Protocol (Applied Biosystems). The TaqMan Universal PCR Master Mix and Microamp reaction tubes were supplied by Applied Biosystems. The starting copy number of the unknown samples was determined using the comparative Ct method, as previously described [Ariani et al., 2004].

RESULTS

We performed array-CGH experiments using an oligonucleotides array with an averaged spatial resolution of approximately 75–100 kb. The analysis identified a chromosome 2 interstitial deletion of 12.75–13.47 Mb [46,XY,del(2)(q31.2q32.3)] (Fig. 2A). The proximal breakpoint is mapped in 2q31.2, with the last oligonucleotide presently located in 179.91 Mb and the first deleted in 180.13 Mb. The distal breakpoint is located in 2q32.3 between 192.88 and 193.38 Mb (last oligonucleotide deleted and first present, respectively). To confirm array data, Real-Time Quantitative PCR experiments were performed in the patient and his parents. The deletion was confirmed in the patient, while parents showed a normal result (Fig. 2B).

DISCUSSION

A small number of patients with a chromosome 2 deletion partially overlapping the deletion identified in our patient has been reported and reviewed until today [Ramer et al., 1989, 1990; Van Buggenhout et al., 2005] (Table I). Most of them the characterization of the deletion was performed through classical cytogenetic techniques and thus shows an inadequate definition of breakpoints based on the current standards. On the contrary, the four cases recently reported by Van Buggenhout were studied by array-CGH with a resolution of about 1 Mb [Van Buggenhout et al., 2005].

In Ramer et al., the authors highlight the common manifestations of seven cases with a del(2)(q31q33) and of other patients with partially overlapping 2q deletions [Ramer et al., 1989]. Successively Ramer describes the clinical phenotype of five additional related patients with a del(2)(q31q33) [Ramer et al., 1990]. The common clinical features include: growth failure, developmental delay, mental retardation, microcephaly, and eye and ear abnormalities [Ramer et al., 1989, 1990].

Recently Van Buggenhout et al. [2005] reported the clinical and molecular characterization of four patients with a del(2)(q32.2q33). Only two of them have a deletion partially overlapping the deletion identified in our patient (Patients 2 and 3, Fig. 3). Comparing the clinical phenotype of these three cases we can appreciate common features such as long face, high forehead, abnormal teeth and ears, midface hypoplasia, high palate, micrognathia, transparent and thin skin, high frequency of inguinal hernia, severe development impairment, and behavioral problems (Table II). Microcephaly is a constant feature of all reported cases with an interstitial 2q deletion. However, Patient 2 reported by Van Buggenhout has an OFC around the 75th centile and height and weight below the 3rd centile. This finding is more close to our patient who is macrocephalic like his father. Our propositus has never shown fits even if he has been treated with antiepileptic drugs given the EEG abnormalities. Instead, epileptic seizures are present in Patient 2 and are not reported in Patient 3. Both patients reported by Van

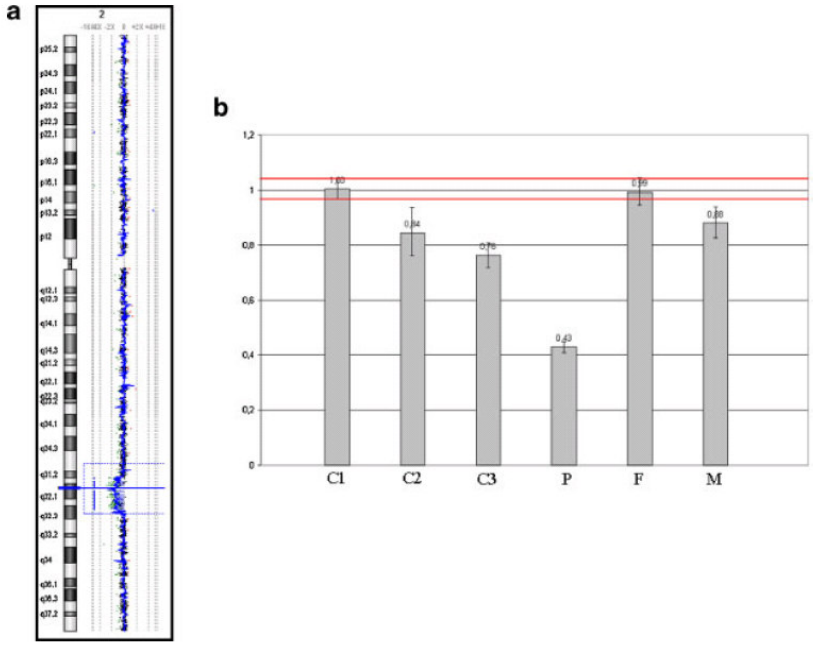

FIG. 2. Molecular data of the patient. **a**: Array-CGH ratio profile. On the left, the chromosome 2 ideogram. On the right, the log2 ratio of chromosome 2 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left. **b**: Real-time quantitative PCR validation experiment. *CALCRL* ddCT ratios and standard deviations of three different controls and of the patient and his parents. The patient shows a ddCT ratio of about 0.5 indicating the presence of a single copy of *CALCRL* (deletion), while the parents like the controls show ddCT ratios of about 1.0, that indicates a double copy of the gene (normal). C1-3, control samples; P, proband; F, father; M, mother. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Buggenhout presented a specific behavioral phenotype with hyperactivity and motor restlessness, chaotic behavior, happy-personality but with periods of aggression and anxiety, sleeping problems and self-mutilation. Behavioral problems are evident in our propositus as well: in some periods he is particularly hyperactive and aggressive with self-mutilation; he also presents sleep disorder with frequent awakening, recurrent apnoeas and screams.

The common deleted region among our case and Patients 2 and 3 reported by Van Buggenhout is about 2.9 Mb and contains 14 genes (Fig. 3). A possible candidate gene for the behavioral phenotype may be the *GLS* gene (glutaminase, phosphate-activated), encoding the major enzyme yielding glutamate from glutamine. Significance of the enzyme derives from its possible implication in behavior disturbances in which glutamate acts as a neurotransmitter [Prusiner, 1981; Sahai, 1983]. Other relevant genes included in the common deleted region are *MYO1B*, that seems to participate in processes critical to neuronal development and function such as cell migration, neurite outgrowth and vesicular transport [Lee, 1997] and the *TMEFF2* gene which is a novel survival factor for hippocampal and mesencephalic neurons [Horie et al., 2000]. The *ZNF533* gene, which is not included in the common deleted region, can be involved in the neurological phenotype of our patient. In fact, mutations in other ZNF genes have been already associated with mental retardation [Lugtenberg et al., 2006]. Deletion involving band 2q32 was associated with WSS, characterized by wrinkled skin of palms and soles, poorly developed skeletal musculature and hypotonia with winging of the scapulas and visible venous pattern over the anterior thorax [Gazit et al., 1973]. Hyperextensible joints, particularly of the hands, intrauterine growth retardation, postnatal failure to thrive, mental and developmental delay and an old appearance were reported as characteristics of the syndrome [Casamassima et al., 1987; Kreuz and Wittwer, 1993]. A 2q32 deletion was found in two sibs and their mother who presented a complex phenotype characterized by small size at birth, retarded growth and development, craniofacial dysmorphisms and skeletal and ocular anomalies together with symptoms of the WSS [Kreuz and Wittwer, 1993]. The identification of this deletion in a WSS familial case prompted the authors to consider this region as the candidate region for WSS and to hypothesize the involvement of the *COL3A1* and *COL5A2* genes

TABLE I. Comparison of the Main Clinical Findings in Patients With a Partially Overlapping 2q31.2-q32.3 Deletion

Clinical findings	Ramer et al. [1989]											
Deletion	(2)(q22q31)	(2)(q31q33)	(2)(q23q34)	(2)(q31q33)	(2)(q31q33)	(2)(q31q33)	(2)(q32)	(2)(q32)	(2)(q31q33)	(2)(q31q35)	(2)(q31q33)	(2)(q31q33)
Sex	F	F	F	F	F	F			M		F	M
Age	Died at birth	7 years	20 years	8 months					4 months	Died at 4 days	Died at 8 days	10 months
Birth weight<5th	+	−	−	+	+		+	−	−	+		+
Growth failure		+	+	+	+				+			+
Developmental delay/mental retardation		+	+	+	+	+	+	+	+			+
Microcephaly	−	+	+	+	+		+	+	+	+		+
Ocular abnormalities		−	+	+	−	+	+	+	+		+	+
Abnormal ears	+	+	+	−	+	−	+	+	+	+	+	+
Cleft palate	+	−	+	−	+	+	−	−	−	+	+	+
Syndactyly		−		−	−	+		+	+		+	−
Camptodactyly		−		+	+	+			+		+	−
Cardiac anomalies	+	+	+	+	−	−	−	−	−	+	+	−
Other anomalies	Scaphocephaly, occipital meningomyelocele, internal hydrocephalus			Acetabular hypoplasia, absent ribs	Seizures, abnormal dentition		Short neck, wide mouth			Hypertelorism, hypoplastic right leg	Renal hypoplasia, equinovarus deformities	Partial agenesis of corpus callosum, nystagmus, optic nerve hypoplasia

Clinical findings	Ramer et al. [1990]					Van Buggenhout et al. [2005]				Present case
Deletion		(2)(q31q33)				(2)(q31q33.3)	(2)(q32q33)	(2)(q32q33)	(2)(q32.2q34)	(2)(q31.2q32.3)
Sex	M	F	F	F	M	M	M	M	M	M
Age	2 years and 3 months	1 year and 10 months	Died at 15 days	6 months	Died at 31 days	11 years	20 years	3 years and 8 months	11 years 11 months	13 years 8 months
Birth weight <5th		+	+	+	−	−	−	+	+	−
Growth failure	+	+		+	−	+	+	+	+	−
Developmental delay/mental retardation	+	+		+		+	+	+	+	+
Microcephaly	+	+	+	+	−	+	+	+	+	−
Ocular abnormalities	−	−	−			+	−	+	−	−
Abnormal ears	−	+	−		+	+	+	−	+	+
Cleft palate	−	−	−	−	−	+	+	−	−	−
Syndactyly		+	−	+	+					−
Camptodactyly		+								−
Cardiac anomalies	−	−	−		+	−	−	+	−	−
Other anomalies	Hypospadias, bilateral ectrodactyly	Myoclonic seizures	Extrahepatic biliary atresia		Seizures	Behavioral problems	Behavioral problems	Behavioral problems		Behavioral problems

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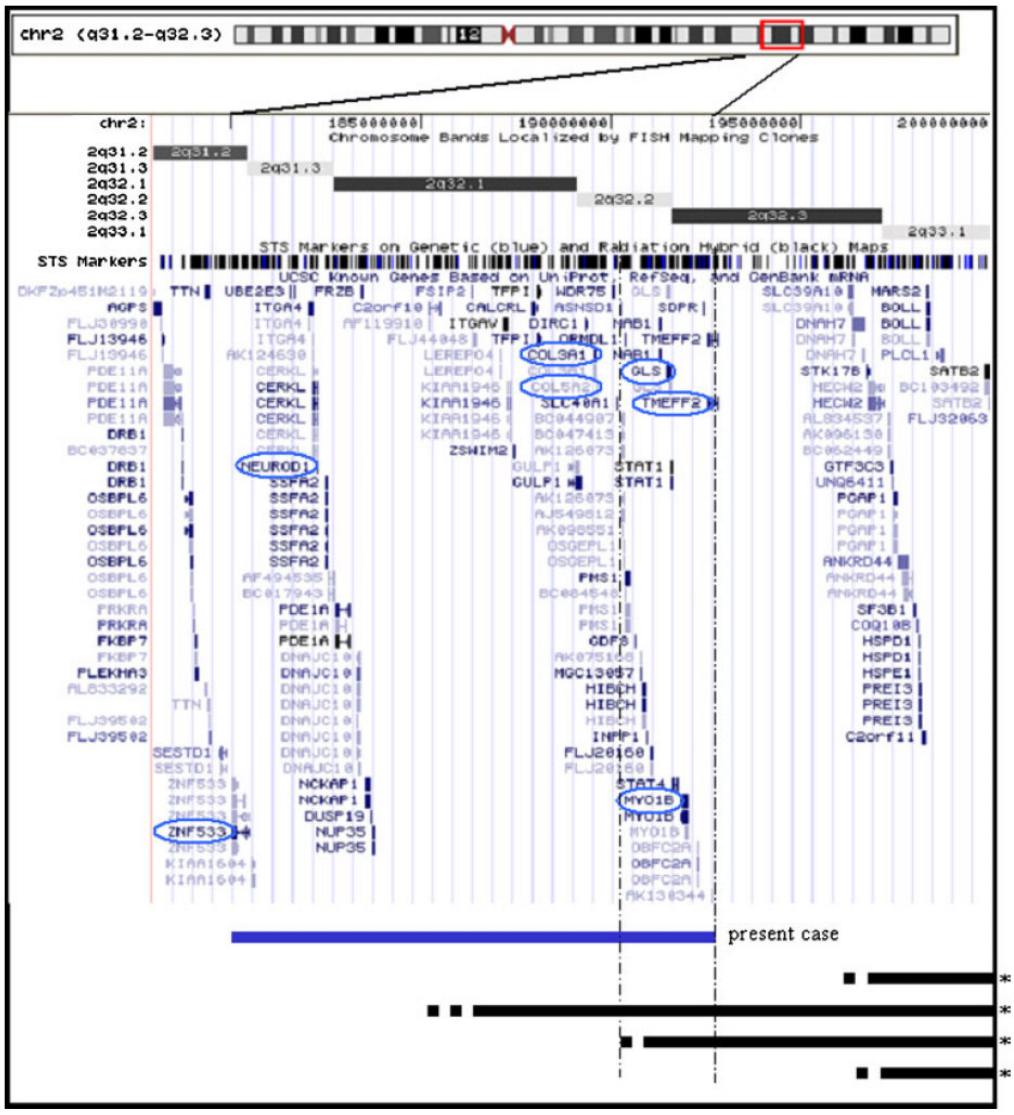

FIG. 3. Gene content of the deleted region (UCSC Genome Browser; http://genome.ucsc.edu). Circled genes are commented on in the text. In the bottom part of the figure the deletion of our patient is compared with the deletions of four patients reported by Van Buggenhout (labeled by an asterisk). Deletion extent of this four cases is more approximate than our since it is characterized by BAC array with a resolution of about 1 Mb (dotted lines). The overlapping region is delimited by vertical dotted lines. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Kreuz and Wittwer, 1993]. However, the few reported cases with a deletion involving band 2q32 including our patient, do not present WSS typical signs such as wrinkly skin on the abdomen, increased number of creases on the palm and soles and hypermobility of joints. The only sign in common with the WSS is a thin and transparent skin with a prominent venous pattern, which is present in our case and reported in other cases with a 2q32 deletion [Van Buggenhout et al., 2005]. In addition, our patient shows thick hairs and eyebrows and abnormal teeth suggesting that genes included in the deletion could have an important role in the development of ectodermal derivatives.

The *COL3A1* and *COL5A2* genes are known disease genes and are responsible for the Ehlers–

TABLE II. Comparison of the Clinical Features of the Present Case With Two Cases With an Overlapping Deletion Identified by Array-CGH, Reported by Van Buggenhout et al. [2005]

	Present case	Van Buggenhout Patient 2	Van Buggenhout Patient 3
Age	13 years and 8 months	20 years	3 years and 8 months
Sex	M	M	M
Pregnancy	29 weeks	39 weeks	39 weeks-cesarean
Birth weight	1,560 g (75th–90th centile)	2,750 g (10th–25th centile)	1,610 g (<3rd centile)
Birth length	ND	48 cm (25th–50th centile)	ND
Birth OFC	ND	ND	29 cm (<3rd centile)
Postnatal growth retardation	No	Yes	Yes
Mental retardation	Severe	Severe	Severe
Seizures	EEG abnormalities	Yes	
CT/MRI	Mild enlargement of lateral ventricles	ND	Corpus callosum agenesis
Microcephaly	No	No	Yes
Craniofacial	Long	Long	Long, asymmetry
Hair	Thick and coarse	Sparse and thin	Sparse and thin
Forehead	High	High	High
Eyes	Deep-set	Deep-set, downward slanting	Bilateral iris coloboma, right microphthalmia with retinal coloboma
Ears	Dysmorphic right ear	Low-set, dysplastic	
Nose	Bifid nasal tip		High and broad nasal bridge
Midface	Midface hypoplasia	Midface hypoplasia	
Cleft lip	No	No	No
Cleft palate	High palate	Yes	High palate
Teeth	Broad and overcrowded teeth	Diastema, short and broad teeth	Abnormal teeth
Micrognathia	Yes	Yes	Yes
Hands	Tapering fingers	Small	
Feet	Small with bilateral sandal gap	Small	
Skin	Thin and transparent	Thin and transparent	Thin and transparent
Thorax	Kyphoscoliosis		Thoracic kyphosis
Genitalia	Cryptorchidism	Small genitalia, shawl scrotum	
Inguinal hernia	Yes	Yes	Yes
Speech	No active speech		Donald Duck speech
Behavioral problems	Difficult behavior, self mutilation	Difficult behavior, self mutilation	Difficult behavior, hyperactive, autistic-like

Danlos syndrome. The *COL5A2* gene is responsible for at least some cases of classic EDS (EDS type I, OMIM #130000) [Malfait et al., 2005]. The haploinsufficiency of *COL3A1* gene causes either EDS type IV (OMIM #130050) or EDS, hypermobility type (type III, OMIM #130020) [Narcisi et al., 1994; Pepin et al., 2000]. Given that our patient does present scant subcutaneous tissue with easily visible veins, a possible diagnosis of EDS cannot be completely excluded [Schwarze et al., 2001]. Biochemical tests on cultures dermal fibroblast are planned in order to define a surveillance especially for bowel and/or vascular ruptures. In the deleted region, another disease gene is present, NEUROD1. Inactivating mutations of NEUROD1 have been associated with the development of maturity-onset diabetes of the young, type VI (MODYVI) [Malecki et al., 1999]. At the age of 14, our patient does not present hyperglycemia, but we can not exclude that he will develop signs of MODYVI later in life.

Finally, the delineation of a 2q deletion syndrome involving 2q31 and 2q32 bands is still premature. When comparing phenotypes of patients presenting a chromosomal deletion, several aspects have to be considered. Usually the deletions have different extensions and often, especially in the past, the breakpoints have been poorly defined given the resolution of the employed techniques. In addition, the phenotype comparison has to take into consideration the different age of patients at the time of the evaluation and the fact that different cases are evaluated by several clinicians. Furthermore, in different reports some clinical details are missing making it difficult to ascertain if a sign is absent or has not been evaluated. Given all the issues discussed above, the description of more cases well characterized both by a clinical and molecular point of view will be essential in order to define a possible interstitial 2q deletion syndrome.

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REFERENCES

Ariani F, Mari F, Pescucci C, Longo I, Bruttini M, Meloni I, Hayek G, Rocchi R, Zappella M, Renieri A. 2004. Real-time

quantitative PCR as a routine method for screening large rearrangements in Rett syndrome: Report of one case of MECP2 deletion and one case of MECP2 duplication. Hum Mutat 24:172–177.

Beighton P. 1988. Hypermobility scoring. Br J Rheumatol 27:163.

Casamassima AC, Wesson SK, Conlon CJ, Weiss FH. 1987. Wrinkly skin syndrome: Phenotype and additional manifestations. Am J Med Genet 27:885–893.

Gazit E, Goodman RM, Katznelson MB, Rotem Y. 1973. The wrinkly skin syndrome: A new heritable disorder of connective tissue. Clin Genet 4:186–192.

Horie M, Mitsumoto Y, Kyushiki H, Kanemoto N, Watanabe A, Taniguchi Y, Nishino N, Okamoto T, Kondo M, Mori T, Noguchi K, Nakamura Y, Takahashi E, Tanigami A. 2000. Identification and characterization of TMEFF2, a novel survival factor for hippocampal and mesencephalic neurons. Genomics 67:146–152.

Kreuz FR, Wittwer BH. 1993. Del(2q)—cause of the wrinkly skin syndrome? Clin Genet 43:132–138.

Lee JE. 1997. NeuroD and neurogenesis. Dev Neurosci 19:27–32.

Lugtenberg D, Yntema HG, Banning MJ, Oudakker AR, Firth HV, Willatt L, Raynaud M, Kleefstra T, Fryns JP, Ropers HH, Chelly J, Moraine C, Gecz J, Reeuwijk J, Nabuurs SB, de Vries BB, Hamel BC, de Brouwer AP, Bokhoven H. 2006. ZNF674: A new Kruppel-associated box-containing zinc-finger gene involved in nonsyndromic X-linked mental retardation. Am J Hum Genet 78:265–278.

Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T, Saad M, Warram JH, Montminy M, Krolewski AS. 1999. Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. Nat Genet 23:323–328.

Malfait F, Coucke P, Symoens S, Loeys B, Nuytinck L, De Paepe A. 2005. The molecular basis of classic Ehlers-Danlos syndrome: A comprehensive study of biochemical and molecular findings in 48 unrelated patients. Hum Mutat 25:28–37.

Narcisi P, Richards AJ, Ferguson SD, Pope FM. 1994. A family with Ehlers-Danlos syndrome type III/articular hypermobility syndrome has a glycine 637 to serine substitution in type III collagen. Hum Mol Genet 3:1617–1620.

Pepin M, Schwarze U, Superti-Furga A, Byers PH. 2000. Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. N Engl J Med 342:673–680.

Pescucci C, Caselli R, Grosso S, Mencarelli MA, Mari F, Farnetani MA, Piccini B, Artuso R, Bruttini M, Priolo M, Zuffardi O, Gimelli S, Balestri P, Renieri A. 2007. 2q24-q31 deletion: Report of a case a review of the literature. Eur J Med Genet 50:21–32.

Prusiner SB. 1981. Disorders of glutamate metabolism and neurological dysfunction. Annu Rev Med 32:521–542.

Ramer JC, Ladda RL, Frankel CA, Beckford A. 1989. A review of phenotype-karyotype correlations in individuals with interstitial deletions of the long arm of chromosome 2. Am J Med Genet 32:359–363.

Ramer JC, Mowrey PN, Robins DB, Ligato S, Towfighi J, Ladda RL. 1990. Five children with del (2)(q31q33) and one individual with dup (2)(q31q33) from a single family: Review of brain, cardiac, and limb malformations. Am J Med Genet 37:392–400.

Sahai S. 1983. Glutaminase in human platelets. Clin Chim Acta 127:197–203.

Schwarze U, Schievink WI, Petty E, Jaff MR, Babovic-Vuksanovic D, Cherry KJ, Pepin M, Byers PH. 2001. Haploinsufficiency for one COL3A1 allele of type III procollagen results in a phenotype similar to the vascular form of Ehlers-Danlos syndrome, Ehlers-Danlos syndrome type IV. Am J Hum Genet 69:989–1001.

Van Buggenhout G, Van Ravenswaaij-Arts C, Mc Maas N, Thoelen R, Vogels A, Smeets D, Salden I, Matthijs G, Fryns JP, Vermeesch JR. 2005. The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients. Eur J Med Genet 48:276–289.

Case 3:

A 2.6 Mb deletion of 6q24.3-25.1 in a patient with growth failure, cardiac septal defect, thin lip and asymmetric dysmorphic ears.

Caselli R, Mencarelli MA , Papa FT, Uliana V, Schiavone S, Pescucci C, Ariani F, Rossi V, Longo I, Meloni I, Renieri A, Mari F.

