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Case Study
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Fine-mapping of 1p36 deletion, related to the manifestation of hirsutism

Dimova I.[1], Nikolova D.[1], Nesheva D.[1]

Department of Medical genetics, Medical University – Sofia, Bulgaria

Abstract: Rearrangements of 1p36 resulting in deletion are observed in 1 in 5,000 live births. Molecular characterisation of deletions, along with phenotypic correlations, have yielded regions in which to search for genes related to specific features of the syndrome. We report a child with sub-telomeric interstitial 1p36 deletion, spanning 3,683,364-8,822,150 bp (1p36.33-1p36.23) region. The capability of aCGH to provide high-resolution mapping of variation in copy number has been demonstrated. Determining the smallest region of overlap, we narrowed the common region between cases with hirsutism on chromosome 1 band 1p36.23 between 7,500,000 and 8,500,000 bp. Since our patient has overlapped symptom of hirsutism with the patients of proximal 1p36 deletion syndrome, we therefore searched the database to identify potential candidate genes for this feature. We suggest a new candidate-gene for hirsutism – *PARK7*, which is related to the function of androgens and hyperandrogenaemia. The suggestion is based on the location of this gene in the narrowed deleted 1p36.23 region in patients, affected by hirsutism together with other features of 1p36 monosomy syndrome.

Key words: mental retardation, array CGH, 1p36 deletion, hirsutism

Introduction

Rearrangements of 1p36 resulting in deletion are observed in 1 in 5,000 live births [1]. All subjects have mental retardation of varying degrees, mostly moderate to severe, as assessed with a number of neuropsychiatric tests depending on the age of the subject. Language skills are commonly delayed in patients with monosomy 1p36.

It was originally believed that there were two separate phenotypes, both characteristic of a 1p36 deletion and sharing some dysmorphic features [2]. The first was characterized by impairment of growth and heart failure, the second associated with obesity and physical characteristics similar to patients with Prader-Willi syndrome. Although a single characteristic phenotype of monosomy 1p36 has since been established, it may be possible that different phenotypic subgroups exist relating to the size of the deletion and location of the deletion breakpoint [2]. For example, a correlation between the severity of the neurological deficit and the size of the terminal deletion has been previously proposed [3].

It is not possible to detect these deletions with banded karyotypes by routine chromosome analysis at the 400- to 550-band resolution. [4, 5]. Using FISH analysis in addition to routine cytogenetic analysis in children with mental retardation [6], many more terminal deletions of 1p36 are currently being identified. However, in cases with interstitial deletions and complex rearrangements, a targeted FISH approach may be necessary [7]. Array-based comparative genomic hybridization (CGH) is the most powerful tool which can be used to define the type of rearrangement and the extent of the imbalances [8].

Array CGH detects abnormalities by comparing DNA content from two differently labeled genomes [9]. It has the ability to detect any genomic imbalance including deletions, duplications, aneuploidies, and amplifications [reviewed in 9]. The primary advantage of array CGH, compared with FISH, is that the array is capable of simultaneously detecting DNA copy changes at multiple loci over the whole genome [9].

Several studies have taken advantage of the higher resolution afforded by aCGH to map more precisely the boundaries of altered regions in different diseases [10-12]. Once these are known, candidate disease-related genes that map within the region will be readily identified from the genome sequence database.

Case report. A 8-years old girl was referred for genetic analysis because of developmental delay, severe mental retardation, precocious puberty and dysmorphic features. The child was born at 36 weeks of gestation with intrauterine hypotrophy and retardation (weight – 1900 g; height – 40 cm). Six days after delivery the child was manifested by severe respiratory distress and apnea, reflecting in cyanosis and bradicardia. Hydrocephaly with dilatation of all cerebral ventricles has been diagnosed in the first week after birth.

Ivanka Dimova (Correspondence)
idimova73@yahoo.com

At age of 1 month, epilepsy was diagnosed, which is treated till now. There was muscle hypotony and motoric failure. The girl has not developed any speech skills, at age of 8 she is with alalia. The IQ was estimated below 50.

At age of 2 years, pubic hirsutism was noted together with other puberty features.