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Chromosomal imbalance letter

A 2.6 Mb deletion of 6q24.3–25.1 in a patient with growth failure, cardiac septal defect, thin lip and asymmetric dysmorphic ears

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Abstract

We report a female patient with neurodevelopmental delay and peculiar facial features. She has postnatal growth failure and a trial septal defect. Patent duct arteriosis and tricuspidal insufficiency were also noted at birth. Characteristic facial features include medial flare eyebrows, dysmorphic helix of the right ear, cupshaped left ear, anteverted nares, long and smooth philtrum, thin upper lip, high vaulted palate. Array-CGH analysis demonstrated the presence of a 2.6 Mb deletion in 6q24.3–25.1. The phenotypic features of this case are very similar to those previously reported in a patient with a 7 Mb overlapping deletion, pointing to a specific new syndrome. Twenty-two genes are present in the common critical deleted region. Among them, there is the PPP1R14C gene that encodes for KEPI, a PKC-potentiated inhibitory protein for type-1 Ser/Thr protein phosphatase. Its selective distribution in brain and heart well correlates with developmental delay and cardiac anomalies observed in the patient.

Keywords: Chromosome 6; Array CGH; 6q interstitial deletion; Ears abnormalities; Cardiac septal defect; PPP1R14C

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1. Method of detection

1.1. Array-CGH analysis

Genomic DNA of a normal female control was obtained from Promega. Genomic DNA of the patient was isolated from an EDTA peripheral blood sample by using a QIAamp DNA Blood Kit according to the manufacturer protocol (Qiagen, Hilden, Germany).

Ten micrograms of genomic DNA both from the patient (test sample) and the control (reference sample) were sonicated. Test and reference DNA samples were subsequently purified by using dedicated columns (DNA Clean and Concentrator, Zymo Research) and the appropriate DNA concentrations were determined by a DyNA Quant™ 200 Fluorometer (GE Healthcare).

Array based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 43,000 60-mer probes (Human Genome CGH Microarray 44B Kit, Agilent Technologies, Santa Clara, California) as previously reported [12]. The average resolution is about 75–100 Kb.

1.2. Chromosomal anomaly

Array CGH analysis identified a chromosome 6 interstitial deletion of about 2.6 Mb [46,XX,del(6)(q24.3q25.1)] (Fig. 2a). The proximal breakpoint is mapped in 6q24.3, with the last oligonucleotide present located in 148.70 Mb and the first deleted in 148.78 Mb. The distal breakpoint is located in 6q25.1 between 151.28 Mb and 151.35 Mb (last oligonucleotide deleted and first present, respectively).

1.3. Method of confirmation

Real-time quantitative PCR was performed to confirm array-CGH data. We used a predesigned set of primers and probe specific for the *LATS1* gene (6q25.1 locus) provided by the Assay-by-Design service (Applied Biosystems, Foster City, CA). Primers and probe were designed in exon 6 of the gene: *LATS1*_forward primer: 5′-CGGCAAGATAGCATGGATTT CAGTA -3′; *LATS1*_reverse primer: 5′-GCAGCTCTCCGCTCTAATGG -3′; *LATS1*_TaqMan probe: 5′-TCGATGTGGAGACAGACTG-3′. PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 μl. PCR reactions were prepared from a single Mix consisting of: 2X TaqMan Universal PCR Master Mix, 20X LATS1 Assay Mix, 20X RNAaseP Mix (internal reference) and HPLC pure water. A total of 100 ng of DNA (10 μl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a prerun of 2 min at 50 °C and 10 min at 95 °C. Cycle conditions were 40 cycles at 95 °C for 15 s and 60 °C for 1 min according to the TaqMan Universal PCR Protocol (Applied Biosystems). Control samples were used for the purpose of calibration. The starting copy number of the unknown samples was determined using the comparative C_t method, as previously described [8].

1.4. Causative of the phenotype

Real-time quantitative PCR analysis performed in both healthy parents showed a normal result indicating that the rearrangement is a de novo deletion (Fig. 2b).

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2. Clinical description

The patient, a 8 years and 2 months old girl, is the second child of unrelated parents (Fig. 1a–d). At the time of delivery, mother and father were 23 and 28 years old, respectively. The older sister and younger brother are healthy. Family history is unremarkable. No teratogen exposure during pregnancy was reported. Delivery, at term of uncomplicated pregnancy, was normal. Birth weight was 3120 g (25th–50th centile), length 47 cm (10th –25th centile) and OFC 33 cm (25th centile). In the neonatal period, she was diagnosed with persistence of fetal circulation and respiratory distress. Tricuspidal insufficiency, right ventricular dilatation, patent duct arteriosis, atrial septal defects (ASD) and increase pulmonary resistance were detected. The motor development has been delayed: she began to sit alone at 9 months, to walk independently at 13 months and to use single words at 3 years. Presently, language evaluation discloses a moderate impairment, in particular in explaining and relating. Short attention span, poor concentration and visuospatial disorganization are also evident. Neuropsychatric evaluation made evidence of a mild mental retardation. Abnormalities of respiratory control and feeding difficulties were described. Clinical examination at the age of 8 years and 2 months shows: weight < 5th centile (15.5 kg), height < 5th centile (107 cm), OFC between 25th and 50th centile (51 cm), mild facial asymmetry, medial flare eyebrows, short palpebral fissures, dysmorphic helix of the right ear, cupshaped left ear, anteverted nares, thin upper lip, long and smooth philtrum, high vaulted palate (Fig. 1a, c and d). Hands are small, feet are small and flat with wide plant and increased distance between 1st and 2nd toe (Fig. 1b). Echocardiogram at the age of 8 years demonstrated an atrial septal defect with minimum shunt. Auditory evoked response testing and visually evoked potential test are normal. MRI scan of the brain EEG and abdominal ultrasonographic examination are normal. Conventional chromosome analysis (450 band resolution) was performed and revealed a normal karyotype.

3. Discussion

We describe a patient with a 6q24.3–25.1 deletion. The most remarkable clinical features are: mild mental retardation with prevailing language impairment, congenital heart defect, short stature and peculiar facial characteristics, such as prominent forehead, ears abnormalities, short palpebral fissures, thin upper lip, long philtrum and high vaulted palate. The patient exhibits some clinical features in common with other patients previously described with partially overlapping deletions [1,3,9–11,14]. These features include developmental delay and mental retardation, growth failure, ear anomalies, thin lips, high arched palate and a wide variety of cardiac defects (Table 1). However, the characterization of the deletions through classical cytogenetic techniques, the poor definition of breakpoints and the major size of previously reported deletions do not allow an adequate clinical comparison [14]. In 2006, Bisgaard et al. identified by metaphase CGH a de novo interstitial deletion of 6q25 in a 6 years and 6 months old girl with ventricular septal defect (VSD), failure to thrive, slightly delayed psychomotor development and facial dysmorphic features [1]. This case has the shortest deletion reported so far in this region and the boundaries of the deletion have a quite good definition since they are characterized using real-time quantitative PCR [1]. Considering the clinical description and the pictures, the phenotype of this case is remarkably similar to that of our patient. In particular, we can appreciate a failure to thrive with short stature in both. However, while in the Bisgaard's case the weight-for-height is increased, our case shows both weight and height under the third percentile. In both girls slightly delayed psychomotor development and heart septal defect

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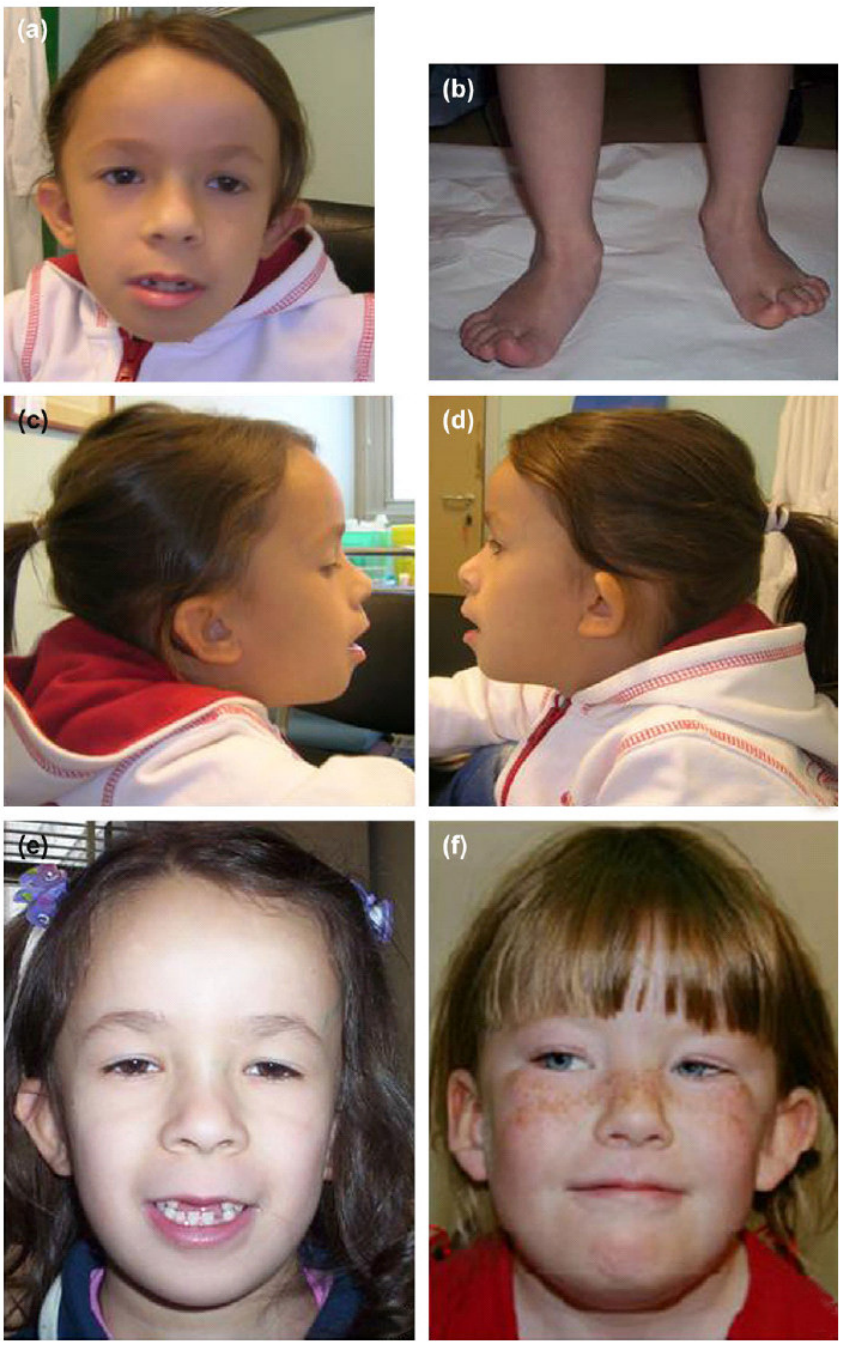

Fig. 1. Clinical features. (a, c and d) Patient at the age of 8 years and 2 months. Frontal view (a) showing medial flare eyebrows, anteverted nares, long and smooth philtrum, thin upper lip. Lateral right (c) and left view (d). Note the asymmetric ears with dysmorphic helix of the right ear and cupshaped left ear. (b) Feet of the proband. Note upturned

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Table 1
Clinical findings in the present patient and other cases with overlapping 6q deletions

Deletion	McLeod 1990 [9]	Narahara 1991 [11] (patient 2)	Meng 1992 [10] (patient 1)	Sukumar 1999 [14] (patient 1)	Bisgaard 2006 [1] (patient 1)	Present case
	q23–25	q25.1–25.3	q24.3–qter	q24.2–25.1	q25.1–25.3	q24.3–25.1
Age	2 years and 6 months	7 months	4 months	1 year	6 years and 6 months	8 years and 2 months
Sex	M	M	F	M	F	F
Low birth weight	+	+	+	+	−	−
Developmental delay	+	NA	+	+	+	+
Mental retardation	+	+	ND	NA	+	+
Hypotonia	−	+	+	+	NA	−
Growth failure	+	+	+	+	+	+
Microcephaly	+	+	+	−	NA	−
Prominent forehead	−			+	NA	−
Epicanthic folds	+	+	+	+	+	−
Short palpebral fissures	NA	NA	NA	NA	+	+
Ear anomalies	+ (Prominent, cupshaped ears)	+	+	NA	+ (Low set, protruding dysplastic left ear)	+ (Dysmorphic helix of the right ear, cupshaped left ear)
Prominent nasal bridge	−	+	+	+	−	−
Thin lips	−	NA	NA	+	+	+
Smooth philtrum	NA	NA	NA	NA	+	+
High arched palate	−	+	+	+	NA	+
Cardiac anomalies	+	−	+ (Triatrial heart)	+(Tricuspid regurgitation)	+ (Ventricular septal defect)	+ (Atrial septal defect, tricuspidal insufficiency)

NA: not available; and ND: not determinable.

(VSD vs ASD in our case) are observed. The facial appearance is very similar: both cases have short palpebral fissures, smooth philtrum, thin upper lip, asymmetric dysplastic ears with left protruding more than right (Fig. 1e, f). These characteristics should be taken into account in order to define a possible new deletion syndrome.

According to UCSC Genome Browser (http://genome.ucsc.edu) on Human March 2006 Assembly, the 2.6 Mb deleted region of our patient contains 23 known genes (Fig. 2c). The deletion reported by Bisgaard overlaps most of this region with the exception of about 400 Kb from the proximal breakpoint (Fig. 2c) [1]. The resulting critical deleted region contains 22 genes. Among them, there is the *PPP1R14C* gene that encodes for KEPI (kinase-enhanced PP1 inhibitor), a PKC-potentiated inhibitory protein for type-1 Ser/Thr protein phosphatase (PP1). Its selective distribution in brain and heart well correlates with cardiac anomalies and developmental delay observed in our patient [7]. Studies on rabbits demonstrated that PP1 in

halluces. (e, f) Our patient (e) at the age of 6 years and 6 months and the case described by Bisgaard (f) at the age of 6 years [1]. Note the phenotypical similarities: short palpebral fissures, smooth philtrum, thin upper lip. (Image f from [1], reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.).

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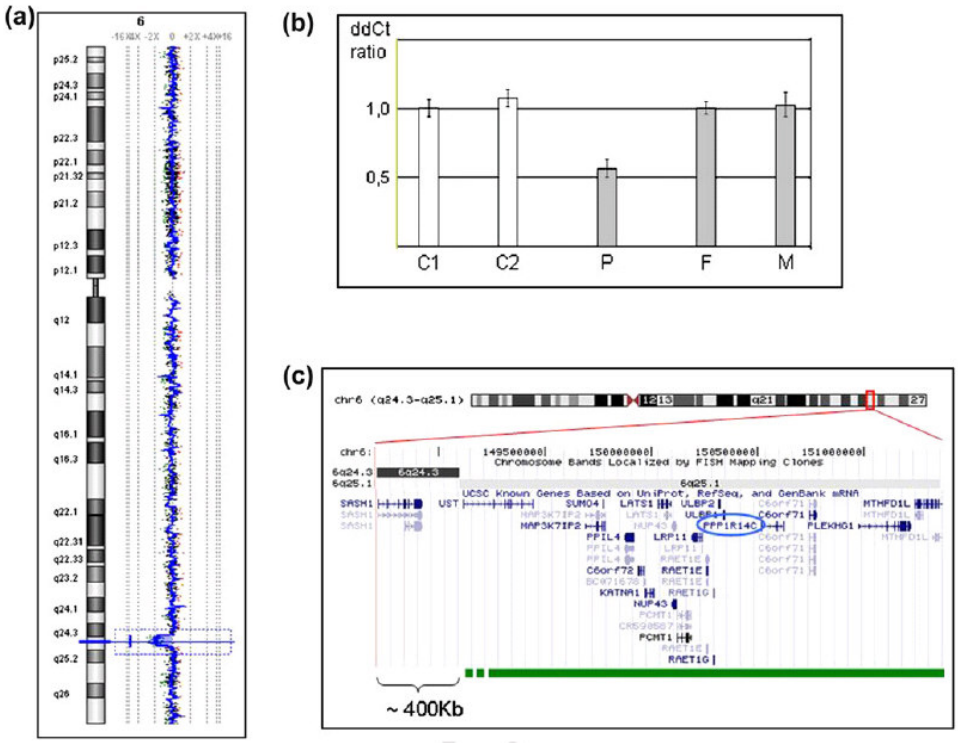

Fig. 2. Molecular results. (a) Array CGH ratio profile. On the left, the chromosome 6 ideogram. On the right, the log 2 ratio of chromosome 6 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left. (b) Real-time quantitative PCR validation experiment. *LATS1* ddCT ratios and standard deviations obtained for the patient (P), her parents (F, father; M, mother) and two different control samples (C1 and C2). The patient shows a ddCT ratio of about 0.5, indicating the presence of a single copy of *LATS1* (deletion), while the parents and the controls show ddCT ratios of about 1.0, indicating a double copy of the gene (normal). (c) Gene content of the deleted region (UCSC Genome Browser, Human March 2006 Assembly; http://genome.ucsc.edu). The circle indicates a gene that may be involved in the clinical phenotype of the patient (see Section 3). In the bottom part of the figure, it is shown the size of the deletion reported by Bisgaard [1].

heart is expressed at much higher levels in newborn and decline to lower levels in adult ventricular myocytes [6]. These data indicate that PP1 has an important role in immature heart cells and suggest that it may be involved in heart growth and development. Given these results, a tight regulation of PP1 activity by KEPI may be important for proper cardiac development.

In a recent study, Gong et al. studied KEPI expression in mouse CNS [4]. They showed widespread, multifocal pattern of KEPI expression and colocalization with PP1 catalytic subunits, pointing to possible roles for KEPI in regulating protein dephosphorylation by inhibiting PP1 activities in a number of brain pathways [4]. PP1 is enriched in neural postsynaptic densities and acts in neurons to regulate phosphorylation of many neurotransmitter receptors, voltage gated ion channels, ion pumps and transcription factors [2,5,13].

At present, considering the small number of reported cases and the large extension of the previously described deletions, the delineation of a deletion syndrome involving 6q25 band

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is still premature. However, considering literature data and the clinical and molecular features of the patients described here, we suggest to accurately evaluate the region 6q25 in cases presenting a syndromic condition with neurodevelopmental delay, cardiac defects and the above described facial features. The description of new cases well characterized both by a clinical and a molecular point of view will be essential in order to define a possible interstitial 6q25 deletion syndrome.

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Bibliography

[1] A.M. Bisgaard, M. Kirchhoff, Z. Tumer, B. Jepsen, K. Brondum-Nielsen, M. Cohen, B. Hamborg-Petersen, T. Bryndorf, N. Tommerup, F. Skovby, Additional chromosomal abnormalities in patients with a previously detected abnormal karyotype, mental retardation, and dysmorphic features, Am. J. Med. Genet. A 140 (20) (2006) 2180–2187.

[2] A.A. Fienberg, N. Hiroi, P.G. Mermelstein, W. Song, G.L. Snyder, A. Nishi, A. Cheramy, J.P. O'Callaghan, D.B. Miller, D.G. Cole, R. Corbett, C.N. Haile, D.C. Cooper, S.P. Onn, A.A. Grace, C.C. Ouimet, F.J. White, S.E. Hyman, D.J. Surmeier, J. Girault, E.J. Nestler, P. Greengard, DARPP-32: regulator of the efficacy of dopaminergic neurotransmission, Science 281 (5378) (1998) 838–842.

[3] J.P. Fryns, K. Vandenberghe, H. Van den Berghe, Prune-belly anomaly and large interstitial deletion of the long arm of chromosome 6, Ann. Genet. 34 (2) (1991) 127.

[4] J.P. Gong, Q.R. Liu, P.W. Zhang, Y. Wang, G.R. Uhl, Mouse brain localization of the protein kinase C-enhanced phosphatase 1 inhibitor KEPI (kinase C-enhanced PP1 inhibitor), Neuroscience 132 (3) (2005) 713–727.

[5] P. Greengard, P.B. Allen, A.C. Nairn, Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade, Neuron 23 (3) (1999) 435–447.

[6] R. Kumar, R.W. Joyner, Expression of protein phosphatases during postnatal development of rabbit heart, Mol. Cell. Biochem. 245 (1–2) (2003) 91–98.

[7] Q.R. Liu, P.W. Zhang, Q. Zhen, D. Walther, X.B. Wang, G.R. Uhl, KEPI, a PKC-dependent protein phosphatase 1 inhibitor regulated by morphine, J. Biol. Chem. 277 (15) (2002) 13312–13320.

[8] K. Livak, ABI Prism 7700 Sequence Detection System, 1997.

[9] D.R. McLeod, S.B. Fowlow, A. Robertson, D. Samcoe, I. Burgess, J.J. Hoo, Chromosome 6q deletions: a report of two additional cases and a review of the literature, Am. J. Med. Genet. 35 (1) (1990) 79–84.

[10] J. Meng, H. Fujita, N. Nagahara, A. Kashiwai, Y. Yoshioka, M. Funato, Two patients with chromosome 6q terminal deletions with breakpoints at q24.3 and q25.3, Am. J. Med. Genet. 43 (4) (1992) 747–750.

[11] K. Narahara, K. Tsuji, Y. Yokoyama, H. Namba, M. Murakami, T. Matsubara, R. Kasai, Y. Fukushima, T. Seki, K. Wakui, et al., Specification of small distal 6q deletions in two patients by gene dosage and in situ hybridization study of plasminogen and alpha-L-fucosidase 2, Am. J. Med. Genet. 40 (3) (1991) 348–353.

[12] C. Pescucci, R. Caselli, S. Grosso, M.A. Mencarelli, F. Mari, M.A. Farnetani, B. Piccini, R. Artuso, M. Bruttini, M. Priolo, O. Zuffardi, S. Gimelli, P. Balestri, A. Renieri. 2q24–q31 Deletion: report of a case and review of the literature, Eur. J. Med. Genet., in press.

[13] A.T. Sim, E. Ratcliffe, M.C. Mumby, E. Villa-Moruzzi, J.A. Rostas, Differential activities of protein phosphatase types 1 and 2A in cytosolic and particulate fractions from rat forebrain, J. Neurochem. 62 (4) (1994) 1552–1559.

[14] S. Sukumar, S. Wang, K. Hoang, C.M. Vanchiere, K. England, R. Fick, B. Pagon, K.S. Reddy, Subtle overlapping deletions in the terminal region of chromosome 6q24.2–q26: three cases studied using FISH, Am. J. Med. Genet. 87 (1) (1999) 17–22.

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Case 4:

Delineation of 7q36.1-36.2 deletion syndrome: long-QT, renal hypoplasia and mental retardation.

Caselli R, Mencarelli MA, Papa FT, Ariani F, Longo I, Meloni I, Vonella G, Hayek G, Renieri A, Mari F.

In preparation for the Am J Med Genet

Delineation of 7q36.1q36.2 deletion syndrome: long QT, renal hypoplasia and mental retardation.

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Terminal deletions of long arm of chromosome 7 are well known and are frequently associated with hypotelorism/holoprosencephaly due to the involvement of SHH gene located on 7q36.3. These deletions are easily detectable with routinely subtelomeric MLPA analysis. Deletions affecting a more proximal part of 7q36, namely bands 7q36.2-36.2 are less known, and are missed by subtelomeric MLPA analysis. We report here a case of a 9 year-old female with 5.27 Mb deletion in 7q36.2-36.2. The comparison of the clinical data with those reported in the literature allows to delineate a common phenotype characterized by mental retardation, fetal phenytoin syndrome face, renal hypoplasia and long QT due to loss of KCNH2 gene. These characteristics should be taken into account in order to identify the syndromes on clinical grounds.

CASE DESCRIPTION

The proband is a 9-year-old girl, first child of non consanguineous parents (Fig. 1b). At the time of delivery the mother and the father were 27-year-old. At the age of 14 years, the mother required surgery for an intracerebral haematoma due to a frontal brain angiomata. Eight years later convulsive episodes occurred and antiepileptic therapy with phenytoin was undertaken. During the first 6 weeks of gestation she took pheniytoin and for the following period of gestation she was treated with phenobarbital and carbamazepine. The girl was born after an uncomplicated pregnancy at 39 weeks of gestation with normal vaginal delivery. Her birth weight was 2,950 g (25°-50° centile), length 47 cm (25°-50° centile), OFC 33.5 cm (25°-50° centile) and Apgar score 10 at 1' and 10 at 5'. In the first day of life she presented convulsions due to antiepileptic drugs withdrawal. Cardiac evaluation revealed a heart murmur midsystolic and soft, grade II/VI, best heard underneath the left clavicle and radiated to the axilla. Doppler echocardiography showed a mild pulmonary stenosis. ECG referred only ventricular tachycardia (160 bpm).