The patient was with dysmorphic features in the delivery, including short eyes fissures, broad filtrum, large open anterior fontanel reaching the mid of the forehead, distant skull bones, hyperthelorism, strabismus, flat and wide nasal bridge, thin upper lip, and proximally implanted thumbs.
The parents didn't have any chronic disorders and the mother was not exposed to industrial or other adversities during pregnancy. The family history did not reveal other affected persons.
Since cytogenetic analysis showed normal female karyotype, the patient was subjected to high resolution whole genome array CGH testing in order to study copy number imbalances. Terminal 1p36 deletion was detected. The aim was to delineate most precisely the breakpoints and boundaries of the deleted region and to suggest the potential candidate genes for specific symptoms, observed in our patient.

Materials And Methods

Dna extraction and evaluation. DNA was extracted from blood sample of the patient by phenol-chloroform as previously described. The yield was estimated by Nanodrop as the necessary concentration of 100 ng/µl was obtained and 260/280 ratio for protein/RNA free– 1.81 (the range is 1.8-2.0). As an additional quality, DNA was checked running out on a 1% agarose gel: DNA of high molecular weight (>50 kbp) indicated it suitable for use.

Genomic arrays. We have used genomic array CytoChip (BlueGnome, Cambridge, UK), covering the entire genome at a median 565Kb, a resolution optimised to detect pathogenic imbalances while minimizing polymorphisms. In addition, it investigate sub-telomeres at a median 250Kb resolution, reliably detect mosiacism and examine 90 known genetic conditions at a median 100Kb resolution. This resulted in an average density of 1clone/0.5Mb by 4400 clones. For each disease the OMIM entry and primary literature were tiled with a minimum of three RPCI clones using Ensembl Release 45, NCBI build 36.

Array-CGH probe labeling, hybridization, image capture and data analysis. Test and sex-matched reference genomic DNA (400 ng) was labeled by random-priming, using BlueGnome Fluorescent Labelling System. The labeled products were purified by AutoSeqTM G50 columns, and incorporation of dyes was evaluated by Nanodrop as the incorporation in range 6-15 pmol/ µl and DNA yield in 180-325 ng/µl were considered suitable for further analysis. A mix Cy5 and Cy3 labeled probes and a mix of COT-1 and Herring sperm DNA were ethanol precipitated at -80^0C for at least 30 min. Hybridization processing was done dissolving precipitated probes in hybridization buffer. Arrays were washed in SSC solutions with decreasing concentrations and scanned by a GenPix 4100A. The images were analyzed by BlueFuse for Microarrays 3.5 software (BlueGnome, Cambridge, UK). In data processing $\log_2$ ratios of Cy3 and Cy5 intensities are generated for all hybridized clones. Normal copy numbers are considered in $\log_2$ ratio between -0.3 and +0.3, values above +0.3 were evaluated as gain/amplification and these ones under -0.3 – as losses (deletions). Genomic profiles were represented with $\log_2$ ratios in Y-axis and along the 23 chromosomes in X-axis. Individual chromosomal profiles are represented with clone positions in Y axis and $\log_2$ ratios in X axis.

Results

We have investigated genomic imbalances in a child with unexplained mental retardation, precocious puberty and dysmorphism by whole genome array CGH analysis. For more reliable results dye-swap experiment was conducted, so normalization was performed in at least 4 replicas. More than 85% of genomic clones were successfully hybridized. Figure 1 represents genomic profile of the patient across all the chromosomes.

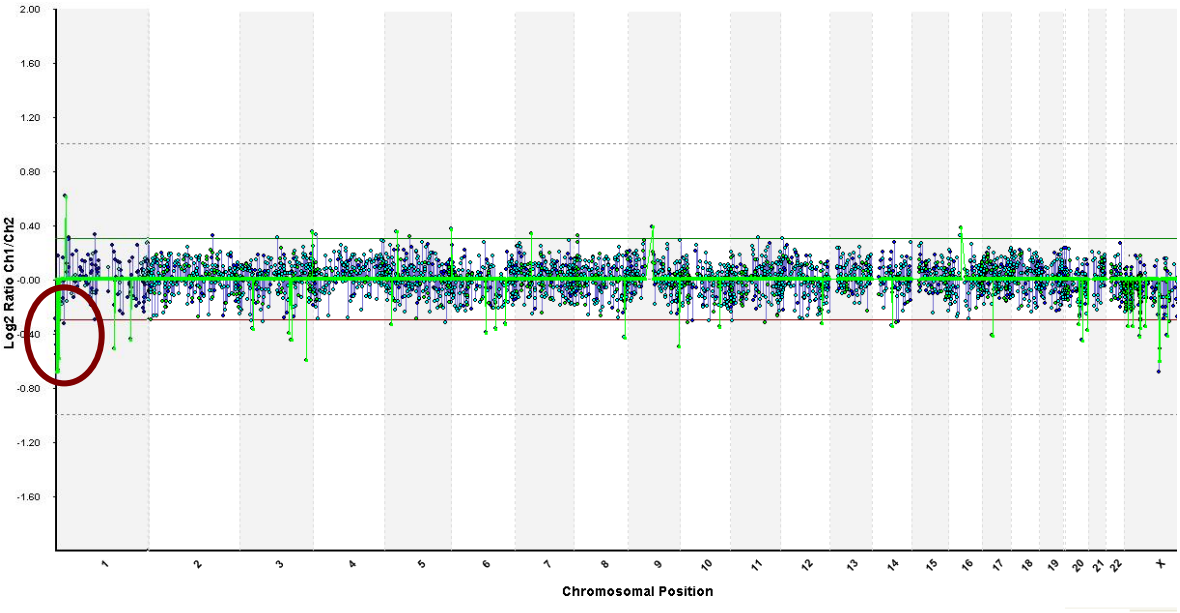

Figure 1. Genomic profile of the patient across all the chromosomes (the aberration is shown in the red circle). Array CGH analysis detected cryptic deletion of 1p36 region in the short arm of chromosome 1, expanding from 1p36.33 to 1p36.23 and involving 28 BAC clones. The size of the deleted region was estimated to be ~ 7.5 Mb (figure 2).

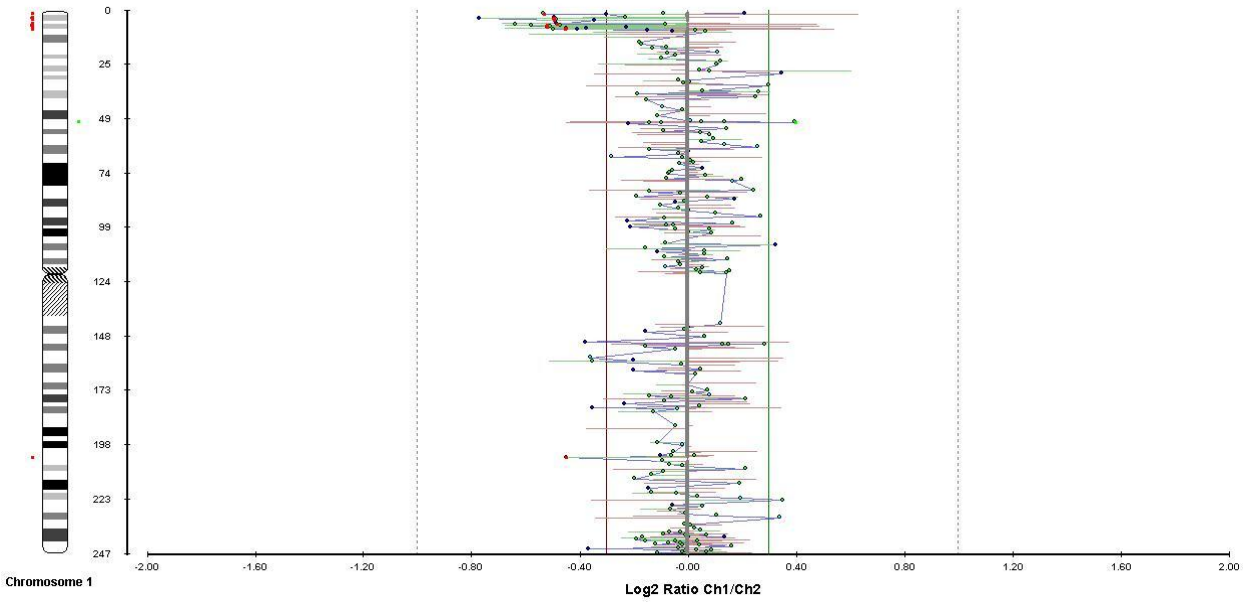

Figure 2. Genomic deletion in 1p36.23 - 1p36.33, detected in the patient.