She presented a delayed psychomotor development: she sat upright at 12 months, was able to walk after 24 months, and began to use her first words at the age of 3 years. She presents verbal dispraxia, her adaptive behavior revealed mild deficits in the areas of communications, self help skills and socialization. Although she exhibits delays in both receptive and expressive language, relative to overall cognition, her expressive language skills are significantly more delayed. There is history of difficulties with feeding and of frequent gastroesophageal reflux. She presents also sleep disturbances with frequent awakenings during night.

At the age of 5 months, right vesicoureteral reflux was diagnosed for the recurrence of urinary tract infections. At 9 years Hippuran Renal Scintigraphy evidenced hypodysplasia of the right kidney with important decrease of renal function (clearence = 40mL/min/1.73 m^2 BSA). Ophthalmological and audiological evaluations resulted normal. A brain MRI at the age of 1 year revealed a mild enlargement of ventricula and a mild hypoplasia of corpus callosum. EEG resulted normal. Karyotype (320 bands) resulted female normal.

Physical examination at 9 years and 2 months shows: height 130 cm (50° centile), weight 32 kg (75° centile), OFC 53 cm (50°-75° centile), blonde, thick and coarse hair, prominent forehead, deep set eyes, posteriorly angulated ears with simple helix, bilateral epicanthal folds, flat nasal bridge, bulbous nasal tip, flat malar region, pointed chin, pectus excavatum. Hands and feet are small (3° centile). Generalized hypertrichosis is noted.

MOLECULAR RESULTS

Array-CGH analysis

Genomic DNA of a normal female control was obtained from Promega. Genomic DNA of the patient was isolated from an EDTA peripheral blood sample by using a QIAamp DNA Blood Kit according to the manufacturer protocol (Qiagen, Hilden, Germany).

Ten micrograms of genomic DNA both from the patient (test sample) and the control (reference sample) were sonicated. Test and reference DNA samples were subsequently purified by using dedicated columns (DNA Clean and Concentrator, Zymo Research) and the appropriate DNA concentrations were determined by a DyNA Quant™ 200 Fluorometer (GE Healthcare).
Array based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 43.000 60-mer probes (Human Genome CGH Microarray 44B Kit, Agilent Technologies, Santa Clara, California) as previously reported. [Pescucci et al., 2007] The average resolution is about 75-100 Kb.

Chromosomal anomaly

Array CGH analysis identified a chromosome 7 subtelomeric deletion of about 5.27 Mb [46,XX,del(7)(q36.1-q36.2)] (Fig. 2a). The proximal breakpoint is mapped in 7q36.1, with the last oligonucleotide present located in 147.55 Mb and the first deleted in 147.70 Mb. The distal breakpoint is located in 7q36.2 between 152.80 Mb and 153.00 Mb (last oligonucleotide deleted and first present, respectively).

Method of confirmation

Real-time quantitative PCR was performed to confirm array-CGH data. We used a pre-designed set of primers and probe specific for the SMARCD3 gene (7q36.1 locus) provided by the Assay-by-Design service (Applied Biosystems, Foster City, CA). Primers and probe were designed in exon 2 of the gene: *SMARCD3*_forward primer: 5'- CCATCTATGACTCCAGGTCTTCAG-3'; *SMARCD3*_reverse primer: 5'- GGGCCCCAGAGCTTCT -3'; *SMARCD3*_TaqMan probe: 5'-CCACCGTGGTACAGGTAG-3'. PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 µl. PCR

reactions were prepared from a single Mix consisting of: 2X TaqMan Universal PCR Master Mix, 20X SMARCD3 Assay Mix , 20X RNAaseP Mix (internal reference) and HPLC pure water. A total of 100 ng of DNA (10 µl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a prerun of 2 min at 50°C and 10 min at 95°C. Cycle conditions were 40 cycles at 95°C for 15 sec and 60°C for 1 min according to the TaqMan Universal PCR Protocol (Applied Biosystems). Control samples were used for the purpose of calibration. The starting copy number of the unknown samples was determined using the comparative Ct method, as previously described [Livak, 1997].

Causative of the phenotype

Real-time quantitative PCR analysis performed in both healthy parents showed a normal result indicating that the rearrangement is a de novo deletion (Fig. 2b).

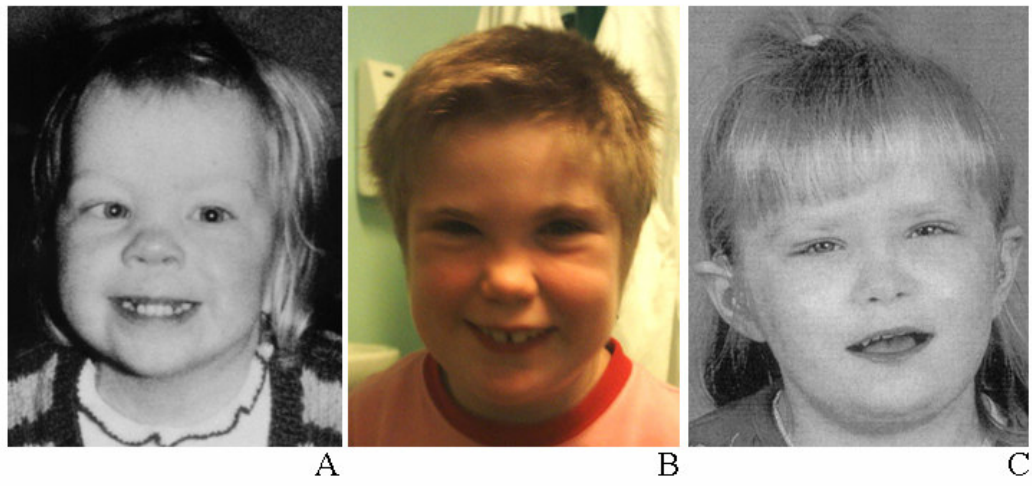

Fig. 1 Comparison of facial characteristics between Fetal Phenytoin Syndrome at the age of 18 months (a), present case at 9 years and 2 months (b) and one of the two twins reported by Bisgaard at 6 ½ years (c). Case (a) by [Moore et al., 2000]; case (c) by [Bisgaard et al., 2006].

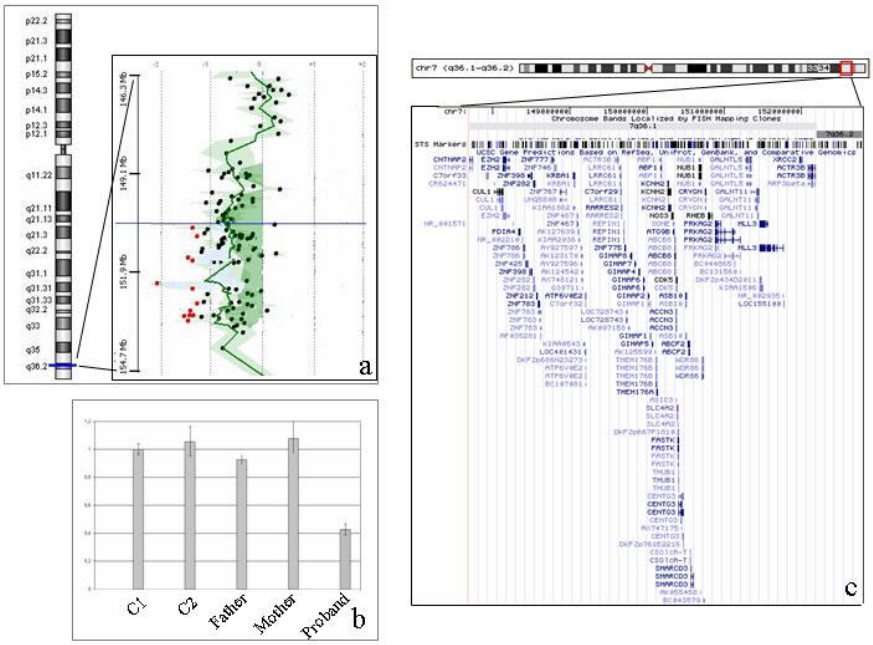

Fig. 2 Molecular results. a) Array CGH ratio profile. a) On the left, the chromosome 7 ideogram. On the right, the log2 ratio of chromosome 7 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left. b) Real-time quantitative PCR validation experiment. *SMARCD3* ddCT ratios and standard deviations obtained for the patient, her parents and two different control samples (C1 and C2). The patient shows a ddCT ratio of about 0.5, indicating the presence of a single copy of *SMARCD3* (deletion), while the parents and the controls show ddCT ratios of about 1.0, indicating a double copy of the gene (normal). c) Gene content of the deleted region (UCSC Genome Browser, Human March 2006 Assembly; http://genome.ucsc.edu).

DISCUSSION

Given the clinical history of fetal anticonvulsant exposure, phenytoin in the first 6 weeks of gestation and phenobarbital and carbamazepine successively, we first hypothesized a fetal phenytoin syndrome. In fact, infants exposed in utero to phenytoin seem to have a two- to three-fold increased risk over to general population of a congenital anomaly. They show an increase of different abnormalities, especially heart defects, facial clefts digital hypoplasia and anomalies of external genitalia [Ornoy, 2006]. In utero exposition to phenytoin is also associated with abnormalities of growth, developmental delay and peculiar facial features such as hypertelorism, epicanthic folds, short nose with flat nasal bridge and anteverted nares, long and shallow philtrum, thin upper lip, hirsutism [Moore et al., 2000; Ornoy, 2006] (Fig. 1a). Some similarities may be recognized between our patient and fetal phenytoin syndrome: epicanthic folds, short nose with flat nasal bridge, long and shallow philtrum, thin upper lip (Fig. 1a, b). Hypertrichosis is one additional common sign.

Given the association of peculiar facial features and multiple congenital anomalies, we decided to perform the Array CGH analysis in the patient, and we found a de novo deletion of the terminal cytoband of the chromosome 7q. More than 50 cases with a deletion of the distal part of the long arm of the chromosome 7 have been reported and reviewed [Verma et al., 1992; Bisgaard et al., 2006]. Most reported cases present a terminal deletion, identified by standard cytogenetic analysis, and many patients have a deletion of the SHH gene, located in 7q36.3, with consequent holoprosencephaly.

Recently Bisgaard reports a pair of female twins with a deletion of 7q34-36.2 identified by HR-CGH. They present prenatal growth retardation, developmental delay, feeding problems, agenesis of the right kidney, long QT syndrome (LQTS) and a similar facial phenotype characterized by round face, flat malar region, deep-set eyes, narrow palpebral fissures, low set ears, bulbous nasal tip, smooth philtrum and thin upper lip (Fig 1 b, c) [Bisgaard et al., 2006]. The deletion identified in our patient is completely included in that of Bisgaard, and although our is smaller, some clinical features such as developmental delay, renal dysgenesis, feeding problems are common (Tab. 1). Also the facial gestalt of the three patients is very similar: all shows flat malar region, narrow palpebral fissures, deep-set eyes, low set ears, bulbous nasal tip, smooth philtrum and narrow upper lip.

The patients reported by Bisgaard present a LQTS type 2, due to haploinsufficiency of the KCNH2 gene, mapped in 7q36.1, which encodes for a pore-forming (alpha) subunit of voltage-gated inwardly rectifying potassium channel. The revaluation of the electrocardiograms previously performed by our patients revealed long QT intervals with QTc 470 msec (QTc prolonged if > 460 msec) in addiction to ventricular tachycardia already reported. LQTS is a potentially life-threatening condition in which QT prolongation and T-wave abnormalities on the ECG, associated with tachyarrhythmias and ventricular tachycardia, may degenerate into ventricular fibrillation and cause cardiac arrest or sudden death [Priori et al., 1999]. Asymptomatic children and adults under the age of 40 years at the time of diagnosis with the LQTS should be treated prophylactically with beta-blockers [Schwartz et al., 2006]. In a genetic counseling perspective is important to emphasize the relevance of the characterization of chromosomal abnormality, which has led to the diagnosis of a potentially life-threatening condition permitting a prophylactic treatment to prevent syncope and sudden death.

In conclusion, the common emerging phenotype of deletion involving 7q36.1-36.2 is characterized by mental retardation, fetal phenytoin syndrome face, renal hypoplasia and long QT due to loss of KCNH2 gene. These characteristics should be taken into account in order to identify the syndromes on clinical ground.

Table 1. Common clinical findings in the present patient and in the twins reported by Bisgaard.

Case	Bisgaard et al. 2006	Present case
Deletion	7q34q36.2	7q36.1q36.2
Mental retardation	+	+
Growth retardation	+	-
Feeding problems	+	+
Renal dysgenesis	+	+
LQTS	+	+
Seizures	+	-
Hearing impairments	+ sensorineural	-
Ear anomalies	+	+
Flat malar region	+	+
Narrow palpebral fissures	+	+
Deep-set eyes	+	+
Ocular involvement	+ coloboma; hypermetropia	-
Bulbous nasal tip	+	+
Smooth philtrum	+	+
Narrow upper lip	+	+

REFERENCES

Bisgaard AM, Rackauskaite G, Thelle T, Kirchhoff M, Bryndorf T. 2006. Twins with mental retardation and an interstitial deletion 7q34q36.2 leading to the diagnosis of long QT syndrome. Am J Med Genet A 140(6):644-8.

Livak K. 1997. ABI Prism 7700 Sequence Detection System. no.2 UB, editor.

Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T, Dean JC. 2000. A clinical study of 57 children with fetal anticonvulsant syndromes. J Med Genet 37(7):489-97.

Ornoy A. 2006. Neuroteratogens in man: an overview with special emphasis on the teratogenicity of antiepileptic drugs in pregnancy. Reprod Toxicol 22(2):214-26.

Pescucci C, Caselli R, Grosso S, Mencarelli MA, Mari F, Farnetani MA, Piccini B, Artuso R, Bruttini M, Priolo M, Zuffardi O, Gimelli S, Balestri P, Renieri A. 2007. 2q24-q31 deletion: report of a case and review of the literature. Eur J Med Genet 50(1):21-32.

Priori SG, Barhanin J, Hauer RN, Haverkamp W, Jongsma HJ, Kleber AG, McKenna WJ, Roden DM, Rudy Y, Schwartz K, Schwartz PJ, Towbin JA, Wilde AM. 1999. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management part III. Circulation 99(5):674-81.

Schwartz PJ, Spazzolini C, Crotti L, Bathen J, Amlie JP, Timothy K, Shkolnikova M, Berul CI, Bitner-Glindzicz M, Toivonen L, Horie M, Schulze-Bahr E, Denjoy I. 2006. The Jervell and Lange-Nielsen syndrome: natural history, molecular basis, and clinical outcome. Circulation 113(6):783-90.

Verma RS, Conte RA, Sayegh SE, Kanjilal D. 1992. The interstitial deletion of bands q33-35 of long arm of chromosome 7: a review with a new case report. Clin Genet 41(2):82-6.

4.1.2 Known deletions in atypical patients

Unpublished results

4.1.2 Known deletion in atypical patients

In this section I will describe a series of patients turned to be affected by a well known microdeletion syndrome. The atypical signs that led to miss the diagnosis on a clinical ground are stressed.

Case5.

Clinical summary
Male
4y 2m
Hypotonia, seizures, ataxia, sleep disturbances, gastroesophageal reflux, dolichocolon, hypospadia, monolateral inguinal hernia, bilateral 2-3 syndactyly of toes. Severe mental retardation.

Array-CGH result (44K, Agilent)
15q11-13.1 deletion
normal copy oligo 21250794
first rearranged oligo 21290451
last rearranged oligo 26198996
normal copy oligo 26999000
beakpoint position 21.29-26.19 Mb
deletion size: 5 Mb

Method of confirmation
Southern blot with SbaI and NotI enzymes

Result of parents analysis (method)
Both normal (Southern blot with SbaI and NotI enzymes)

Atypical signs
Presence of hypospadia and dolichocolon

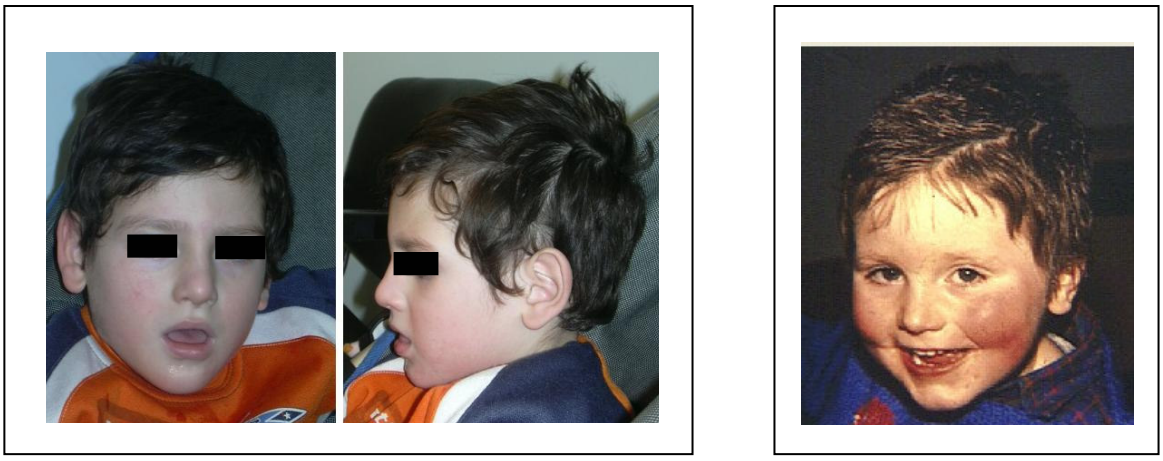

Fig 4a. Picture of case 5 (#MR174) at the age of 4y2m on the left. Photo of a typical case from LDM database on the right.

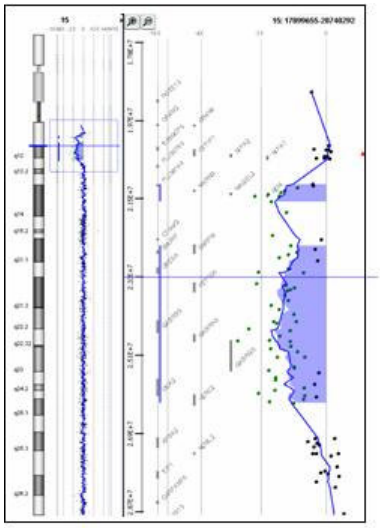

Fig 4b. Array-CGH ratio profile. On the left, the chromosome 15 ideogram. On the right, the log2 ratio of chromosome 15 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about _2X).

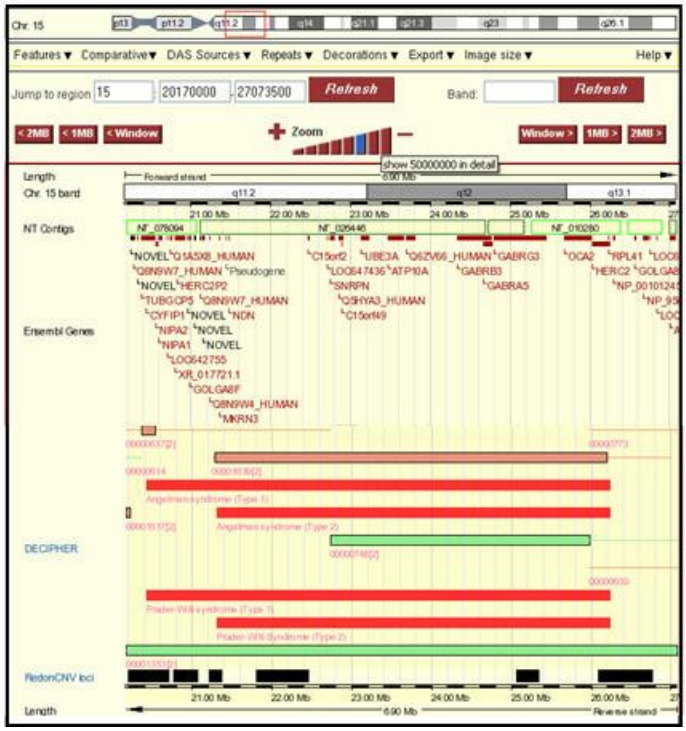

Fig 4c. Cytoview part of the Decipher database on 15q11.2 region. This section of the database includes information on the genomic context based on the Ensemble browser. It shows the index imbalance and eventually similar imbalances inserted in the Decipher. It is possible to see the phenotype of each patient by clicking on the bar corresponding to the deletion. Red bars indicate the typical deleted region in Prader-Willi/Angelman syndrome.

Case6.

Clinical summary
Female
16y
Bilateral corneal leukoma, iris and retinal coloboma, cleft lip and palate, VSD, postaxial polydactyly of hands and feet. Short stature, ataxic gait, no speech. Long nose, prognathism, hypotelorism. Severe mental retardation.

Array-CGH result (44K, Agilent)
22q11.21 deletion
first rearranged oligo 17.398 Mb
last rearranged oligo 20.123 Mb
deletion size: 2.8 Mb

Method of confirmation
MLPA (Salsa P023 Kit, MCR Holland)
Result of parents analysis (method)
Both normal (MLPA)

Atypical signs
Absence of "soft" dysmorphic features. Presence of severe mental retardation, bilateral corneal leucoma and hands/feet postaxial polydactyly.

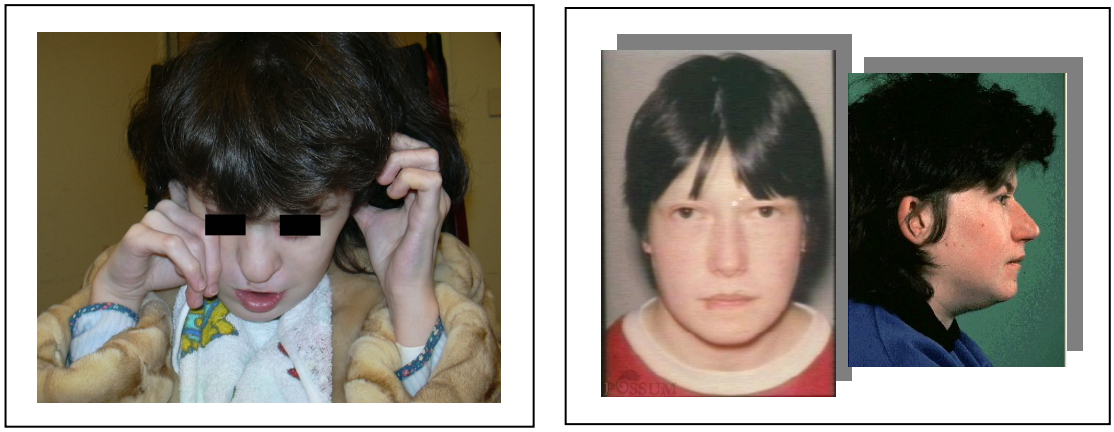

Fig 5a. Picture of case 6 (#RET900) at the age of 16 on the left. Note the very dimorphic features. Photos of a typical case from POSSUM database on the right with typical "soft" dysmorphic features.

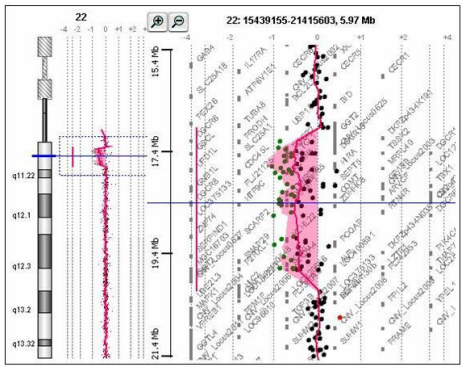

Fig 5b. Array-CGH ratio profile. On the left, the chromosome 22 ideogram. On the right, the log2 ratio of chromosome 22 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about _2X).

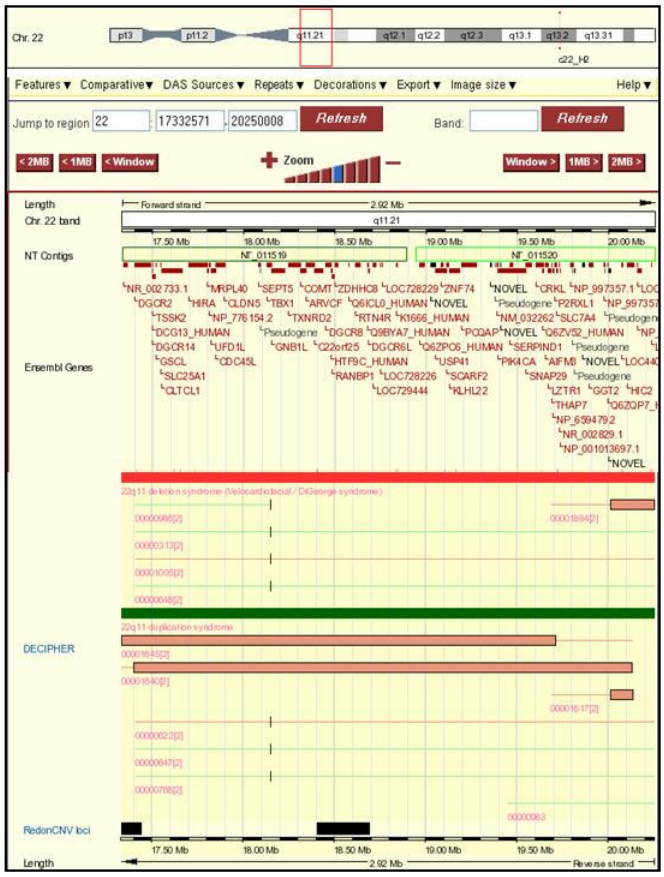

Fig 5c. Cytoview part of the Decipher database on 22q11.21 region. This section of the database includes information on the genomic context based on the Ensemble browser. It shows the index imbalance and eventually similar imbalances inserted in the Decipher. It is possible to see the phenotype of each patient by clicking on the bar corresponding to the deletion. Red and green bars indicate the typical deleted and duplicated region in DiGeorge syndrome/velocardiofacial syndrome, respectively.

Case7.

Clinical summary
Female
4y 2m
Psychomotor delay, obesity, round facies, macroglossia and macrostomia, prognathism, synophris, thick eyebrows, strabismus, small feet. Height at 97th centile. Quiet and sociable behaviour. Moderate mental retardation.

Array-CGH result (44K, Agilent)
17p11.2 deletion
normal copy oligo 16806648
first rearranged oligo 16833126
last rearranged oligo 20133702
normal copy oligo 20162228
beakpoint position 16.80-20.16 Mb
deletion size: 3.6 Mb

Method of confirmation
MLPA (Salsa P064B MR1, MCR Holland)

Result of parents analysis (method)
Both normal (MLPA)

Atypical signs
Absence of short stature, absence of self-destructive behaviour, absence of onychotillomania, absence of polyebolokoilomania, absence of self hugging, absence of sleep disturbances.

Fig 6a. Picture of case 7 (#MR334) at the age of 8y on the left. Photos of typical case from [23] on the right.

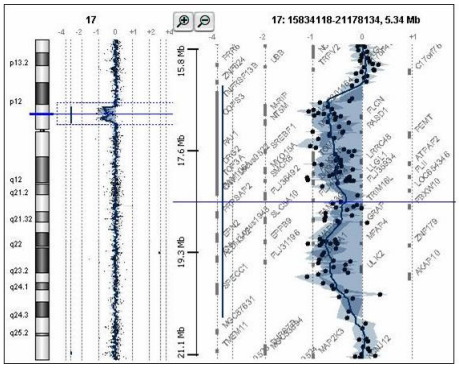

Fig 6b. Array-CGH ratio profile. On the left, the chromosome 17 ideogram. On the right, the log2 ratio of chromosome 17 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about _2X).

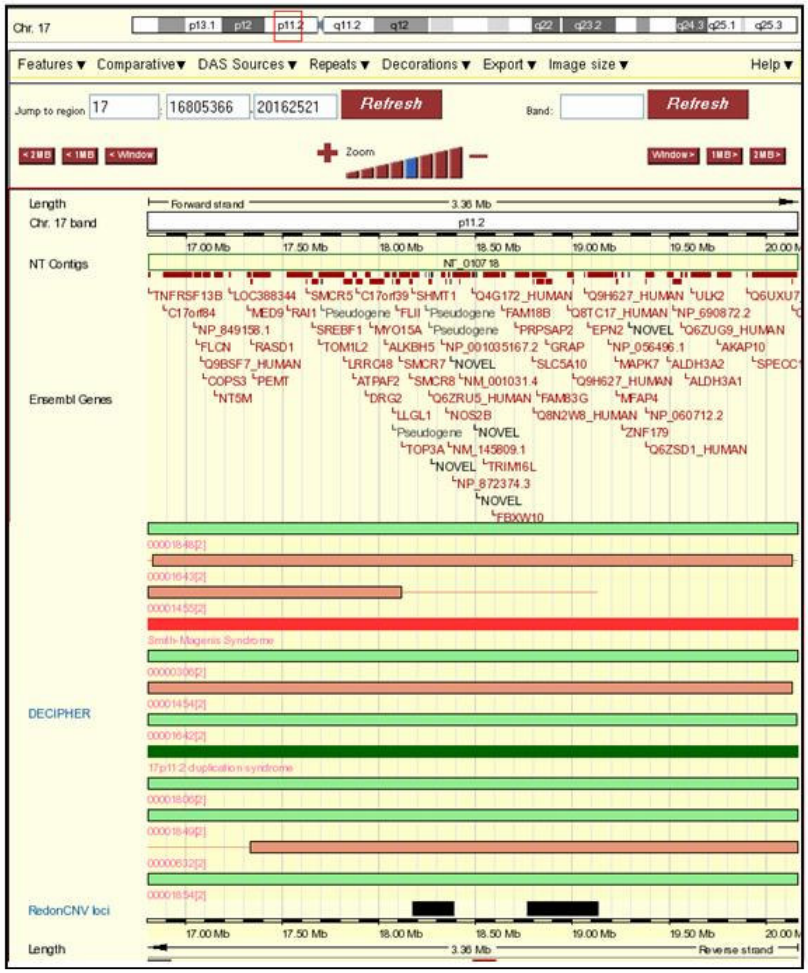

Fig 6c. Cytoview part of the Decipher database on 17p11.2 region. This section of the database includes information on the genomic context based on the Ensemble browser. It shows the index imbalance and eventually similar imbalances inserted in the Decipher. It is possible to see the phenotype of each patient by clicking on the bar corresponding to the deletion. Red and green bars indicate the typical deleted and duplicated region in Smith-Magenis syndrome, respectively.

Case 8:

Expanding the phenotype of 22q11 deletion syndrome: the MURCS association.

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Clin Dysmorphol. In press

Expanding the phenotype of 22q11 deletion syndrome: the MURCS association.

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The MURCS association consists in Müllerian Duct aplasia or hypoplasia, unilateral renal agenesis and cervicothoracic somite dysplasia. We report on a 22 year-old female with bicornuate uterus, right renal agenesis and C2-C3 vertebral fusion (MURCS association) and 22q11.2 deletion. Angio-MRI revealed aberrant origin of arch arteries. Hashimoto thyroiditis, micropolycystic ovaries with a dermoid cyst in the right ovary and mild osteoporosis were also diagnosed. Accurate revision of Xrays allowed us to identify also thoracolumbar and lumbosacral vertebral differentiation defects. Audiometry and echocardiogram were normal. Bone densitometry showed osteoporosis. At our evaluation, she had short stature, obesity (BMI 30.7) and facial features suggestive of 22q11 deletion syndrome. MLPA analysis revealed a de novo 22q11.2 deletion confirmed by Array-CGH analysis. We discuss whether this is a casual association or one additional syndrome due to the well known phenotype extensive variability of the 22q11 deletion syndrome.

INTRODUCTION

The MURCS association has a frequency of 1/50.000 females and consists in Müllerian Duct aplasia or hypoplasia (M), unilateral renal agenesis (R) and cervicothoracic somite dysplasia (CS) [Lin et al., 1996]. In the first 30 cases with vaginal agenesis with normal secondary sexual characteristics described by Duncan et al., in 1979, uterine abnormalities were present in 96% of the cases, renal abnormalities (agenesis/ectopy) in 80%, spinal defects (Klippel-Feil malformations, scoliosis, rib, upper limb and scapular anomalies) in 80% [Duncan et al., 1979]. Other anomalies reported in association with MURCS include cleft lip and palate, ovarian agenesis, abnormal pulmonary fissures, tetralogy of Fallot, anorectal malformations and transmissive deafness [Duncan et al., 1979; Greene et al., 1986; Gunsar et al., 2003; Lin et al., 1996]

The 22q11 deletion is the most frequent known interstitial deletion, with an incidence of 1 out 4000 live births and very high clinical variability [Scambler, 2000]. Beside the well known Di George and velocardiofacial syndrome, the phenotypic spectrum of this deletion includes Opitz G/BBB, Cayler cardiofacial and CHARGE syndromes [Lipson et al., 1991; Digilio et al., 1997; Emanuel et al., 2001; McDonald-McGinn et al., 1999]. A number of malformations have been described, including: cardiac outflow tract anomalies, renal agenesis [Wilson et al., 1993], multicystic renal dysplasia and vescicoureteral junction obstruction [Driscoll et al., 1992], skeletal anomalies and deformities, such as scoliosis [Wilson et al., 1993], limb anomalies, hypoplastic vertebrae, hemivertebrae and vertebral coronal clefts [Ming et al., 1997], development variations of the occiput and cervical spine, such as blatybasia, C1 anomalies, fusion of C2-C3, increased occipitoatlantal and C3-C4 segmental motion [Ricchetti et al., 2004]. Genital anomalies have been rarely reported in 22q11 deletion patients: hypoplastic genitalia, hypospadia and undescended testes in males [Digilio et al., 1997; McDonald-McGinn et al., 1999] and Mayer-Rokitansky-Küster-Hauser syndrome in females [Cheroki et al., 2006; Devriendt et al., 1997]. Most patients have hemizygous deletion of 3 Mb, while others might present either a common approximately 1,5 Mb deletion or atypical deletions. No correlation between the length of the deletion and severity of the disease has been found [Lindsay, 2001; Scambler, 2000; Yamagishi, 2002]; intrafamilial variability, even in monozygotic twins, has been found, as well [Singh et al., 2002]. This suggests that other factors might be involved in the expression of these malformations, including genetic and environmental factors.

The pathogenesis of MURCS association is not clear, but it was suggested that it could be the result of a non-random event at the end of the fourth weeks of gestation (days 25-28), when the blastemas of the pronephric buds and cervicothoracic somite buds are relatively close in location [Duncan et al., 1979]. The pathogenesis of 22q11 deletion syndrome is to be related to an abnormal neural crest derived cell development and function. In fact, the structures primarily affected, palate, brachial arch arteries and face, are all derivatives of the branchial arch/pharyngeal pouch system and each of the main tissue involved receives a contribution from the rostral neural crest during embryogenesis [Bockman et al., 1984]. It is not clear if neural crest derived cells might play somewhat role in the development of both Müllerian and Wolffian structures. Similarly, explanations of the cervical spine anomalies in 22q11 deletion syndrome is unclear [Ricchetti et al., 2004].

CLINICAL REPORT

The proband, a girl, is the second child of healthy, non consanguineous parents. The mother was 40 years old at the birth and the father 50. She was born at term, with caesarean section for maternal uterine fibromas. Birth length was 45 (<10° cnt), weight was 3100 kg (50° cnt). She was able to speak with phrases at 3 years. At 4 years old, a delayed bone age (3 years) was noted. Her parents reported frequent infections and fractures occurred when she was a child. Auxological parameters were all below the 3° percentile from 3 to 10 years. At 11 years, obesity was evident (height<3° cnt, weight 75-90° cnt). She presented menarche at 11, followed by irregular menses with dysmenorrhoea.

When she was 12, she was admitted to the hospital for acute abdomen due to an abnormal uterine bleeding retention. In this occasion, pelvic MRI revealed uterus bicornis unicollis with double right cervical canal. Dermoid cyst in right ovary

(surgery treated) and right renal agenesis were also diagnosed. At 14 years, a hypothyroidism due to Hashimoto thyroiditis was diagnosed and at 17 micropolycystic ovaries was observed through pelvic ultrasound examination. At 18 years old, she was admitted to the Department of Endocrinology and discharged with the diagnosis of "Short stature, hypothiroidism, class I obesity, osteopeny". At 19 years old vertebral radiography identified cervical lordosis, lumbar scoliosis, and C2-C3 fusion (Klippeil-Feil anomaly) (Fig 1). Pansystolic heart murmur resulted from heart auscultation, but echocardiography was normal. Brain MRI showed left carotid artery hypoplasia and the following angio-MRI identified complex aortic arch anomalies: aberrant origin of arch arteries with brachiocephalic vessels agenesis and hypoplastic left carotid artery originating from the aortic arch, left subclavian artery originating from descending thoracic aorta and hypoplastic left vertebral artery. Audiometry was normal and her IQ was 89, with dyscalculia. She was discharged from the Department of Endocrinology with the diagnosis of MURCS.

When she was 22, she was admitted to the Department of Internal Medicine, because of an in-depth study of bone metabolism. Bone densitometry showed osteoporosis, in particular at the level of lumbar spine (T-score L1-L4: -2.82). The biochemical parameters of bone metabolism showed normal values of serum total calcium, phosphorus, alcaline phosphatase, intact parathyroid hormone (PTH), vitamin D3, calcitonin, serum Insulin-like growth factor 1(IGF-1) and Insulin-like growth factor binding protein 3 (IGFBP-3).

On this occasion, she was eventually suggested for genetic counselling. At our clinical examination, short stature (142 cm, <5° cnt), microcephaly (52 cm, about 3° cnt), and obesity (weight 62 kg, BMI 30.7) were evident. Typical facial gestalt for 22q11 deletion has been observed: long face, tubular nose with bulbous tip, high nasal bridge, and swollen eyelids (Fig. 1C). Nasal speech was also noted. Her ears were small (2 cm, <-2DS) and posteriorly rotated with narrow external auditory meatus. Tapering hands, pet planus, feet clinodactyly and normal secondary sexual characteristics were also evidenced. Accurate revision of vertebral radiography performed at 19 years identified spinal abnormalities, other than C2-C3 fusion, cervical lordosis and lumbar scoliosis, in particular thoracolumbar regional vertebral differentiation defects with lumbar ribs and lumbosacral regional vertebral differentiation defects with sub-total S1 lumbarization (Fig 1 A, B).

Standard karyotype at 12 years old was normal. As the proband presented some features suggestive of 22q11 deletion syndrome, in particular aortic arch anomalies, nasal voice and typical facial gestalt, we performed molecular analysis in order to confirm clinician suspect.

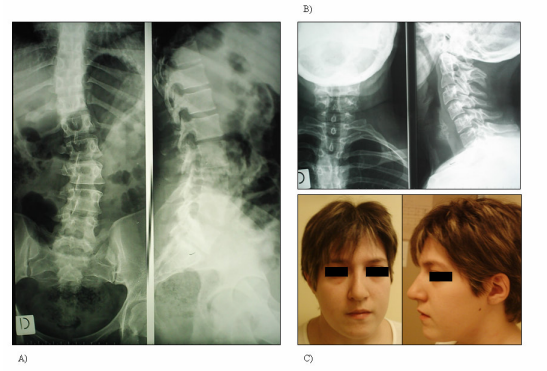

Fig 1. Vertebral radiographs of the patients at the age of 19 years: A) Whole vertebral imaging showing lumbar scoliosis, thoracolumbar regional vertebral differentiation defects with lumbar ribs and lumbosacral regional vertebral differentiation defects with sub-total S1 lumbarization. B) Cervical imaging showing fusion of C2-C3 vertebrae and cervical lordosis C) Front view (left) and lateral view (right) of the proband face.

MOLECULAR ANALYSIS

Multiplex Ligation-dependent Probe Amplification (MLPA) analysis (SALSA P023 kit, MRC-Holland, Amsterdam, Netherlands; http://www.mrc-holland.com) on patient's DNA extracted from blood showed a diminished normalized value corresponding to probes identifying the common deleted critical region in DGS located inside HIRA, CLDN5, FLJ14360, and PCQAP genes. The KIAA1652 probe was deleted, as well. The BID gene seemed to be preserved in our patient. According to Shaikh TH, et al, the present case belongs to the 2% of cases whose deletion extends in between LCR22-A and LCR22-B and ends in LCR22-D [Shaikh et al., 2000]. The analysis was performed in her healthy parent, as well, and resulted normal.

Oligo array-CGH analysis was performed to confirm MLPA and search other genetic variations (Human Genome CGH Microarray 44B Kit, Agilent Technologies, Santa Clara, California) [Pescucci et al., 2007]. This analysis confirmed the chromosome 22 interstitial deletion of about 2.7 Mb [46,XX,del(22)(q11.21)] (Fig. 2). The proximal breakpoint is mapped in 22q11.21, with the last oligonucleotide present located in 170.01 Mb and the first deleted in 172.27 Mb. The distal breakpoint is located in 22q11.21 between 19.70 Mb and 20.12 Mb (last oligonucleotide deleted and first present, respectively). As expected by MLPA results HIRA, CLDN5, FLJ14360, and PCQAP are deleted while the BID gene is not included in the deleted interval. No additional rearrangements were present at the resolution of 75-100 kb.

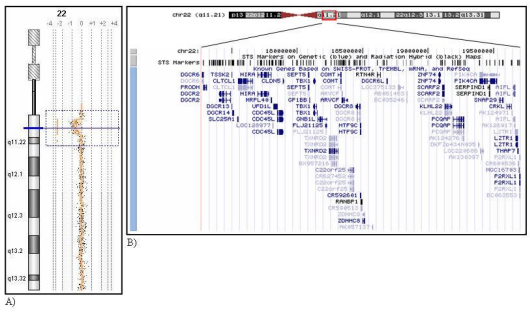

Fig 2. Molecular results. A) Array CGH ratio profile. On the left, the chromosome 22 ideogram. On the right, the log2 ratio of chromosome 22 probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left. B) Gene content of the deleted region (UCSC Genome Browser; http://genome.ucsc.edu).

DISCUSSION

Our patient has several anomalies described in MURCS association. She has the typical renal anomaly (unilateral renal agenesis) and the typical vertebral anomaly (Klippel-Feil anomaly). Although she does not have the typical vaginal agenesis described in the first report [Duncan et al., 1979], she does have uterine malformations and uterine anomalies without vaginal aplasia were described later as typical MURCS cases [Lopez et al., 2002].

She also presents many features of 22q11 deletion phenotype: aortic arch anomalies, typical facial gestalt, nasal voice, mild learning difficulties, renal agenesis, autoimmune disease and cervical spine anomaly. In fact, when investigated many 22q11 deletion patients present spine anomalies, [Ricchetti et al., 2004]. Vertebral defects described in patients with 22q11 deletion syndrome, ranges from scoliosis, supernumerary ribs, hypoplastic vertebrae, hemivertebrae, to vertebral coronal clefts [Ming et al., 1997]. Although regional vertebral differentiation defects are never openly described, those cases with supernumerary ribs may fall within as well.

To our knowledge osteoporosis has never been described in 22q11 deletion syndrome. It is worth of noting that she doesn't present hypoparathyroidism and

hypocalcemia that in the course of time may account for body mass density anomalies.

While from a clinical point of view, uterine malformations are infrequently reported in the 22q11 deletion syndrome (despite the fact that uterine and renal anomalies are often associated), recent molecular results connect 22q11 deletion to uterine aplasia and renal defects. In 1997, Denvriedt K et al., reported a 19-week fetus with Potter sequence, MKRHS and a 22q11 deletion [Devriendt et al., 1997]. In 2006, Cheroki et al. reported a patient with 22q11 deletion, about 4 Mb in size, associated with MRKHS, facial features suggestive of 22q11 deletion, Hashimoto thyroiditis and renal, cardiac and skeletal defects [Cheroki et al., 2006]. The Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) consists in aplasia of the Müllerian duct, often associated with urinary tract, skeletal and cardiac abnormalities [Griffin et al., 1976; Guerrier et al., 2006; Willemsen, 1982]. About 13-16% of patient with MRKHS fulfilled criteria for MURCS, suggesting a link between the 2 conditions [Pittock et al., 2005; Strubbe et al., 1994]. Our patient strengthens the link between three different conditions : MURCS, MRKHS and 22q11 deletion syndrome (Fig. 3).

The patient here reported present a 2,7 Mb deletion, overlapping to the typical 3 Mb deletion. One MRKHS patient reported with 22q11 deletion presents a larger one, 4Mb in size [Cheroki et al., 2006]. Our results make unlikely that MRKHS features might be caused by deletion of genes flanking the typical 22q11 interval and further confirm that no correlation between the length of the deletion and severity of the disease exists.

The proband may present a 22q11 deletion and, independently from it, MURCS-like anomalies. Alternatively, MURCS association can be one additional feature due to 22q11 deletion.

Considering the broad clinical spectrum of both the disorders, this case could be consistent with the extended phenotype of either. In particular, patients with MURCS association and evocative facial features might be valuated for 22q11 deletion genetic test; on the other hand, patients with 22q11 deletion might be valuated for genital anomalies, as well.

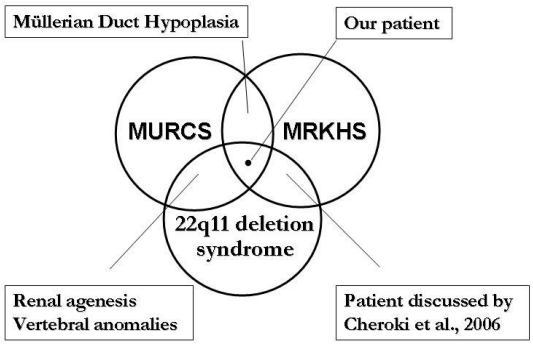

Fig 3. Phenotypic overlap between the three phenotypes: MURCS, MRKHS and the 22q11 deletion syndrome.

REFERENCES

Bockman DE, Kirby ML. 1984. Dependence of thymus development on derivatives of the neural crest. Science 223(4635):498-500.

Cheroki C, Krepischi-Santos AC, Rosenberg C, Jehee FS, Mingroni-Netto RC, Pavanello Filho I, Zanforlin Filho S, Kim CA, Bagnoli VR, Mendonca BB, Szuhai K, Otto PA. 2006. Report of a del22q11 in a patient with Mayer-Rokitansky-Kuster-Hauser (MRKH) anomaly and exclusion of WNT-4, RAR-gamma, and RXR-alpha as major genes determining MRKH anomaly in a study of 25 affected women. Am J Med Genet A 140(12):1339-42.

Devriendt K, Moerman P, Van Schoubroeck D, Vandenberghe K, Fryns JP. 1997. Chromosome 22q11 deletion presenting as the Potter sequence. J Med Genet 34(5):423-5.

Digilio MC, Giannotti A, Marino B, Guadagni AM, Orzalesi M, Dallapiccola B. 1997. Radial aplasia and chromosome 22q11 deletion. J Med Genet 34(11):942-4.

Driscoll DA, Spinner NB, Budarf ML, McDonald-McGinn DM, Zackai EH, Goldberg RB, Shprintzen RJ, Saal HM, Zonana J, Jones MC, et al. 1992. Deletions and microdeletions of 22q11.2 in velo-cardio-facial syndrome. Am J Med Genet 44(2):261-8.

Duncan PA, Shapiro LR, Stangel JJ, Klein RM, Addonizio JC. 1979. The MURCS association: Mullerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia. J Pediatr 95(3):399-402.

Emanuel BS, McDonald-McGinn D, Saitta SC, Zackai EH. 2001. The 22q11.2 deletion syndrome. Adv Pediatr 48:39-73.