Except for clinically relevant deletion detected in the patient, several polymorphic copy number variations were established.

Figure 3 represents ideogram of deleted region of chromosome 1 and thirty of totally 87 mapped genes in this region. Among the genes, considered to be associated with disease phenotype, were *DVL1* (dishevelled-1 gene), *GABRDA* (γ-Aminobutiric acid receptor, delta) and *KCNAB2* (Potassium Channel B-subunit gene). There were also some dose-dependent genes as *MTHFR*, *TNFRSF4* and *CYP4A11*.

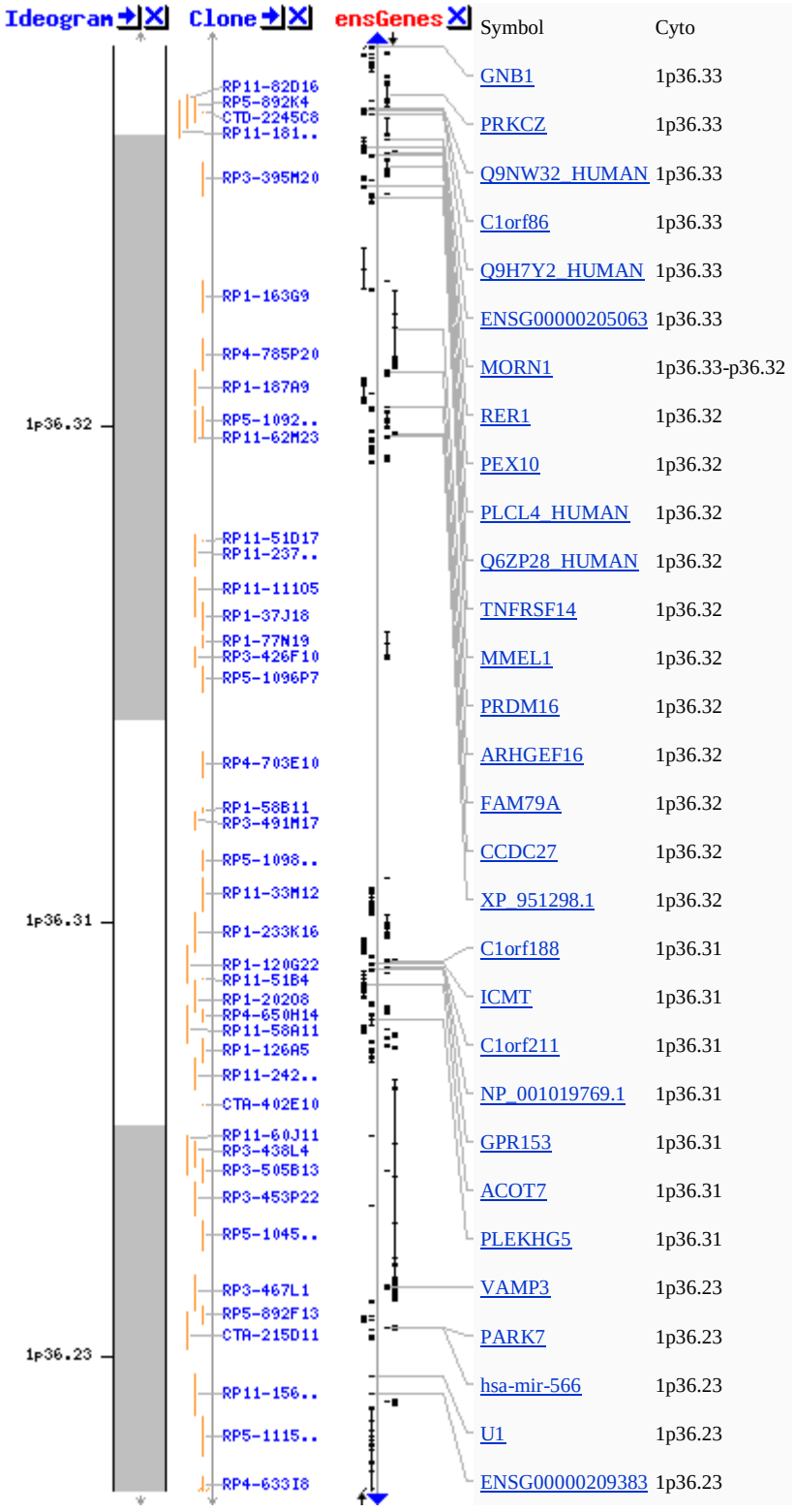

Figure 3. Ideogram of deleted region of choromosome 1 and 30 of totally 87 mapped genes in this region.