Greene RA, Bloch MJ, Huff DS, Iozzo RV. 1986. MURCS association with additional congenital anomalies. Hum Pathol 17(1):88-91.

Griffin JE, Edwards C, Madden JD, Harrod MJ, Wilson JD. 1976. Congenital absence of the vagina. The Mayer-Rokitansky-Kuster-Hauser syndrome. Ann Intern Med 85(2):224-36.

Guerrier D, Mouchel T, Pasquier L, Pellerin I. 2006. The Mayer-Rokitansky-Kuster-Hauser syndrome (congenital absence of uterus and vagina)--phenotypic manifestations and genetic approaches. J Negat Results Biomed 5:1.

Gunsar C, Genc A, Sencan A, Daglar Z, Alparslan O, Mir E. 2003. MURCS association and rectovestibular fistula: case report of a patient treated with one-stage posterior sagittal anorectoplasty and sigmoid loop vaginoplasty. J Pediatr Surg 38(2):262-4.

Lin HJ, Cornford ME, Hu B, Rutgers JK, Beall MH, Lachman RS. 1996. Occipital encephalocele and MURCS association: case report and review of central nervous system anomalies in MURCS patients. Am J Med Genet 61(1):59-62.

Lindsay EA. 2001. Chromosomal microdeletions: dissecting del22q11 syndrome. Nat Rev Genet 2(11):858-68.

Lipson AH, Yuille D, Angel M, Thompson PG, Vandervoord JG, Beckenham EJ. 1991. Velocardiofacial (Shprintzen) syndrome: an important syndrome for the dysmorphologist to recognise. J Med Genet 28(9):596-604.

Lopez AG, Fryns JP, Devriendt K. 2002. MURCS association with duplicated thumb. Clin Genet 61(4):308-9.

McDonald-McGinn DM, Kirschner R, Goldmuntz E, Sullivan K, Eicher P, Gerdes M, Moss E, Solot C, Wang P, Jacobs I, Handler S, Knightly C, Heher K, Wilson M, Ming JE, Grace K, Driscoll D, Pasquariello P, Randall P, Larossa D, Emanuel BS, Zackai EH. 1999. The Philadelphia story: the 22q11.2 deletion: report on 250 patients. Genet Couns 10(1):11-24.

Ming JE, McDonald-McGinn DM, Megerian TE, Driscoll DA, Elias ER, Russell BM, Irons M, Emanuel BS, Markowitz RI, Zackai EH. 1997. Skeletal anomalies and deformities in patients with deletions of 22q11. Am J Med Genet 72(2):210-5.

Pescucci C, Caselli R, Grosso S, Mencarelli MA, Mari F, Farnetani MA, Piccini B, Artuso R, Bruttini M, Priolo M, Zuffardi O, Gimelli S, Balestri P, Renieri A. 2007. 2q24-q31 deletion: report of a case and review of the literature. Eur J Med Genet 50(1):21-32.

Pittock ST, Babovic-Vuksanovic D, Lteif A. 2005. Mayer-Rokitansky-Kuster-Hauser anomaly and its associated malformations. Am J Med Genet A 135(3):314-6.

Ricchetti ET, States L, Hosalkar HS, Tamai J, Maisenbacher M, McDonald-McGinn DM, Zackai EH, Drummond DS. 2004. Radiographic study of the upper cervical spine in the 22q11.2 deletion syndrome. J Bone Joint Surg Am 86-A(8):1751-60.

Scambler PJ. 2000. The 22q11 deletion syndromes. Hum Mol Genet 9(16):2421-6.

Singh SM, Murphy B, O'Reilly R. 2002. Monozygotic twins with chromosome 22q11 deletion and discordant phenotypes: updates with an epigenetic hypothesis. J Med Genet 39(11):e71.

Strubbe EH, Cremers CW, Willemsen WN, Rolland R, Thijn CJ. 1994. The Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome without and with associated features: two separate entities? Clin Dysmorphol 3(3):192-9.

Willemsen WN. 1982. Renal--skeletal--ear- and facial-anomalies in combination with the Mayer--Rokitansky--Kuster (MRK) syndrome. Eur J Obstet Gynecol Reprod Biol 14(2):121-30.

Wilson DI, Burn J, Scambler P, Goodship J. 1993. DiGeorge syndrome: part of CATCH 22. J Med Genet 30(10):852-6.

Yamagishi H. 2002. The 22q11.2 deletion syndrome. Keio J Med 51(2):77-88.

4.1.3 Reciprocal duplication difficult to recognise

Unpublished results

4.1.3 *Reciprocal duplication difficult to recognize*

In this section I will describe a patient (***case 9***) turned to be affected by Potocki-Lupski syndrome. It is due to the reciprocal duplication of the region deleted in the Smith-Magenis syndrome. The identification of the syndrome on a clinical ground is still very difficult.

Clinical summary
Female
8y 6m
Neonatal hypotonia, moderate mental retardation, visuo-spatial deficit, sociable attitude, hoarse voice, strabismus, telecantus, epicantus, down-slanting palpebral fessures, medial flared eyebrows.

Array-CGH result (44K, Agilent)
17p11.2 duplication
normal copy oligo 16473461
first rearranged oligo 16543855
last rearranged oligo 20162228
normal copy oligo 20374551
beakpoint position 16.54-20.16 Mb
deletion size: 3.6 Mb

Method of confirmation
MLPA (Salsa P064B MR1, MCR Holland)

Result of parents analysis (method)
Both normal (MLPA)

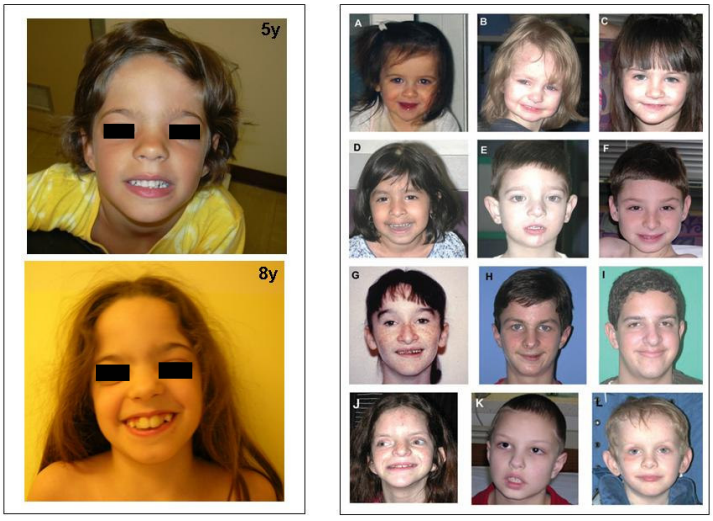

Fig 7. Picture of case 8 (WS27) at different ages (5y ad 8y) on the left. Faces of affected patients modified from [24] on the right. Note the triangular face in younger patient (F) and the oval faces in older patient (H), the relatively long nasal tip (G), the gentle down-slating of palbebral fissures (I) and the broad forehead (K).

Comment to *case 9*:

Concerning the facial phenotype, which appears not characteristic, our case in comparison with those previously reported has more clear down slanting palpebral fissures and a broader forehead. The patient presents also telecanthus. However, telecanthus is also present in the father and thus, does not correlate with the disease.

Table 1 reports a comparison between our case and previously reported cases in respect to cognitive, physical and behavioral phenotype. It is worth noting that autistic features are reported in 10/11 patients [(24)]. On the contrary, our patient does not show any autistic traits and has a friendly behavior and sociable attitude. The Childhood Autism Rating Scale (CARS) showed a score of 17 (autistic score above 30). Cognitive assessment by the WISC scale revealed moderate mental retardation. Vineland adaptive behavior scale scores were higher for "motor abilities" and "communication" respect to "daily living skills" and "socialization".

Tab 1: Comparison of clinical features in patients with 17p11.2 duplication

Trait	***Case9***	***Patients from Potocki et al., 2007***
Developmental history:		
Poor feeding as infant	+	18/19
Hypotonia as infant	+	19/21
Developmental delay	+	24/24
Neuropsichiatric and language evaluation:		
Autistic features	-	10/11
Language impairment	+	10/13
Articulation difficulties	+	18/29
Central and/or obstructive apnea	?	8/9
EEG abnormality	-	11/17
Hypermetropia seen on ophthalmic examination	+	9/16

4.1.4.Inherited imbalances

Unpublished results

4.1.4 Inherited imbalances

In this section I will describe patients with a rearrangement present also in one or more healthy relatives.

Case 10

Clinical summary
male
17y 5m
Gynoid obesity, high stature, multiple deficit of coagulation factors (II, V, VII), deafness, severe mental retardation.

Array-CGH result (44K, Agilent)
Xq25.1 deletion (Fig. 9)
normal copy oligo 123895310
first rearranged oligo 124790379
last rearranged oligo 127525412
normal copy oligo 127668712
breakpoint position 124.79-127.52 Mb
deletion size: 3 Mb

Method of confirmation
qPCR on the *WDR40C* gene (probe 5' CCAGCACCAACCCCA 3')

Result of family analysis (method)
A deletion involving the *WDR40C* gene was present in the mother and one healthy brother (qPCR)
Same deletion extent in the proband and in the healthy brother (marker analysis, personal communication from Prof. Ruth Chiquet-Ehrismann)

Fig 8. Picture of case 10 (#MR116) at the age of 17y 5m

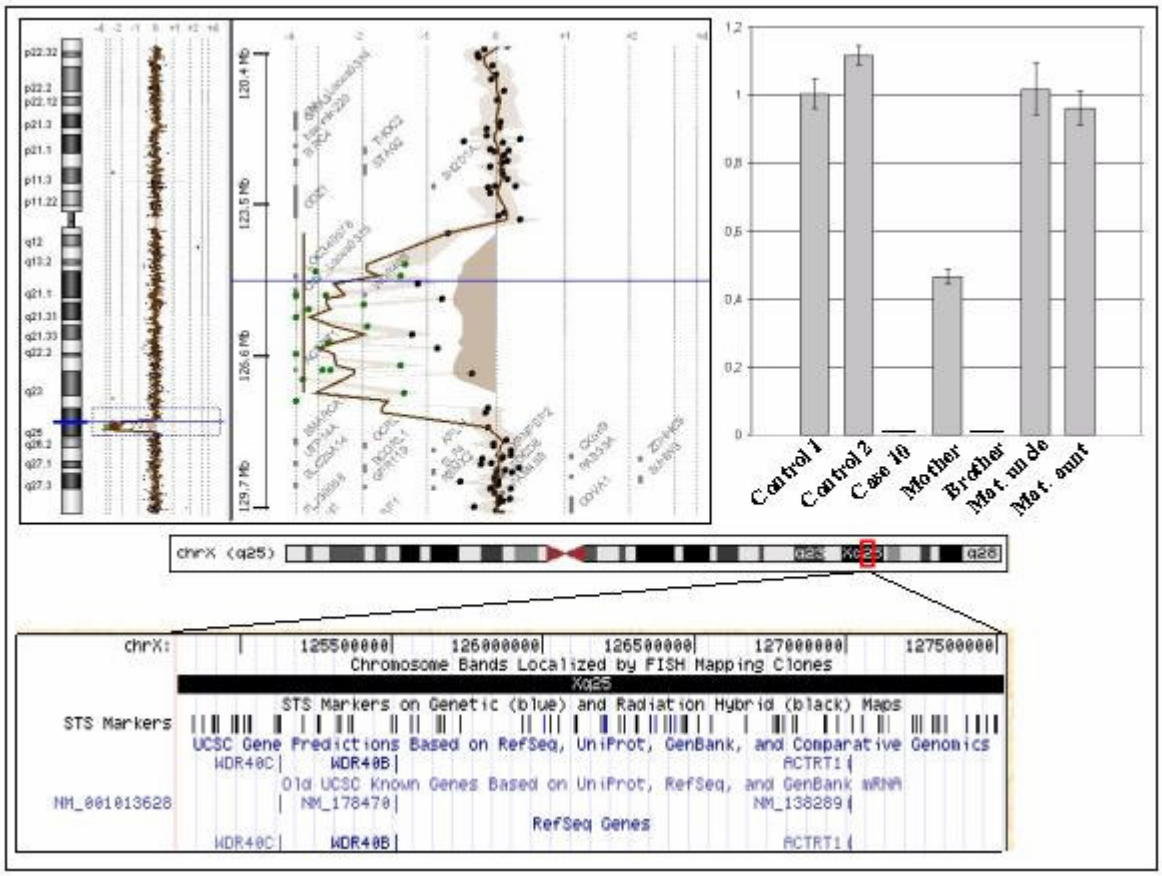

Fig.9 Molecular data of the case 10. (A) Array-CGH ratio profile. On the left, the chromosome X ideogram. On the right, the log2 ratio of chromosome X probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about _2X). (B) Realtime quantitative PCR validation experiment. Wdr40c ddCT ratios and standard deviations of two different controls and of the patient and her relatives. The patient and the healthy brother show a ddCT ratio of 0.0 that indicates the absence of a copy of Wdr40c (deletion), while the carrier mother shows ddCT ratios of about 0.5, that indicate a single copy of the gene. (C) Gene content of the deleted region (UCSC Genome Browser; http://genome.ucsc.edu).

Case 11

Clinical summary
male (46,XX karyotype)
2y 3m
Sex reversal (small penis with absence of testis), ocular anomalies (Peters' anomaly, microphtalmia, glaucoma), renal cysts, cleft soft palate, interatrial defect, mild brachydactyly and V° finger clinodactyly, severe mental retardation.

Array-CGH result (44K, Agilent)
17q12 duplication (Fig. 11)
normal copy oligo 31891535
first rearranged oligo 31925650
last rearranged oligo 33726698
normal copy oligo 33728116
breakpoint position 31.92-33.72 Mb
deletion size: 1.8 Mb

Method of confirmation
qPCR on the *AP1GB1* gene (probe 5' GCATATTAGGCTGCATGACAGAGA3')

Result of family analysis (method)
Same deletion extent in the father and healthy sister (qPCR, array-CGH 105K Agilent)

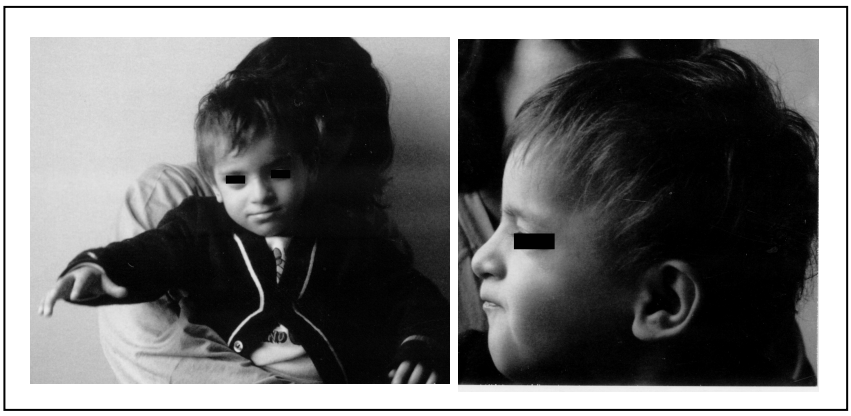

Fig.10. Picture of case 11 (#XXM1) at the age of 2y3m.

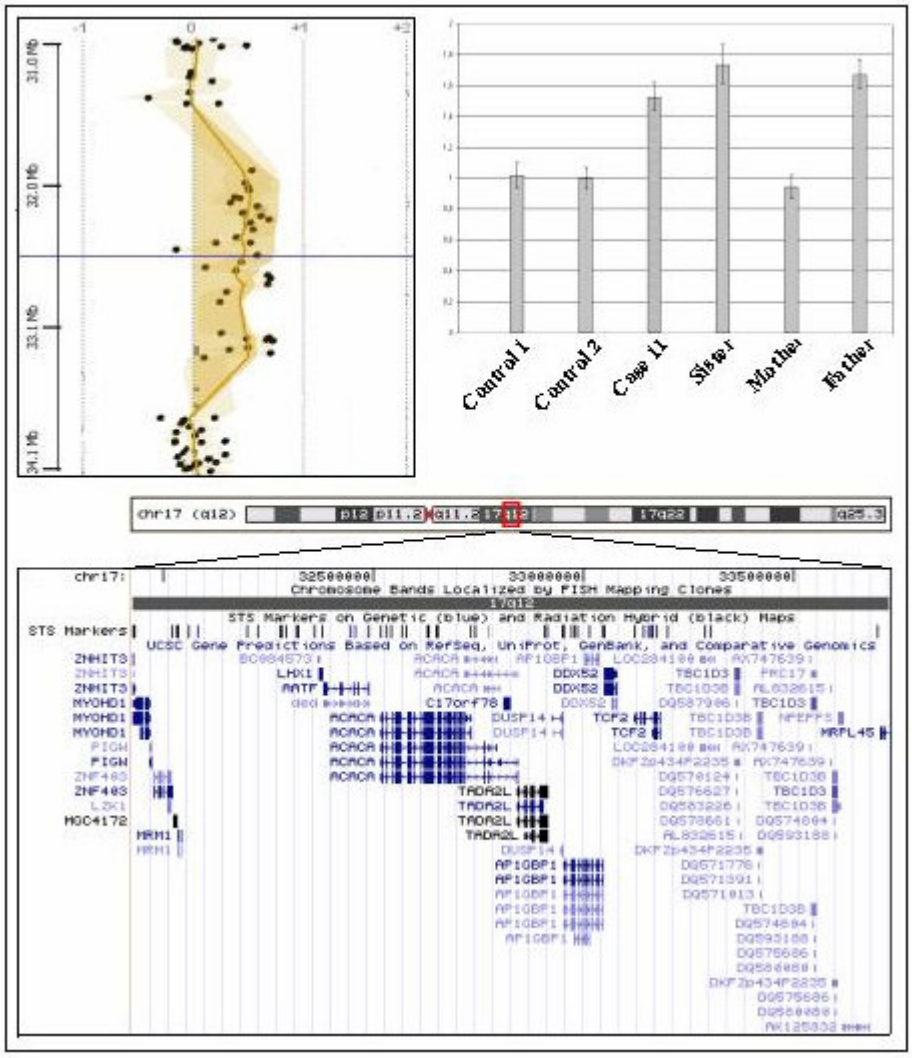

Fig.11 Molecular data of the case 11. (A) Array-CGH ratio profile. The log2 ratio of chromosome 17 probes plotted as a function of chromosomal position. Oligos with a value ofzero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number gains shift the ratio to the right (value of about 2X). (B) Real time quantitative PCR validation experiment. AP1GB1 ddCT ratios and standard deviations of two different controls and of the patient and her parents. The patient, the father and the sister show a ddCT ratio of about 1.5 that indicates the presence of an extra copy of Ap1gb1 (duplication), while the mother as controls show ddCT ratios of about 1.0, that indicates a double copy of the gene. (C) Gene content of the duplicated region (UCSC Genome Browser; http://genome.ucsc.edu).

4.2 Array-CGH as a tool for identification of MR genes

Case 12.

Clinical summary
male
24y
X-linked ichthyosis, deafness, epilepsy, severe mental retardation.

Array-CGH result (105K, Agilent)
Xp22.31 deletion
normal copy oligo 6410891
first rearranged oligo 6488000
last rearranged oligo 7351325
normal copy oligo 7415028
breakpoint position 6.48-7.35 Mb
deletion size: 0.9 Mb

Method of confirmation
PCR on the *STS* gene.

Result of family analysis (method)
Maternal uncle with isolated X-linked ichthyosis carries a larger deletion (array-CGH)

Case 13 "Maternal uncle"

Clinical summary
11y
X-linked ichthyosis, trombocitopenia

Array-CGH result (105K, Agilent)
Xp22.31 deletion
normal copy oligo 6304998
first rearranged oligo 6317139
last rearranged oligo 7941487
normal copy oligo 7962612
breakpoint position 6.31-7.94 Mb
deletion size: 1.63 Mb

Method of confirmation
PCR on the *STS* gene.

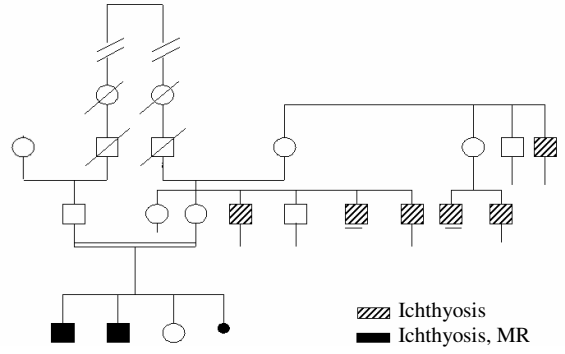

Fig.12 Pedigree of case 12 (#STS18).

Case 14

Clinical summary
male
7y10m
X-linked ichthyosis, Kallman syndrome

Array-CGH result (105K, Agilent)
Xp22.32-p22.31 deletion
normal copy oligo 5741596
first rearranged oligo 5754165
last rearranged oligo 8569445
normal copy oligo 8607194
breakpoint position 5.75-8.56 Mb
deletion size: 2.83 Mb

Method of confirmation
PCR on the *STS* gene.

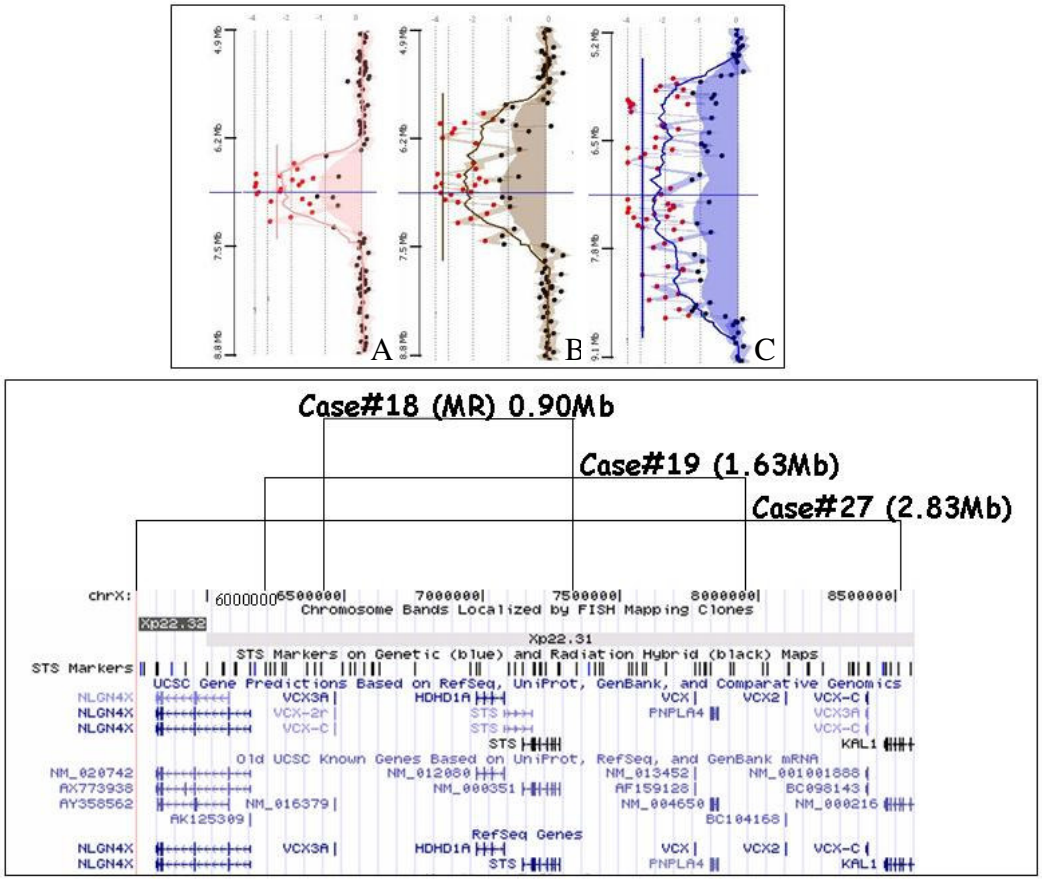

Fig.13. Molecular data of case 12 (#STS18), case 13 (#STS19) and case 14 (#STS27). (A) (B) (C). The log2 ratio of chromosome X probes plotted as a function of chromosomal position in case 12, 13 and 14, respectively. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference DNAs. Each dot represents a single probe (oligo) spotted on the array. Copy number losses shift the ratio to the left (value of about -2X). (C) Gene content of the deleted region (UCSC Genome Browser; http://genome.ucsc.edu).

Cases 15-18:

Retinoblastoma and mental retardation microdeletion syndrome: clinical and molecular dissection using array CGH

Caselli R, Speciale C, Pescucci C, Uliana V, Sampieri K, Bruttini M, Longo I, De Francesco S, Pramparo T, Zuffardi O, Frezzotti R, Acquaviva A, Hadjistilianou T, Renieri A, Mari F.

J Hum Genet. 2007;52(6):535-42

J Hum Genet
DOI 10.1007/s10038-007-0151-4

ORIGINAL ARTICLE

Retinoblastoma and mental retardation microdeletion syndrome: clinical characterization and molecular dissection using array CGH

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Received: 28 February 2007 / Accepted: 31 March 2007

Abstract We describe three patients with retinoblastoma, dysmorphic features and developmental delay. Patients 1 and 2 have high and broad forehead, deeply grooved philtrum, thick anteverted lobes and thick helix. Patient 1 also has dolicocephaly, sacral pit/dimple and toe crowding; patient 2 shows intrauterine growth retardation and short fifth toe. Both patients have partial agenesis of corpus callosum. Patient 3 has growth retardation, microcephaly, thick lower lip and micrognathia. Using array-comparative genomic hybridization (CGH), we identified a 13q14 de novo deletion in patients 1 and 2, while patient 3 had a 7q11.21 maternally inherited deletion, probably not related to the disease. Our results confirm that a distinct facial phenotype is related to a 13q14 deletion. Patients with retinoblastoma and malformations without a peculiar facial phenotype may have a different deletion syndrome or a casual association of mental retardation and retinoblastoma. Using array-CGH, we defined a critical region for mental retardation and dysmorphic features. We compared this deletion with a smaller one in a patient with retinoblastoma (case 4) and identified two distinct critical regions, containing 30 genes. Four genes appear to be good functional candidates for the neurological phenotype: *NUFIP1* (nuclear fragile X mental retardation protein 1), *HTR2A* (serotonin receptor 2A), *PCDH8* (prothocaderin 8) and *PCDH17* (prothocaderin 17).

Keywords 13q14 deletion syndrome · Developmental delay · Mental retardation · Retinoblastoma · Array-CGH

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Introduction

Retinoblastoma is the most common intraocular tumor of early childhood, with an incidence of 1/15,000–28,000 live births. Tumor development is caused by inactivation of both alleles of the *RB1* gene located in 13q14.2. In 68% of cases *RB1* is inactivated by point mutations, in 5% *RB1* complete gene deletions have been found, while gross-sized molecular deletions have been found in 10% of cases (Albrecht et al. 2005; Dahiya et al. 2000; Kloss et al. 1991; Lohmann and Gallie 2004; Sampieri et al. 2006). When the deletion involves part of the *RB1* surrounding genome it causes a contiguous gene deletion syndrome characterized by retinoblastoma, developmental abnormalities and peculiar facial dysmorphisms. The first author to suggest a specific facial phenotype associated with 13q14 deletion was Motegi in 1983 (Motegi et al. 1983). He described two patients with retinoblastoma and common facial features including prominent eyebrows, broad nasal bridge, bulbous nasal tip,

large mouth, thin upper lip and long philtrum (Motegi, et al. 1983). A few years later, he described an additional patient, and was able to improve the clinical definition of the syndrome (Motegi et al. 1987). In 1999, Baud et al. (1999) defined the dysmorphic features of 13q14 deletion syndrome. He described a cohort of 22 patients with the following common dysmorphic abnormalities: high and broad forehead, thick and everted ear lobes, short nose, prominent philtrum and thick everted lower lip (Baud et al. 1999). In 2001, Bojinova et al. (2001) extended the facial phenotype associated with the 13q14 deletion syndrome with the description of additional 13 patients characterized by cranial anomalies, frontal bossing, deeply grooved and long philtrum, depressed and broad nasal bridge, bulbous tip of the nose, thin upper lip, broad cheeks, and large ears and lobules. Afterward, a patient with a X:13 translocation and phenotypic features peculiar to the 13q14 deletion syndrome was described (Dries et al. 2003). Finally, in 2004, a patient with retinoblastoma, pinealoma and mild multiple congenital anomalies/mental retardation syndrome (MCA/MR) and a germline 13q14 deletion were reported (Skrypnyk and Bartsch 2004).

All these reported cases were studied by means of cytogenetic analysis. We investigated using array-based comparative genomic hybridization (array-CGH) three patients with retinoblastoma and MCA/MR. Using the same method, we analyzed an additional patient with isolated retinoblastoma and a previously identified RB1 deletion (Sampieri et al. 2006) to attempt to define a minimal critical region for MCA/MR. Patients were selected among the cohort of retinoblastoma cases collected in the biobank of the Medical Genetics Unit of the University of Siena (http://www.biobank.unisi.it). Here we report an accurate clinical and molecular characterization of these patients.

Materials and methods

Array-CGH analysis

Array-CGH analysis was performed using commercially available oligonucleotide microarrays containing approximately 43,000 60-mer probes (Human Genome CGH Microarray 44B Kit. Agilent Technologies, Santa Clara, California), as previously reported (Pescucci et al. 2006). The average resolution is about 75–100 kb.

Real-time quantitative PCR

Real-time quantitative polymerase chain reaction (PCR) was performed to confirm array-CGH data. We used TaqMan Gene Expression Assays by design (Applied Biosystems, http://www.products.appliedbiosystems.com), which provides a pre-designed primers-probe set for real-time PCR experiments. In order to validate the presence of the 13q deletion in cases 1, 2 and 4, we used the TaqMan probe and primers in exon 17 of RB1, as previously described (Sampieri et al. 2006). For validating the presence of the 7q deletion in case 3, we designed the probe in the BC066990 sequence related to the 7q11.21 locus. Forward primer: 5′-GTG CTG TAG TGC AGA ATG TAA CAA A-3′; reverse primer: 5′-CAG AAA GCC AAG AAT AAC-3′; TaqMan probe: 5′-AGG GTG AAC AAA ACC AGT TGA GTT-3′. PCR was carried out using an ABI prism 7000 (Applied Biosystems) in a 96-well optical plate with a final reaction volume of 50 µl. A total of 100 ng (10 µl) was dispensed in each of the four sample wells for quadruplicate reactions. Thermal cycling conditions included a pre-run of 2 min at 50°C and 10 min at 95°C. Cycle conditions were 40 cycles at 95°C for 15 s and 60°C for 1 min, according to the TaqMan Universal PCR Protocol (ABI). The TaqMan Universal PCR Master Mix and Microamp reaction tubes were supplied by Applied Biosystem. The starting copy number of the unknown samples was determined using the comparative Ct method, as previously described (Livak 1997).

RB1 mutation analysis

Genomic DNA was amplified by PCR. Primers and PCR conditions for single exons and promoter analysis have been described previously (Hogg et al. 1992; Houdayer et al. 2004; Scheffer et al. 2000). PCR products were mixed with an equal volume of formamide, denatured by heating at 95°C for 5 min, followed by immediate chilling on ice. Single-strand conformational polymorphism (SSCP) was performed on a Genephor apparatus (Pharmacia Amersham, Little Braunschweig, Germany) using a GeneGel Excel 12.5/24 Kit (Pharmacia Amersham).

Results

Clinical description

Case 1

Patient number 1, a 1-year and 2-month-old female, is the first and only child of healthy unrelated parents (Fig. 1a). At birth, the mother and father were 28 and 33 years old, respectively. Their family history was unremarkable. No teratogen exposure during pregnancy had been reported. The child was born on term by means of caesarean delivery.

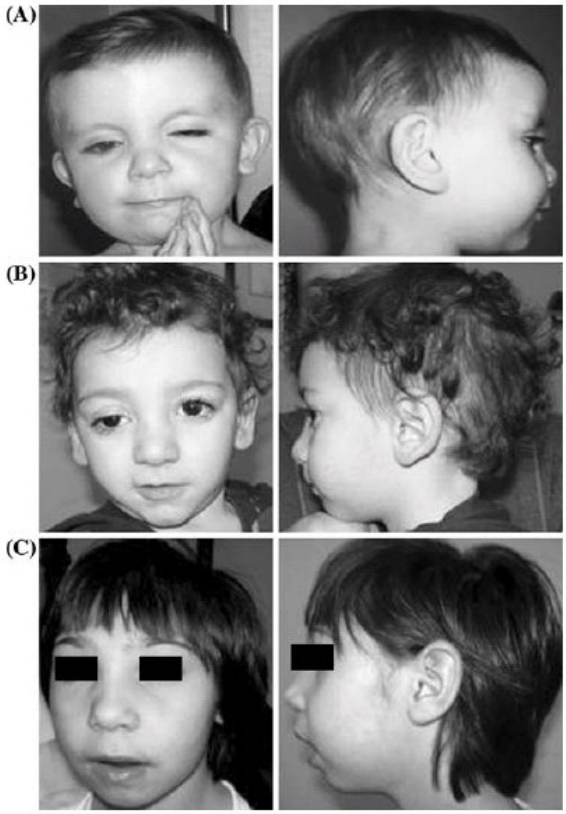

Fig. 1 Face and profile views of the patients. **a** Case 1: patient no. 1 at the age of 1 year 2 months. *Frontal view* showing high and broad forehead, deeply grooved philtrum. *Side view* showing dolicocephaly and thick anteverted lobes and helix. **b** Case 2: patient no. 2 at the age of 2 years 7 months. *Frontal view* showing hypotelorism, long palpebral fissures, epichantic folds, slight unilateral ptosis and thick and everted lower lip. Thick anteverted lobes and helix are showed on the *side view*. **c** Case 3: patient no. 3 at the age of 7 years 6 months. *Frontal view* showing sparse eyebrows in the medial third broad nasal bridge, bulbous tip of the nose, long philtrum, thick and everted lower lip. *Side view* showing large ears and micrognathia

Birth weight was 3,130 g (50th percentile), length was 51 cm (50th percentile) and head circumference was 36 cm (>90th percentile). Bilateral retinoblastoma was diagnosed at 5 months of age. At that time, MRI revealed corpus callosum hypoplasia. ABR and ankle ultrasonography were normal. At our first examination (6 months), psychomotor development was slightly delayed. Her weight was 7.850 g (75th percentile), length 68 cm (90th percentile) and head circumference 46 cm (97th percentile) with dolicocephaly. The patient presented scalp anomalies including widely open fontanelles and an alopecic area on the right temporo-parietal region. High and broad forehead, deeply grooved philtrum, and thick anteverted lobes and thick helix were noted. In addition, she showed sacral dimple and

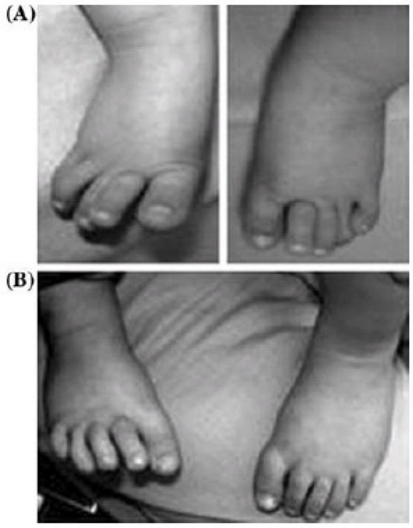

Fig. 2 Toe anomalies in cases 1(a) and 2(b). **a** View of the right feet showing toe crowding. Note clinodactyly of the 5th toe on the left foot. **b** Note short 5th toe with hypoplastic toe nail

clinodactyly of the 5th toe on the left and toe crowding on the right (Fig. 2a). At the age of 11 months, she presented with a relapse in the right eye treated by chemotherapy. Two months later, the right eye was enucleated. In the following months, a relapse in the left eye occurred, which was treated successfully by means of radiotherapy. At our second clinical examination (14 months), her weight was 9 kg (10–25th percentile), length 76–77 cm (50th percentile) and head circumference 49 cm (>97th percentile). Psychomotor delay persisted. Ultrasound cardiac examination was normal (Table 1).

Case 2

Patient number 2, a 2-year and 7-month-old boy, is the third-born of healthy and non-consanguineous parents (Fig. 1b). Intrauterine growth retardation was noted at the 36th week of gestation. He was born on term. At birth, his weight was 2,300 g (<3rd percentile), length was 47 cm (10–25th percentile) and OFC was 32 cm (3rd–10th percentile). He demonstrated a deficit of thermoregulation. On the 11th day, he presented with enterococcus sepsis. During the first months of life, the parents noted iris bilateral heterochromia. Right eye retinoblastoma was diagnosed at 10 months of age. At 1 year of age, a MRI was performed and hypoplasia of the corpus callosum was noted. By 1 year and 5 months of age, he suffered a relapse, which was treated by enucleation. At our first examination (2 years of age) his height was 78 cm (<3rd percentile), weight 8,250 kg (<<3rd percentile) and OFC 44 cm (<<3rd

Table 1 Clinical findings in patients with retinoblastoma, dysmorphic features and developmental delay

Dysmorphic features	Age		
	Case 1 (≠ 133) 1 year 2 month	Case 2 (≠ 129) 2 year 7 month	Case 3 (≠ 76) 7 year 6 month
Thick anteverted lobes	+	+	–
Thick helix	+	+	–
High and broad forehead	+	+	–
Deeply grooved philtrum	+	+/–	–
Short nose	–	+	+
Thick everted lower lip	–	+	+
Cardiac anomaly	–	–	–
Brain anomaly	Partial corpus callosum agenesis	Corpus callosum hypoplasia	–
Skeletal abnormality	Toe crowding	Short V toe	–
Growth retardation	–	+(<3 cnt)	+/–
Other	Dolicocephaly Alopecia Sacral pit	Iris heterochromya	Microcephaly (<<3 cnt) Cutis mormorata epicanthic folds

percentile). He showed hypotonia and particular facial features including high and broad forehead, deeply grooved philtrum, thick and everted lower lip, thick and everted auricular lobes, and thick helix. Moreover, he had short 5th toe with hypoplastic toenail (Fig. 2b). A second clinical examination 7 months later confirmed growth delay: 80 cm in height (<5th percentile), weight 9.0 kg (<<3rd percentile) and OFC 45 cm (<<3rd percentile). The previously noted facial features were still present. There were no abnormalities of other organs and systems. He reached self-governing deambulation at 2 years and 6 months. Presently, he is able to say only few words and he has no sphincter control. An echocardiogram showed minimum aortic reflux, probably due to the infantile infection. X-rays of hands and toes indicated no abnormalities (Table 1).

Case 3

Patient number 3, a 7-year and 11-month-old female, is the first-born of healthy, non-consanguineous parents (Fig. 3c) at birth, the mother was 35 years and the father 47 years old. After bearing this child, the mother later had a spontaneous abortion. However, this patient does have a 19-year-old maternal half-sister suspected to have Gilles De La Tourette syndrome; the mother had a spontaneous abortion after her birth. During gestation, ultrasound study revealed microcephaly. This patient was born on term and her weight was 2,780 g (10–25th percentile); data on length and OFC are not available. Development has been slightly delayed: she reached self-governing deambulation at 20 months of age and was able to say her first words when she was 2 years old. At 2.5 years of age, her mother noted right leucoria. Unilateral retinoblastoma was diagnosed and treated with eye enucleation. At our first examination (5 years and 7 months of age), her weight was 14 kg (<5th percentile), height 109.5 cm (25–50th percentile) and OFC 41 cm (<<3rd percentile). Physical examination showed sparse eyebrows in the medial third, epichantic folds, broad nasal bridge, bulbous nasal tip, long philtrum, thick and everted lower lip, large ears, micrognathia and cutis marmorata. She also showed a moderate mental retardation (Table 1). A second clinical examination at 7 years and 11 months of age confirmed short stature (115 cm, <5th percentile), the same previously described facial features and microcephaly (OFC 42 cm, <<3rd percentile).

Case 4

Patient number 4, a 5-year and 4-month-old female, is the second child of unrelated parents. At birth, the mother was 20 years and the father 26 years old. Paternal history was unremarkable and the first child of the couple is healthy. The mother and a younger brother, however, have been affected by retinoblastoma. The mother presented with retinoblastoma in the left eye at 11 months of age and enucleation was immediately performed. In the younger brother, the diagnosis was made at 40 days of life. In the second child, patient number 4, the gestation of the proband was unremarkable and no teratogen exposure was reported. Multifocal retinoblastoma in the left eye was diagnosed at 2 years and 7 months. After four cycles of chemotherapy, the tumors showed good regression but three relapses occurred and enucleation was performed.

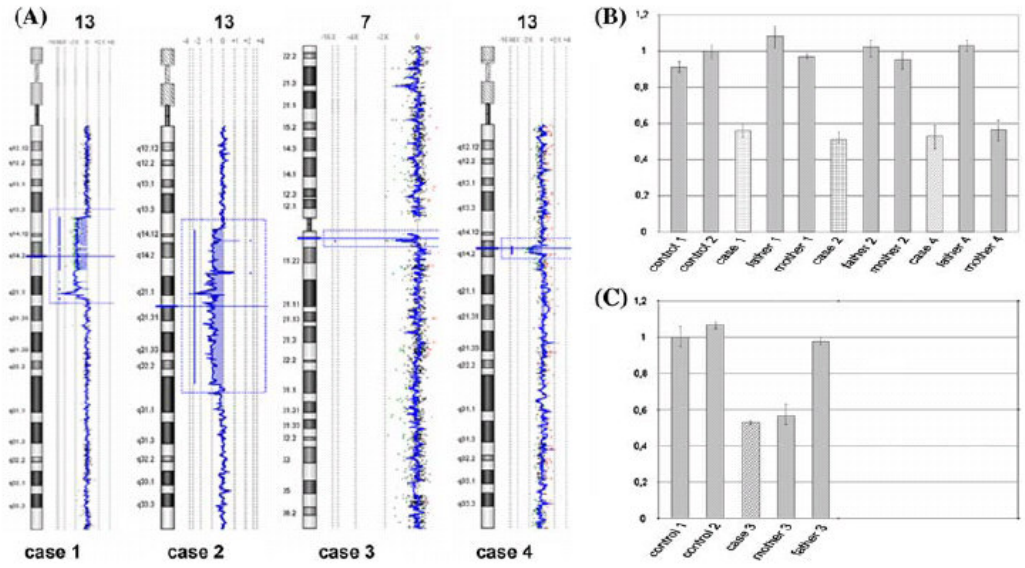

Fig. 3 Patients' molecular data. **a** Array CGH ratio profiles. On the left, chromosome ideogram for each case. On the right, the log2 ratio of the chromosome probes plotted as a function of chromosomal position. Oligos with a value of zero represent equal fluorescence intensity ratio between sample and reference. Each *dot* represents a single probe (oligo) spotted on the array. Copy number loss shifts the ratio to the left (value of about –2X). **b** and **c** Real-time quantitative PCR validation experiment. **b** RB1 ddCT ratios and standard deviations of two different controls and of patients 1, 2 and 4 and their parents. Patients 1, 2 and 4 and the mother of 4 show a ddCT ratio of about 0.5, indicating the presence of a single copy of RB1 (deletion). The parents of patients 1 and 2 and the father of patient 4, as the controls, show ddCT ratios of about 1.0, which indicates a double copy of the gene. **c** BC066990 sequence ratios and standard deviations of two different controls and of patient 3 and her parents. The patient and the mother show ddCT ratio of about 0.5, indicating the presence of a single copy of BC066990 sequence (deletion); while the father, like the controls, shows ddCT ratios of about 1.0 indicating a double copy of the sequence

During ophthalmological follow-up, no foci were noted in the right eye. On clinical examination, neither mental retardation nor dysmorphisms have been noted. Isolated unilateral retinoblastoma was the unique clinical sign.

Molecular characterization

Point mutation analysis of promoter and coding sequences of the *RB1* gene in the four cases did not reveal any alterations.

Oligonucleotide array-CGH analysis with an averaged spatial resolution of approximately 75 kb was performed on DNA from the four patients. The analysis of ratio profiles in cases 1, 2 and 4 revealed a different sized interstitial deletion in the long arm of chromosome 13. Based on the array findings, the deleted region observed in case 1 was found to extend approximately 19 Mb [46,XXdel(13)(q13.3q21.2)]. The proximal breakpoint is mapped in 13q13.3 (last oligonucleotide present located in 40.34 Mb, first deleted in 40.40 Mb position), while the distal breakpoint is located between 59.29 and 59.36 Mb in 13q21.2 (last oligonucleotide deleted and first present, respectively) (Fig. 3a). The deleted region seen in case 2 is approximately 36 Mb in size [46,Xydel(13)(q14.11q31.1)]. In this case, the proximal breakpoint is mapped in 13q14.11 (last oligonucleotide present located in 43 Mb, first deleted in 43.24 Mb position), while the distal breakpoint is located between 79.27 and 79.80 Mb in 13q31.1 (last oligonucleotide deleted and first present, respectively) (Fig. 3a). In contrast, the array-CGH analysis of case 3 revealed a 200-kb proximal deletion on chromosome 7q [46,XX del(7)(q11.21)](Fig. 3a).

The array-CGH analysis of case 4 allowed us to identify an interstitial deletion of approximately 1.7 Mb [46,XX del(13)(q14.2)]. In this case, both breakpoints are located in 13q14.2 (last oligonucleotide present located in 47.35 Mb, first deleted in 47.44 Mb position while last oligonucleotide deleted in 49.10 Mb position and first oligonucleotide present in 49.17Mb position). In order to confirm array findings, real-time Quantitative PCR experiments were performed in the patients and their parents. In all cases, the deletion was confirmed. Identified deletions of the long arm of chromosome 13 were de novo in patients 1 and 2 and inherited in 4. The 7q microdeletion in patient 3 was inherited from the unaffected mother (Fig. 3b, c).

Discussion

Two distinct syndromes are associated with deletions involving different regions of the long arm of chromosome 13. One of these syndromes is caused by a more distal deletion that involves band q32 and is phenotypically characterized by the presence of severe mental retardation, major malformations and digital anomalies (Brown et al. 1993, 1995). The other one is due to a proximal deletion that involves band q14 and is associated with retinoblastoma and mental retardation. Baud et al. defined the peculiar facial traits of this syndrome that is characterized by anteverted ear lobes, high and broad forehead, and a prominent philtrum (Baud et al. 1999; Motegi et al. 1987, 1983).

The facial features of patients 1 and 2 suggest the 13q14 deletion syndrome described by Baud et al. 1999 (Table 1). In particular, both patients have high and broad foreheads, deeply grooved philtrum, thick and anteverted lobes and thick helices. These characteristics are absent in patient 3. As expected on the basis of clinical examination, array CGH analysis confirmed a 13q14 deletion syndrome in patients 1 and 2 but not in 3. Our findings confirm that 13q14 deletion syndrome is characterized by specific facial features and that this diagnosis may be strongly suspected on a clinical ground even before the genetic test.

A more complex situation is present in case 3. The presence of the small 7q deletion in the unaffected mother suggests that this deletion is not responsible for the clinical phenotype in this patient. In addition, a known copy-number polymorphism (CNP) is located at a distance of 80 kb from our deletion (http://www.projects.tcag.ca/bioxrt/), and the deletion is included in a region of highly homologous duplicated sequences (Sharp et al. 2005). However, three different imprinted regions located on the long arm of chromosome 7 have already been described (http://www.geneimprint.com/site/genes-by-species.Homo+sapiens.imprinted-All). Consequently, additional analyses are necessary to investigate whether the deleted region is imprinted and to definitively rule out the involvement of this small deletion in the clinical phenotype of the patient.