Discussion

We report a child with sub-telomeric interstitial 1p36 deletion, spanning 3,683,364-8,822,150 bp (1p36.33-1p36.23) region. The capability of aCGH to provide high-resolution mapping of variation in copy number has been demonstrated.

The phenotype of the patient covers some symptoms which are typically seen in patients with monosomy 1p36 syndrome, usually involving ~6 Mb from the 1p telomere (13). This phenotype for the classical distal monosomy 1p36.3 includes a characteristic facies, mental retardation, developmental delay, sensorineural hearing loss, seizures, cardiomyopathy, hypothyroidism, and hypotonia. Recently, a new possible syndrome with proximal 1p36 deletion has been reported (14), describing the following features: prenatal growth deficiency, poor post-natal growth/FTT, developmental delay, diverse cardiovascular malformations, and dysmorphic craniofacial features. An additional finding

commonly seen in patients with proximal 1p36 deletions has been hirsutism, that was the most manifested symptom in our case.

Molecular characterisation of deletions, along with phenotypic correlations, have yielded regions in which to search for genes related to specific features of the syndrome.

The 1p36 deletion varies in size from 1 to 10 Mb and appear to have no common sequence homology at the breakpoints (15–16). In an attempt to demonstrate that monosomy 1p36 is consistent with a contiguous gene deletion syndrome, Gajecka et al performed an analysis of the relationship between the deletion size and the number of observed clinical features (17). Mental retardation and developmental delay were excluded because they are present in nearly 100% of cases. They found no correlation between the deletion size and the number of observed clinical features and concluded that even subjects with relatively small deletions (<3 Mb) can present with most of the features associated with monosomy 1p36. In contrast, Kang et al demonstrated that deletions greater than 9 Mb with 3' breakpoints extending proximal to band 1p36.2 have very distinct phenotypes with structural heart defects like ASD, VSD and valvular abnormalities (14). In silico analysis of the 2.24 Mb critical deletion interval in thesee patients identified at least 15 genes which are known to be expressed in the heart, among which were *GPR157*, *H6PD*, *SPSB1*, *RBP7*, *LZIC* and *DFFA*. (UniGene Build #199 database from NCBI accessed 2 April 2007). However, the authors could not offer a hypothesis for an obvious single embryological mechanism that would provide a unifying explanation for the highly diverse cardiac defects.

Since our patient has overlapped symptom of hirsutism with the patients of proximal 1p36 deletion syndrome, we therefore searched the database to identify potential candidate genes for this feature. Determining the smallest region of overlap, we narrowed the common region between cases with hirsutism on chromosome 1 band 1p36.23 between 7,500,000 and 8,500,000 bp. We found among the genes in the region several participants in cell growth and apoptosis (*ERRFI1*, *TNFRSF9*), as well as genes, involved in brain function (*VAMP3*, *PER3*, *UTS2*). There was also a gene, which encodes positive regulator of androgen receptor-dependent transcription – *PARK7*. Defects in this gene are known to cause autosomal recessive early-onset Parkinson disease 7 (PARK7) [MIM:606324, 168600].

Hirsutism is one of the manifestations of the hyperandrogenic syndromes. There are various causes of this condition [18]. Polycystic ovary syndrome was diagnosed in 57.1 %, idiopathic hirsutism in 16 %, adrenal hyperplasia (11-beta hydroxylase and 21-hydroxylase deficiency) in 7.1 %, adrenal carcinoma in 1.8 %, and Cushing's disease in 0.6 % of 168 patients, suffered by hirsutism [19]. The etiology of hyperandrogenemia was not clear in 17.4 % patients and these patients were named as idiopathic hyperandrogenemia.

We suggest a new candidate-gene for hirsutism – *PARK7*, which is related to the function of androgens. The suggestion is based on the location of this gene in the narrowed deleted 1p36.23 region in patients, affected by hirsutism together with other features of 1p36 monosomy syndrome.

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