Deletions identified in cases 1 and 2 partially overlap and allowed us to define a minimal critical region for mental retardation and dysmorphic features of about 16 Mb that includes 39 known genes.

Comparison of this critical region with the 13q14.2 deletion present in case 4 with isolated retinoblastoma allowed us to exclude a central region containing 9 known genes. Consequently, we identified two distinct critical regions, a centromeric sub-region of about 4 Mb and a telomeric one of about 10 Mb. Gene content analysis of the centromeric sub-region showed the presence of 14 known genes (Fig. 4, Table 2). Among them, *NUFIP1* is of particular interest due to its putative role in central nervous system (CNS) development and to its preferential brain expression. The *NUFIP1* gene encodes for a nucleo-cytoplasmatic RNA binding protein: FMRP interacting protein 1. NUFIP1 interacts with FMRP, the protein disrupted in Fragile X Mental Retardation (Bardoni et al. 2003). In particular, *NUFIP1* could be involved in the regulation of local protein synthesis near active synapses in association with FMRP (Bardoni et al. 2003). Due to its role in synaptic plasticity, *NUFIP1* could be a good candidate gene for mental retardation in our patients. The *HTR2A* gene is located within the centromeric deleted sub-region. This gene codifies for a receptor of serotonin. Disturbances in

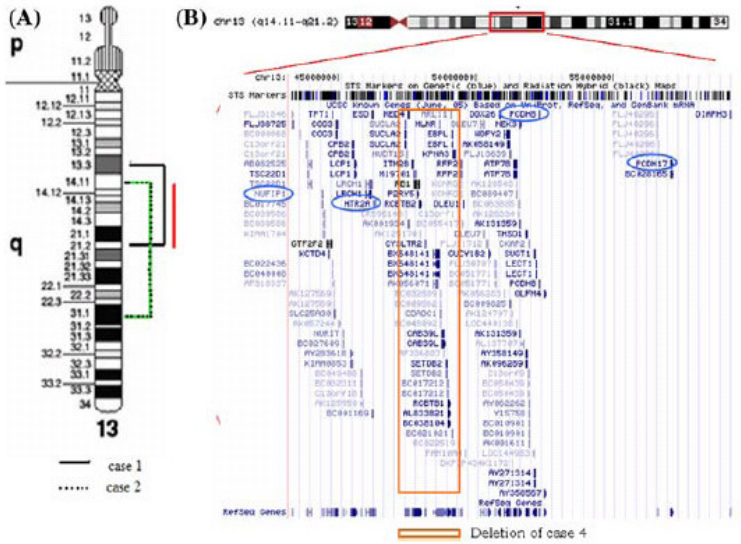

Fig. 4 Gene content of the deleted region (UCSC Genome Browser; http://www.genome.ucsc.edu). **a** Comparison between deletions observed in cases 1 and 2 and minimal critical region. **b** Gene content of the two critical sub-regions. *Orange rectangles* delimit deleted region identified in case 4. *Circled genes* are discussed in the text

Table 2 Centromeric critical region

Gene symbol	Gene name	Note
TSC22D1	TSC22 domain family 1	
NUFIP1	Nuclear fragile X mental retardation protein 1	
GTF2F2	General transcription factor IIF, polypeptide 2	
KCTD4	Potassium channel tetramerisation domain containing protein 4	
TPT1	Histamine-releasing factor	
COG3	Component of Golgi transport complex 3	
NURIT	Testis-specific leucine zipper protein nurit	
SLC25A30	Solute carrier family 25, member 30	
ZC3H13	Zinc finger CCCH-type containing 13	
CPB2	Plasma carboxypeptidase B2 isoform a	
LCP1	Lymphocyte cytosolic protein 1	
LRCH1	Leucine-rich repeats and calponin homology	
ESD	Esterase D	
HTR2A	Serotonin receptor 2A	Polymorphisms have been studied in association with ADHD, Alzheimer disease and behavioral disorders

Table 3 Telomeric critical region

Gene symbol	Gene name	Note
RFP2	Ret finger protein 2 isoform 2	Involved in B-cell chronic lymphocytic leukemia in 13q14 deletion
KCNRG	Potassium channel regulator	Candidate for B-cell chronic lymphocytic leukemia and prostate cancer tumor suppressor
DLEU1	Deleted in lymphocytic leukemia, 1	Leukemia associated protein 1 may act as tumor suppressor
DLEU7	Deleted in lymphocytic leukemia, 7	
DDX26	DEAD/H box polypeptide 26	
WDFY2	WD repeat and FYVE domain-containing protein 2	
GUCY1B2	Guanylate cyclase 1, soluble, beta 2	
ATP7B	ATPase beta polypeptide	Homozygous mutations have been associated with Wilson disease
NEK3	Serine/threonine-protein kinase Nek 3	
THSD1	Thrombospondin type I domain-containing 1	
TMAP	Tumor-associated microtubule-associated protein	
SUGT1	Suppressor of G2 allele of SKP1	
LECT1	Leukocyte cell-derived chemotaxin 1	
PCDH8	Protocadherin 8	
PCDH17	Protocadherin 17	
DIAPH3	Diaphonous protein homolog 3	

the serotonergic neurotransmission system may be responsible for behavioral disorders (Bruce et al. 2005). In particular, a polymorphism in the HTR2A gene was associated with the remission of attention deficit/hyperactivity disorder (ADHD) (Li et al. 2006). Disruption of serotonin receptor activity may contribute to CNS disorders that have been associated with impaired development.

Gene content analysis of the deleted telomeric sub-region showed the presence of 16 known genes (Fig. 4, Table 3). Among them, *PCDH8* and *PCDH17* may be good candidates for the generation of the neurological phenotype in our patients. These genes belong to the protocadherin gene family and codify for integral membrane proteins, which are thought to function in signaling pathways and in cell

adhesion in a CNS-specific manner. The role of Pcdh8 in the nervous system was investigated in rat hippocampus (Yamagata et al. 1999). Antibodies against Pcdh8 attenuate basal synaptic transmission and completely inhibit long-term potentiation in hippocampal slices (Yamagata et al. 1999). The expression and function of the *PCDH17* gene is not well known.

To date, all 48 cases with a 13q14 microdeletion reported in the literature have been characterized at the cytogenetic level. This is the first report of characterization at the molecular level, using array-CGH, of patients with retinoblastoma and mental retardation, and a critical region for mental retardation is defined. Further experiments are necessary to narrow this critical region and to dissect the syndrome, thus identifying the gene(s) responsible for the neurological phenotype in these patients.

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References

Albrecht P, Ansperger-Rescher B, Schuler A, Zeschnigk M, Gallie B, Lohmann DR (2005) Spectrum of gross deletions and insertions in the RB1 gene in patients with retinoblastoma and association with phenotypic expression. Hum Mutat 26(5):437–445

Bardoni B, Willemsen R, Weiler IJ, Schenck A, Severijnen LA, Hindelang C, Lalli E, Mandel JL (2003) NUFIP1 (nuclear FMRP interacting protein 1 is a nucleocytoplasmic shuttling protein associated with active synaptoneurosomes. Exp Cell Res 289(1):95–107

Baud O, Cormier-Daire V, Lyonnet S, Desjardins L, Turleau C, Doz F (1999) Dysmorphic phenotype and neurological impairment in 22 retinoblastoma patients with constitutional cytogenetic 13q deletion. Clin Genet 55(6):478–82

Bojinova RI, Schorderet DF, Addor MC, Gaide AC, Thonney F, Pescia G, Nenadov-Beck M, Balmer A, Munier FL (2001) Further delineation of the facial 13q14 deletion syndrome in 13 retinoblastoma patients. Ophthalmic Genet 22(1):11–18

Brown S, Gersen S, Anyane-Yeboa K, Warburton D (1993) Preliminary definition of a ''critical region'' of chromosome 13 in q32: report of 14 cases with 13q deletions and review of the literature. Am J Med Genet 45(1):52–59

Brown S, Russo J, Chitayat D, Warburton D (1995) The 13q-syndrome: the molecular definition of a critical deletion region in band 13q32. Am J Hum Genet 57(4):859–866

Bruce KR, Steiger H, Joober R, Ng Ying Kin NM, Israel M, Young SN (2005) Association of the promoter polymorphism -1438G/A of the 5-HT2A receptor gene with behavioral impulsiveness and serotonin function in women with bulimia nervosa. Am J Med Genet B Neuropsychiatr Genet 137(1):40–44

Dahiya A, Gavin MR, Luo RX, Dean DC (2000) Role of the LXCXE binding site in Rb function. Mol Cell Biol 20(18):6799–6805

Dries D, Baca K, Truss L, Dobin S (2003) Interstitial deletion of 13q and a 13;X chromosome translocation results in partial trisomy 13 and bilateral retinoblastoma. Ophthalmic Genet 24(3):175–180

Hogg A, Onadim Z, Baird PN, Cowell JK (1992) Detection of heterozygous mutations in the RB1 gene in retinoblastoma patients using single-strand conformation polymorphism analysis and polymerase chain reaction sequencing. Oncogene 7(7):1445–1451

Houdayer C, Gauthier-Villars M, Lauge A, Pages-Berhouet S, Dehainault C, Caux-Moncoutier V, Karczynski P, Tosi M, Doz F, Desjardins L, Couturier J, Stoppa-Lyonnet D (2004) Comprehensive screening for constitutional RB1 mutations by DHPLC and QMPSF. Hum Mutat 23(2):193–202

Kloss K, Wahrisch P, Greger V, Messmer E, Fritze H, Hopping W, Passarge E, Horsthemke B (1991) Characterization of deletions at the retinoblastoma locus in patients with bilateral retinoblastoma. Am J Med Genet 39(2):196–200

Li J, Kang C, Wang Y, Zhou R, Wang B, Guan L, Yang L, Faraone SV (2006) Contribution of 5-HT2A receptor gene -1438A > G polymorphism to outcome of attention-deficit/hyperactivity disorder in adolescents. Am J Med Genet B Neuropsychiatr Genet 141(5):473–476

Livak K (1997) ABI Prism 7700 Sequence detection system

Lohmann DR, Gallie BL (2004) Retinoblastoma: revisiting the model prototype of inherited cancer. Am J Med Genet C Semin Med Genet 129(1):23–28

Motegi T, Ikeda K, Watanabe K, Yanagawa Y, Minoda K (1987) Deletion (13)(q13q14.3) with retinoblastoma: confirmation and extension of a recognisable pattern of clinical features in retinoblastoma patients with 13q deletion. J Med Genet 24(11):696–697

Motegi T, Kaga M, Yanagawa Y, Kadowaki H, Watanabe K, Inoue A, Komatsu M, Minoda K (1983) A recognizable pattern of the midface of retinoblastoma patients with interstitial deletion of 13q. Hum Genet 64(2):160–162

Pescucci C, Caselli R, Grosso S, Mencarelli MA, Mari F, Fametani MA, Piccini B, Artuso R, Bruttini M, Priolo M, Zuffardi O, Gimelli S, Balestri P, Renieri A (2006) 2q24-q31 Deletion: report of a case and review of the literature. Eur J Med Genet

Sampieri K, Hadjistilianou T, Mari F, Speciale C, Mencarelli MA, Cetta F, Manoukian S, Peissel B, Giachino D, Pasini B, Acquaviva A, Caporossi A, Frezzotti R, Renieri A, Bruttini M (2006) Mutational screening of the RB1 gene in Italian patients with retinoblastoma reveals 11 novel mutations. J Hum Genet 51(3):209–216

Scheffer H, Van Der Vlies P, Burton M, Verlind E, Moll AC, Imhof SM, Buys CH (2000) Two novel germline mutations of the retinoblastoma gene (RB1) that show incomplete penetrance, one splice site and one missense. J Med Genet 37(7):E6

Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, Pertz LM, Clark RA, Schwartz S, Segraves R, Oseroff VV, Albertson DG, Pinkel D, Eichler EE (2005) Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 77(1):78–88

Skrypnyk C, Bartsch O (2004) Retinoblastoma, pinealoma, and mild overgrowth in a boy with a deletion of RB1 and neighbor genes on chromosome 13q14. Am J Med Genet A 124(4):397–401

Yamagata K, Andreasson KI, Sugiura H, Maru E, Dominique M, Irie Y, Miki N, Hayashi Y, Yoshioka M, Kaneko K, Kato H, Worley PF (1999) Arcadlin is a neural activity-regulated cadherin involved in long term potentiation. J Biol Chem 274(27):19473–1979

5. DISCUSSION and FUTURE PERSPECTIVES

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The aim of this work was to investigate a selected group of patients with mental retardation and associated anomalies for the presence of submicroscopic chromosomal imbalances. In the last years, several authors demonstrated that array-based Comparative Genomic Hybridisation is a powerful tool for the detection of cryptic chromosomal imbalances in patients with mental retardation and multiple congenital anomalies (MCA/MR). Consequently, we decided to employ this innovative technique to study our group of MCA/MR patients. We have optimised oligonucleotide array-CGH with an average resolution of 75 kb and 16 kb, providing a full coverage of the human genome.

Among 32 patients, we have identified a clear pathogenic rearrangement in 9 cases (28%) (Fig.14). This percentage is likely higher than those previously reported [17 18 25 26]. This difference is likely due to the strict selection criteria we have used, that include mild to severe MR and peculiar facial features and other congenital anomalies in patients with normal karyotype and negative for subtelomeric rearrangements.

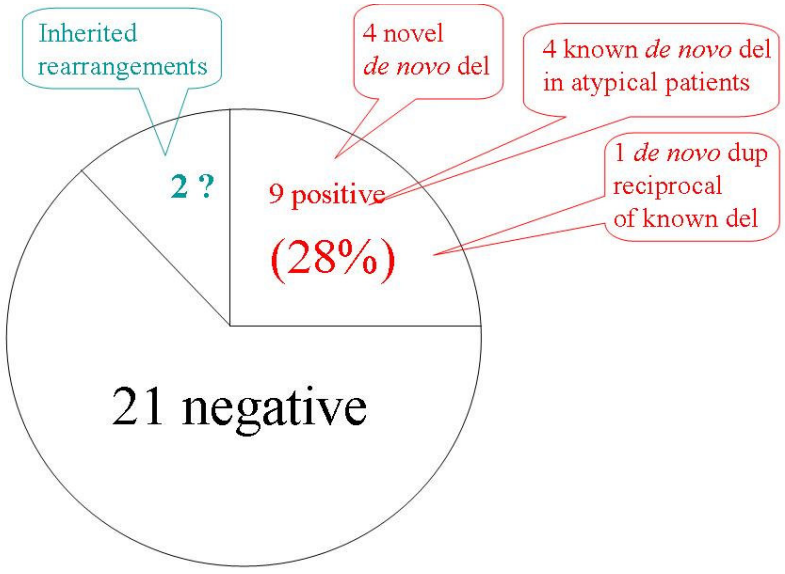

Fig.14. Number and type of rearrangements identified by oligo array-CGH in a cohort of 32 MCA/MR patients.

The nine patients with a pathogenic rearrangement fell in three different categories: 1) novel deletions, 2) known deletions in atypical patients, 3) duplications difficult to recognize on clinical ground (Fig.14). It is worth of note that the fraction of known deletions in atypical patients is relevant and it is numerically similar to the fraction of novel deletions. This quite unexpected finding is reported also by other authors [27-29]. As a consequence, one might expect that the employment of array-CGH on large scale will lead to increase our knowledge on the variability of the phenotype associated with well known syndromes: 15q11-q13, 22q11, 17p11, etc (Fig. 15). As concern for novel deletions, these appear to be rare conditions often not associated with LCR at the breakpoints (Fig. 16).

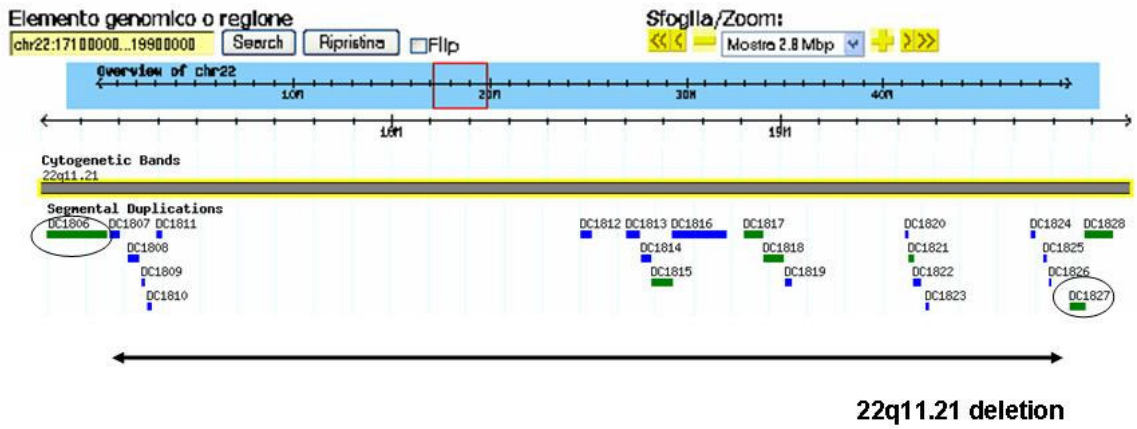

Fig. 15. LCR present in the 22q11.21 region from the Human Genome Segmental Duplication Database (http://projects.tcag.ca/humandup/). Green bars denote interchromosomal and intrachromosomal duplications while blue bars indicate intrachromosomal duplications. The breakpoints of the deletion present in cases 6 and 8 are possibly associated with LCR, indicated with black circles.

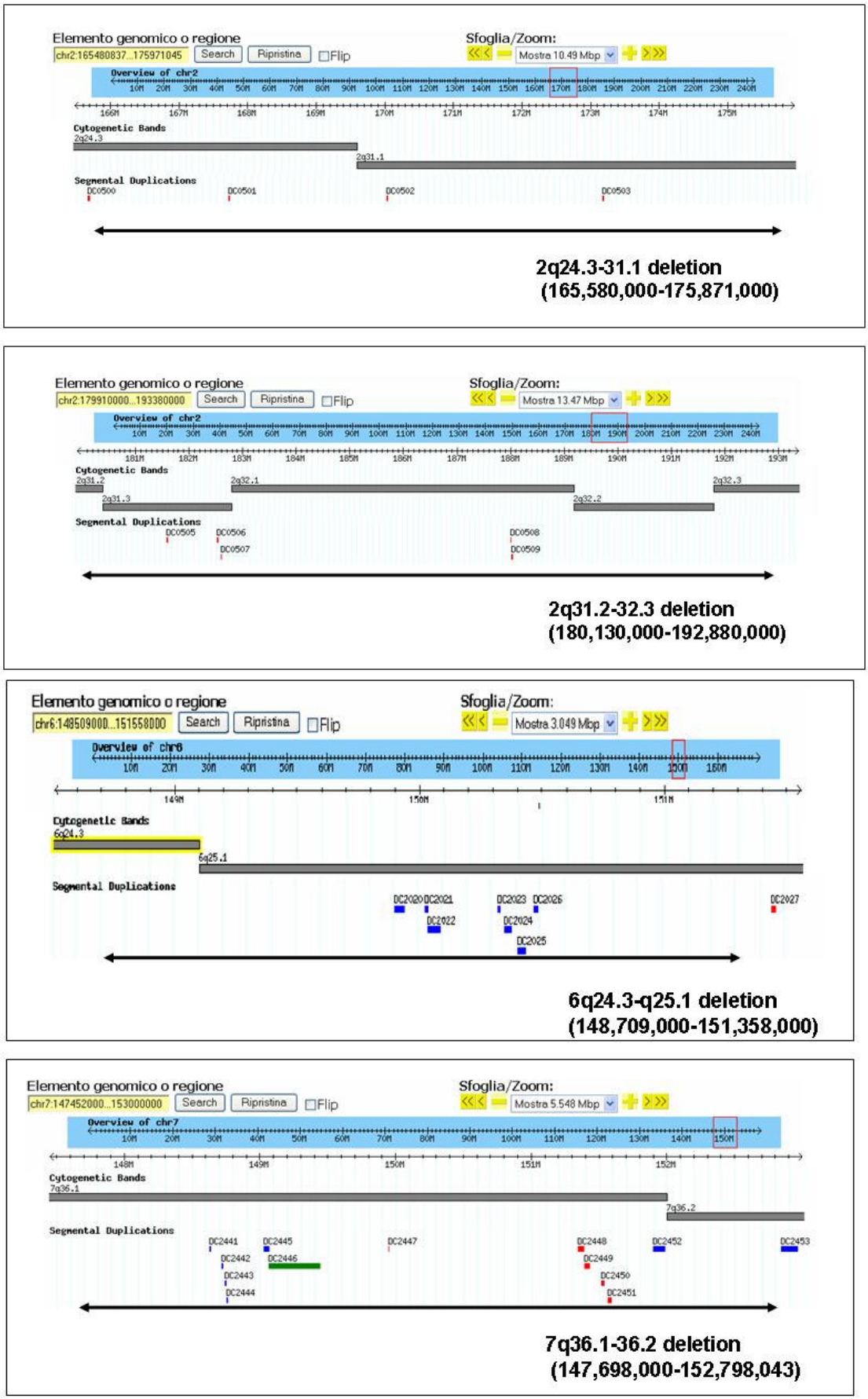

Fig. 16. Image showing absence of LCR at breakpoints of deletions identified in cases 1-4. Green bars denote interchromosomal and intrachromosomal duplications, blue bars indicate intrachromosomal duplications and red bars indicate interchromosomal duplications.

Among the nine rearranged patients only one was a duplication. This low percentage of duplicated patients is likely due to: 1) bias in patients' ascertainment, 2) different molecular mechanisms that generate deletions versus duplications. Generally duplications cause a less severe and faint phenotype. The selection criteria used for patients inclusion in this study may have negatively selected such kind of patients. In addition, the non allelic homologous recombination (NAHR) generates deletions and duplications with different efficiency favoring deletions (Fig. 17).

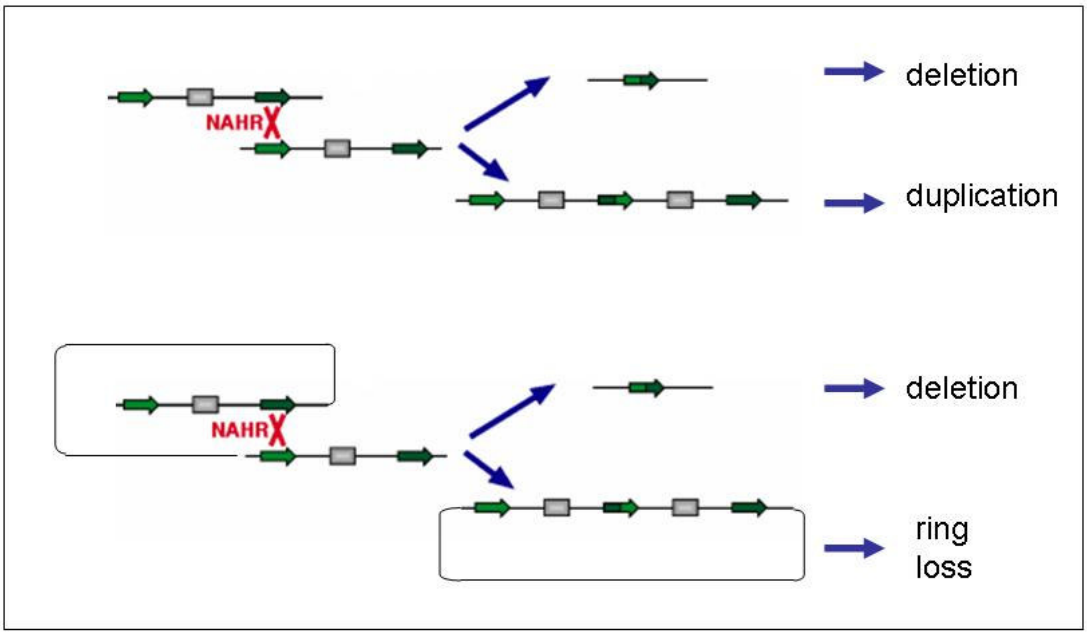

Fig. 17. Non allelic homologous recombination (NAHRs). Interhromatid and interchromosome NAHRs (upper panel) generate both deletion and duplication with the same efficiency. Intrachromatide events (lower panel) generate only deletions since the duplicated ring molecule is lost.

In the above described nine cases the array-CGH analysis led to the diagnosis of the affected child. Although the array-CGH and qPCR analysis indicated that the rearrangements arose "de novo", we performed standard karyotype analysis of both parents in order to exclude the presence of a balanced rearrangement favouring the unbalanced rearrangement in the child. In each case the parents' karyotype resulted normal and during genetic counselling a low recurrence risk was given to the family.

The identification of a de novo event was considered the only criterion for pathogenicity. Following this criterion the two rearrangements on chromosome 17 and on chromosome X would be considered non pathogenic variant. However, a recent paper on TAR syndrome goes against this rule [30]. The authors found a 1q21 deletion in

patients with TAR syndrome as well as in healthy relatives. The absence of the deletion in normal controls induced to consider the hypothesis of a susceptibility locus or an allele with low penetrance. In light of this, we can not completely rule out a pathogenic role for the 17q12 duplication and Xq25 deletion (Fig 18 and 19, respectively, modified by Decipher). In order to establish whether these imbalances are involved in the clinical phenotype of the patients we investigated on the location of CNV. In particular, two CNVs are located in Xq25.1 deletion and include two of three known genes that are present in our deletion [19] (Fig. 18). Only one gene, *WDR40C*, a member of the WD repeat protein family involved in a variety of cellular processes, is not reported as a polymorphism, but its function is not well known. Consequently, we have to investigate further in order to definitively rule out the involvement of this deletion in the clinical phenotype of the patient.

Concerning the 17q12 duplication, the region includes several genes but also three CNVs. The duplication is partially overlapping with a typical deletion responsible for renal cysts and diabetes (RCAD) (Fig. 19). RCAD is caused by deletions or point mutations in the *TCF2* gene that codifies for the transcription factor 2. In particular, defects in *TCF2* are responsible for maturity-onset diabetes of the young type 5 (MODY5) (OMIM#604284) and also for Muellerian aplasia (OMIM#158330). Our patient presents renal cysts and sex reversal, thus, probably, a double dosage effect of the gene could be responsible for the phenotype. Our duplication is larger than the typical deletion responsible for RCAD. However, in the non overlapping regions are located Redon CNVs loci. The presence of CNVs in this region suggests that probably the 17q12 duplication is not responsible for MR in the patient. However, in the Decipher database there seems to be a small region between two Redon CNVs where three genes (*MRPL45*, *GPR179* and *SOCS7*) are located. On the contrary, searching in the database of Genomic Variants (http://projects.tcag.ca/variation/) this small gap seems to be part of the CNV. Given all these contrasting data is still premature to establish a possible genotype-phenotype correlation.

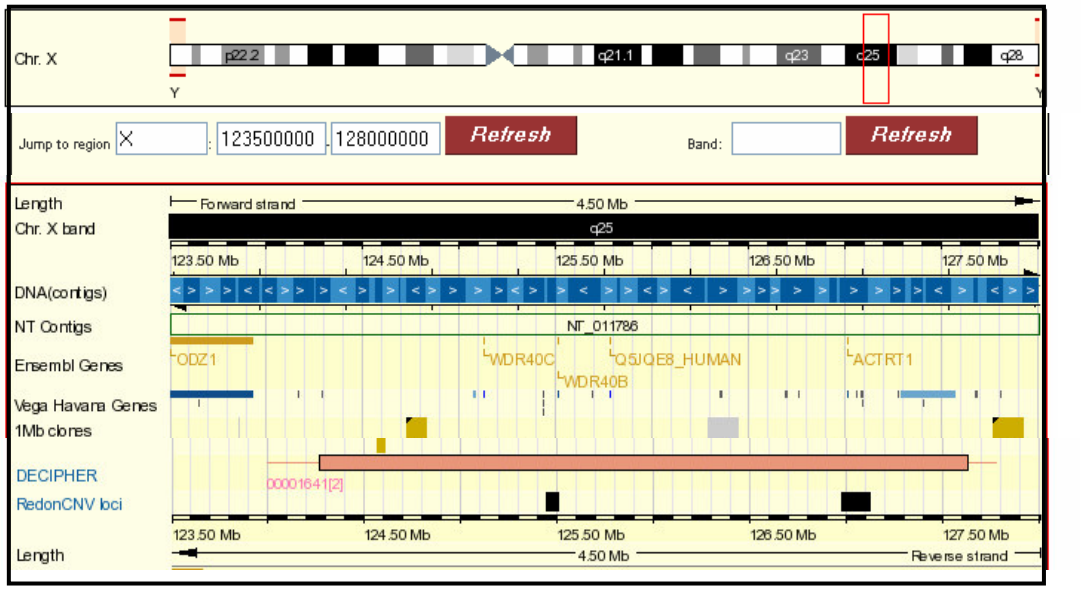

Fig 18. Cytoview part of the Decipher database on Xq25 region. This section of the database includes information on the genomic context based on the Ensemble browser. It shows the index imbalance and eventually similar imbalances inserted in the Decipher. It is possible to see the phenotype of each patient by clicking on the bar corresponding to the deletion.

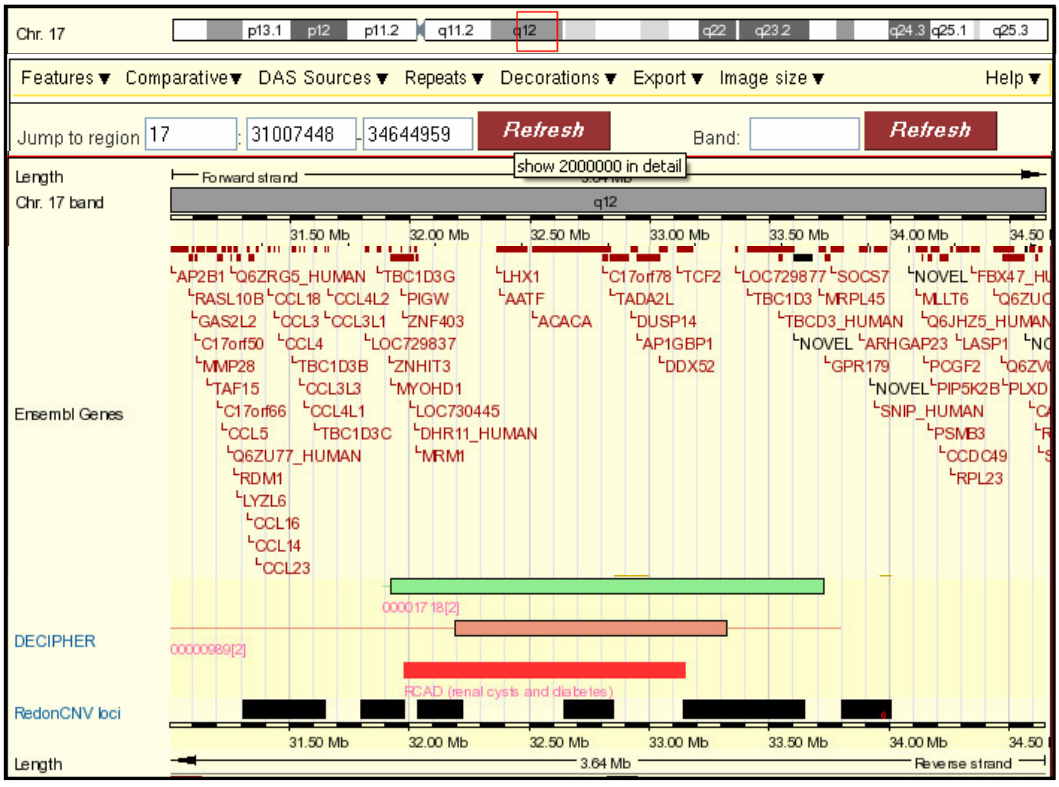

Fig 19. Cytoview part of the Decipher database on 17q12 region.

In addition to the screening of a cohort of 32 patients with unknown diagnosis, we have revaluated, by oligo array-CGH, 7 cases with a clinical and molecular diagnosis, for a more precise definition of deletion breakpoints and for identifying candidate genes possibly responsible for mental retardation.

Among these 7 cases, there was a familial case (cases 12 and 13) with X-linked ichthyosis (XLI) and MR and a sporadic case with XLI and Kallmann syndrome (KAL) (case 14). XLI is caused by deficiency of steroid sulfatase (STS) activity. In 85%-90% of cases it is caused by a deletion which encompasses the STS gene. Typically it is caused by a deletion of 1.5 Mb at Xp22.32 due to non-allelic homologous recombination (NAHR) between the CRI-S232 low-copy repeat regions flanking the STS gene. However, larger deletions were described and may be associated with mental retardation as part of a contiguous gene syndrome.

In the case with XLI and KAL (*case 14*, SIE00001740), array-CGH analysis showed the presence of a large deletion of about 2.83 Mb (Fig 20, modified by Decipher). The deletion includes the *STS* gene, the *KAL1* gene (responsible for KAL) and other genes such as *NLGN4X*, *VCXA*, *VCXB*, *VCXB1* and *VCXC* genes.

In the proband of the familial case with XLI and MR (*case12*, SIE00001719) the analysis revealed the presence of a smaller deletion of about 0.9 Mb (Fig 21, modified by Decipher). The deleted region comprises the *VCX3A* gene, which has been previously suggested to be involved in mental retardation [(31)]. The deletion also involves the *STS* gene responsible for XLI (OMIM#308100) and a third gene, named *HDHD1A*. This gene, also known as *GS1* (OMIM#306480), has been found deleted in patients with isolated XLI. A maternal uncle (*case13*, SIE00001721) has isolated X-linked ichthyosis and carries a larger deletion of 1.63 Mb which comprises also the *VCX1* gene (Fig 21, modified by Decipher).

In order to identify additional patients with an Xp22 deletion a search in the Decipher database has been performed. A female case with severe mental retardation and a small deletion in Xp22.31 of about 0.6 Mb has been found (BWH00001200, Fig.21, modified by Decipher). The deleted region of this case partially overlaps the deletion of case 12 and it contains only *HDHD1A* and *STS* genes. It is interesting to note that the distal breakpoint does not include the *VCX3A* gene and that the majority of the deleted region is represented by CNVs. An additional male patient with a deletion of 7.7 Mb involving Xp22.2-22.3 is reported in the database (00000978) [(32)]. This deletion includes all four VCX genes (*VCX1*, *VCX2*, *VCX3A* and *VCX3B*) and the *NLGN4X* gene.

Functional deficiency of one or more of these genes is most likely associated with the impaired cognitive development in the reported patient. However, at present the mechanism responsible for the onset of MR is not clear. In particular, the role of the VCX genes, and especially of VCX3A, is not firmly established.

The comparison between the Decipher cases and our three patients further complicates the picture. In fact, case 12 with MR bears a deletion involving only the *VCX3A* among the *VCX* gene cluster and excluding the *NLGN4X*. The paternal uncle (case 13) with isolated XLI has a bigger deletion involving also *VCX1*. Unexpectedly, the sporadic patient with ichthyosis and Kallmann syndrome (case 14) has the largest deletion that includes all *VCX* genes and *NLGN4X*. Given all these data it is possible that the presence or absence of MR may result from the location of breakpoints in regulatory elements of one of the known or putative genes included in the regions; the breakage of the regulatory elements could lead to inappropriate gene expression and thus MR, while the complete absence of the gene and its regulatory elements might have no phenotypic effects.

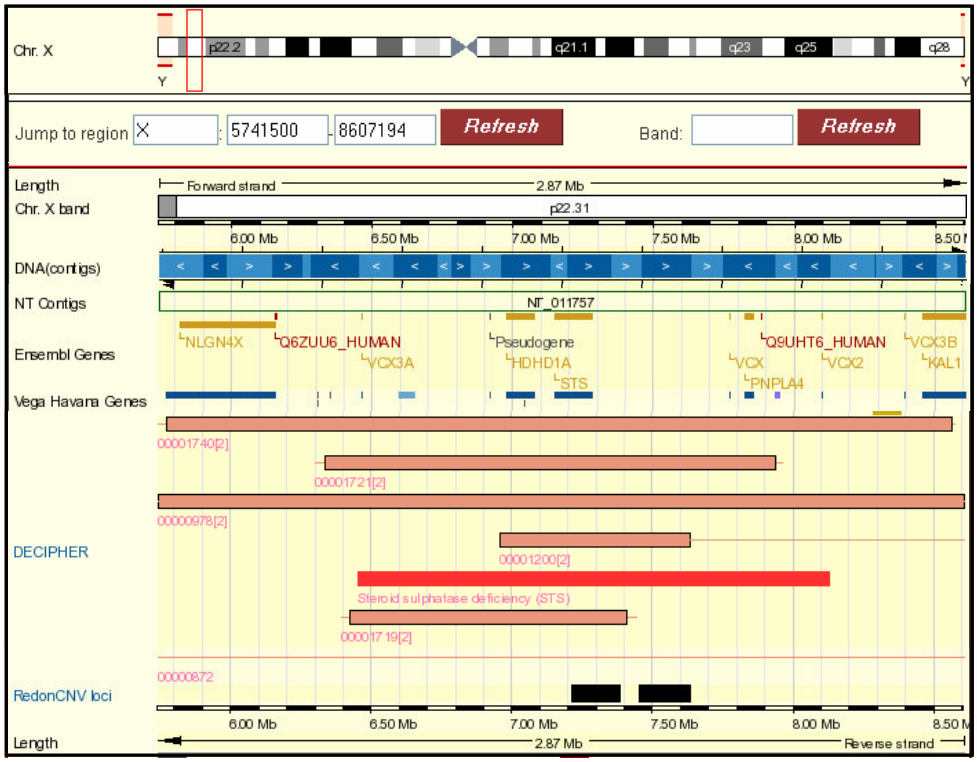

Fig 20. Cytoview part of the Decipher database on Xp22.1 region.

The last cases presented in this study are patients with retinoblastoma, dysmorphic features and developmental delay. We decided to characterise these patients using oligo arrays of 44,000 probes and compare them with another one with isolated retinoblastoma

in order to identify the critical region of the 13q14 deletion syndrome and candidate genes possibly responsible for mental retardation. At present, the critical region that we have identified is still very large and includes several genes (30 genes). From *in silico* analysis four genes seem to be good functional candidates for the neurologic phenotype: *NUF1P1* (nuclear fragile X mental retardation protein 1), *HTR2A* (serotonin receptor 2A) and *PCDH8* and 17 (prothocaderin 8 and 17, respectively).

It is interesting notice that in Decipher database a male case with 13q21.1 deletion, partially overlapping with our critical region, is reported (Fig 21, CAM00000578). The patient presents MR and developmental delay but also microcephaly. The deleted region of this case includes a CNV but its breakpoints are not well characterized (Fig 21, CAM00000578). It could be very interesting to better define the deletion breakpoints of this case and in particular to define if the deletion comprises the *PCDH17* gene, one our candidate gene. This gene belong to the protocadherin gene family and codify for integral membrane proteins, which are thought to function in signalling pathways and in cell adhesion in a CNS-specific manner. Although its expression and function is not well known, the *PCDH17* gene could be responsible for the neurological phenotype of this patient.

Furthermore, it could be useful to have access to the facial phenotype of this patient in order to define if he has the typical facial phenotype of patients with RB microdeletions (see cases 15-18, 4.2 section). Unfortunately, the present version of the Decipher database does not allow to see the pictures of the inserted consented cases. We believe that sharing pictures of patients would be an extremely important improvement for a website that has among its main goals “gene identification and refining molecular dysmorphology”.

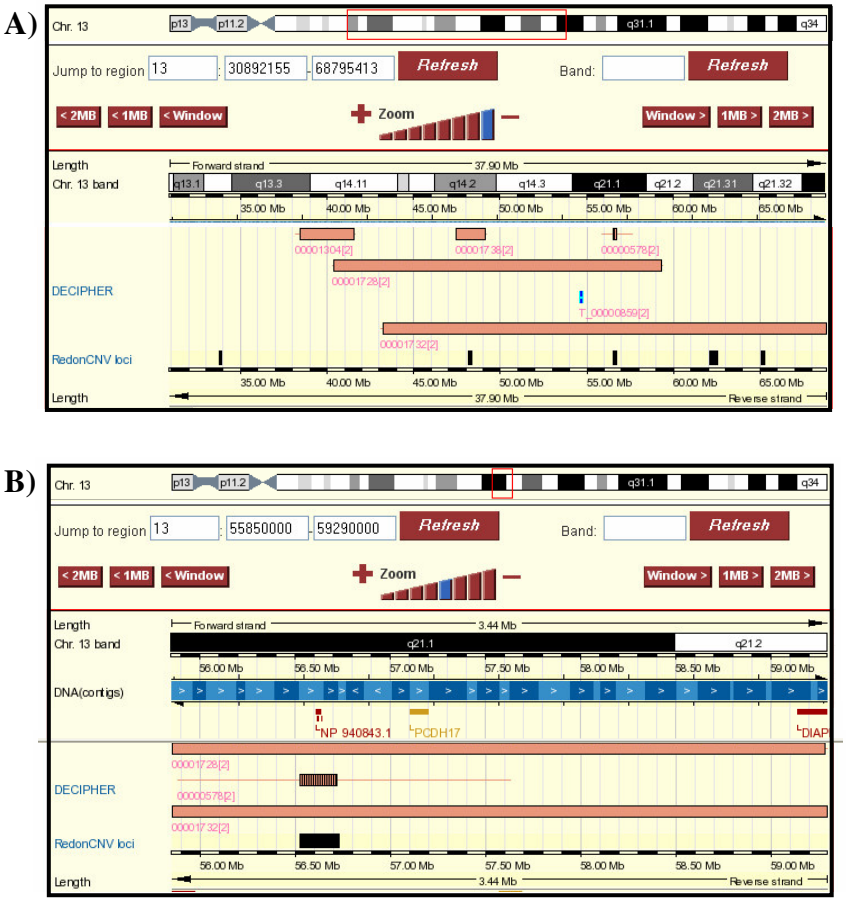

Fig 21. Cytoview part of the Decipher database on 13q12.3-21.33 region (A) and 13q21.1-q21.2 (B).

6. ACKNOWLEDGEMENTS

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7. REFERENCES

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1 Kallioniemi A, Kallioniemi O, Sudar D*, et al.* Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 1992;**258**(5083):818-21.

2 Solinas-Toldo S, Lampel S, Stilgenbauer S*, et al.* Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer* 1997;**20**(4):399-407.

3 Kricka LJ, Fortina P. Microarray technology and applications: an all-language literature survey including books and patents. *Clin Chem* 2001;**47**(8):1479-82.

4 Lockwood WW, Chari R, Chi B*, et al.* Recent advances in array comparative genomic hybridization technologies and their applications in human genetics. *Eur J Hum Genet* 2006;**14**(2):139-48.

5 Veltman JA, Fridlyand J, Pejavar S*, et al.* Array-based comparative genomic hybridization for genome-wide screening of DNA copy number in bladder tumors. *Cancer Res* 2003;**63**(11):2872-80.

6 Weiss MM, Kuipers EJ, Postma C*, et al.* Genome wide array comparative genomic hybridisation analysis of premalignant lesions of the stomach. *Mol Pathol* 2003;**56**(5):293-8.

7 Oostlander AE, Meijer GA, Ylstra B. Microarray-based comparative genomic hybridization and its applications in human genetics. *Clin Genet* 2004;**66**(6):488-95.

8 Speicher MR, Carter NP. The new cytogenetics: blurring the boundaries with molecular biology. *Nat Rev Genet* 2005;**6**(10):782-92.

9 Bignell GR, Huang J, Greshock J*, et al.* High-resolution analysis of DNA copy number using oligonucleotide microarrays. *Genome Res* 2004;**14**(2):287-95.

10 Slater HR, Bailey DK, Ren H*, et al.* High-resolution identification of chromosomal abnormalities using oligonucleotide arrays containing 116,204 SNPs. *Am J Hum Genet* 2005;**77**(5:709-26.

11 Zhao X, Li C, Paez JG*, et al.* An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. *Cancer Res* 2004;**64**(9):3060-71.

12 Carvalho B, Ouwerkerk E, Meijer GA*, et al.* High resolution microarray comparative genomic hybridisation analysis using spotted oligonucleotides. *J Clin Pathol* 2004;**57**(6):644-6.

13 Ylstra B, van den Ijssel P, Carvalho B*, et al.* BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res* 2006;**34**(2):445-50.

14 Brennan C, Zhang Y, Leo C*, et al.* High-resolution global profiling of genomic alterations with long oligonucleotide microarray. *Cancer Res* 2004;**64**(14):4744-8.

15 Barrett MT, Scheffer A, Ben-Dor A*, et al.* Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. *Proc Natl Acad Sci U S A* 2004;**101**(51):17765-70.

16 van den Ijssel P, Tijssen M, Chin SF*, et al.* Human and mouse oligonucleotide-based array CGH. *Nucleic Acids Res* 2005;**33**(22):e192.

17 Stankiewicz P, Beaudet AL. Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. *Curr Opin Genet Dev* 2007.

18 Menten B, Maas N, Thienpont B*, et al.* Emerging patterns of cryptic chromosomal imbalances in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of the literature. *J Med Genet* 2006.

19 Redon R, Ishikawa S, Fitch KR*, et al.* Global variation in copy number in the human genome. *Nature* 2006;**444**(7118):444-54.

20 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning : a laboratory manual,* Second ed. Vol Second Edition: Cold Spring Harbor Laboratory Press, 1989.

21 Livak K. *ABI Prism 7700 Sequence Detection System.*, 1997.

22 Koolen DA, Veltman JA, Renier WO*, et al.* Chromosome 22q11 deletion and pachygyria characterized by array-based comparative genomic hybridization. *Am J Med Genet A* 2004;**131**(3):322-4.

23 Smith AC, Dykens E, Greenberg F. Behavioral phenotype of Smith-Magenis syndrome (del 17p11.2). *Am J Med Genet* 1998;**81**(2):179-85.

24 Potocki L, Bi W, Treadwell-Deering D*, et al.* Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. *Am J Hum Genet* 2007;**80**(4):633-49.

25 de Vries BB, Pfundt R, Leisink M*, et al.* Diagnostic genome profiling in mental retardation. *Am J Hum Genet* 2005;**77**(4):606-16.

26 Schoumans J, Ruivenkamp C, Holmberg E*, et al.* Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). *J Med Genet* 2005;**42**(9):699-705.

27 Krepischi-Santos AC, Vianna-Morgante AM, Jehee FS*, et al.* Whole-genome array-CGH screening in undiagnosed syndromic patients: old syndromes revisited and new alterations. *Cytogenet Genome Res* 2006;**115**(3-4):254-61.

28 Lawson-Yuen A, Wu BL, Lip V*, et al.* Atypical cases of Angelman syndrome. *Am J Med Genet A* 2006;**140**(21):2361-4.

29 Bar-Shira A, Rosner G, Rosner S*, et al.* Array-based comparative genome hybridization in clinical genetics. *Pediatr Res* 2006;**60**(3):353-8.

30 Klopocki E, Schulze H, Strauss G*, et al.* Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet* 2007;**80**(2):232-40.

31 Van Esch H, Hollanders K, Badisco L, *et al.* Deletion of VCX-A due to NAHR plays a major role in the occurrence of mental retardation in patients with X-linked ichthyosis. *Hum Mol Genet* 2005;**14**(13):1795-803.

32 Chocholska S, Rossier E, Barbi G*, et al.* Molecular cytogenetic analysis of a familial interstitial deletion Xp22.2-22.3 with a highly variable phenotype in female carriers. *Am J Med Genet A* 2006;**140**(6):604-10.

8. ERRATA CORRIGE

A reevaluation of a second sample of case 13 (maternal uncle of case 12) demonstrated that the deletion in Xp22.31 region was indeed the same of the case 12. Related discussion has not therefore to be considered.