

Patient with syndromic cleft lip-palate, mosaic karyotype and cytogenetically abnormal brother

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Objective. Cleft lip with or without cleft palate (CLP) is a common congenital abnormality involving genetic and non-genetic factors in its etiology. Although many studies have been made to find the genetic pattern of this malformation, there is still no precise answer. There have been suggested several models of inheritance where a few major loci are influenced by the number of modifiers. The number of modifiers is still unknown and could be scattered all over the genome. Chromosome rearrangements are one of the tools that can help to trace these genes.

Materials and methods. Patient blood samples for cytogenetic analysis were collected. Karyotyping and comparative genomic hybridization analysis was performed according to standard protocols.

Results. The karyotype of the patients was found to consist of 4 different cell clones involving rearrangements of chromosome 21. The patient phenotype involved various congenital abnormalities including cleft lip palate. Chromosome 21 abnormality was detected using both methods. The parents' karyotype was normal, while the brother had karyotype 47, XYY.

Conclusions. Altered chromosome region on chromosome 21 is large enough to involve several dozens of genes. We predict that one or even a few of them could be involved in orofacial morphogenesis, at least as modifiers.

Key words: comparative genomic hybridization, chromosome rearrangement, mosaic karyotype, syndromic cleft lip-palate

INTRODUCTION

Cleft lip with or without cleft palate (CLP) is one of the most common congenital anomalies. The incidence ranges between 1:600 and 1:1000 among Caucasians in Europe with marked racial and geographical disparities (1). CLP in patients could be found in an isolated form as well as along with other anomalies in syndromic form. There are more than 300 syndromes including a number that are either chromosomal or Mendelian in causation, have cleft lip and/or palate as feature(s) (Online Mendelian inheritance in man: <http://www.ncbi.nlm.nih.gov/omim>).

Chromosome abnormalities are one group of causation out of 3 (2) which cause clinically recognizable syndromes with CLP as a feature, and it is well known that cytogenetics could give a great boost in the search of new disease-related genes, but usually they are accidental findings. There are two possible approaches of how

cytogenetics can assist in gene search. The first is balanced chromosome abnormality in patients with a particular, usually isolated, malformation (3, 4). There is a probability that chromosome break has occurred in the gene which was disabled, and it was the basis for anomaly to develop. The second possibility is detection of imbalanced chromosome abnormality, usually partial chromosome aneusomy, in patients with multiple congenital malformations of various severity, including the one of interest (5). Both ways do not ensure that the gene will be located; in the case of balanced chromosome abnormality, the gene disrupted by chromosome break could not be associated with a particular malformations, whereas the unbalanced region, even very small, can embrace dozens of genes. On the other hand, clinical recognition of deletion or duplication specific syndromes supports the assumption that haploinsufficiency, at least for some of the genes, in the aberrant region has a direct effect on specific development processes (6, 7). Trisomy chromosome 13 (Patau syndrome) might be the most known chromosomal abnormality which includes CLP as a common feature, but there are only obscure data about the genetic regions in chromosome 13 contributing to CLP (8).

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There are many methods which could be applied in searching for chromosome abnormalities, and comparative genomic hybridization (CGH) is only one of them; it is a molecular cytogenetic method based on the fluorescence *in situ* hybridization technique to detect chromosomal imbalances which could be missed during conventional karyotyping (9) or could be used to specify marker chromosomes. Higher resolution has made CGH successfully applicable in constitutional karyotype analysis, particularly in cases when no chromosome abnormalities were found by conventional karyotyping, although various dysmorphic phenotype features were purported to this end (10–14).

In the present study, we have applied the CGH method to specify marker chromosome in a patient with syndromic orofacial cleft. Such approach has been successfully applied for other syndromes (15). To find chromosomal rearrangements, we have collected DNA from a patient who suffers from various congenital malformations, including a cleft lip with cleft palate. New genes involved in craniofacial morphogenesis could be located in the abnormal chromosome region (16).

PATIENTS AND METHODS

Family history

The prepositus (Fig. 1), a boy, was 3.5 years old at the time of investigation. He was born at term and weighed 3100 g. He was the second child of unrelated 23-year-old mother who had epilepsy treated with carbamazepine and her healthy 25-year-old partner.

Clinical examination showed a unilateral cleft lip with cleft palate (CLP), dysmorphic features of the face and distal parts of limbs, bilateral inguinal hernias, lymphoedema of both feet, clinodactyly of second toes, micropenis, and undescended testes. His medical course was complicated by seizures, shunt VSD, myelodysplastic syndrome and severe mental retardation. At 4.5 years of age he died from respiratory infection.

His elder sibling (Fig. 2), brother, was 9 years old at the time of investigation. Clinical examination showed a prominent glabella, large ears and teeth, full lower lip, long philtrum. The family genealogy consists of 4 generations, 49 relatives. Karyotype analysis has been performed to parents and sibs, 5 persons in total. There are no data about a chromosome pathology to be detected in other genealogy members except the two described here.

The patient's blood samples, with the permission of their parents, were received for routine cytogenetic analysis at Center for Medical Genetics of Vilnius University Hospital Santariskiu Clinics. His karyotype was investigated using conventional karyotyping after which a DNA sample was collected for CGH analysis for karyotype result specification and confirmation.

Karyotyping and CGH

Metaphase chromosomes for karyotyping were obtained from peripheral blood leucocytes. Cell proliferation was stimulated with PHA-E and was cultivated in RPMI 1640



Fig. 1. The proband at age of 3.5 years

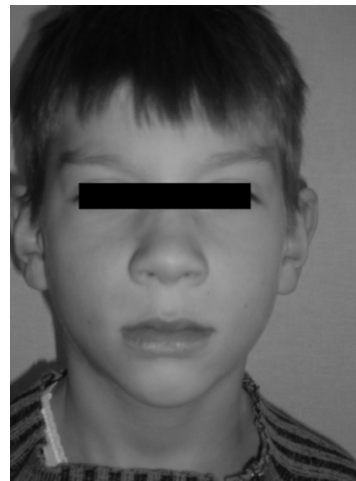


Fig. 2. The brother of the proband at age of 9 years

the medium with fetal calf serum for 72 hours. Proliferation was suspended with colchicin solution. Cells were treated with KCl hypotonic solution and Carnoy fixative. Prepared slides were heated at 62 °C overnight. For G-banding, the slides were treated with trypsin and stained with Giemsa stain. Metaphases were observed with a Nikon Eclipse 600 microscope (Japan); chromosomes were analyzed with MacKtype software (Applied Imaging, UK). At least 10 metaphases of each patient were analysed. Analysis was performed at the ISCN band level 400–550.

CGH was performed as described previously (17), briefly – patient DNA and normal reference DNA were labeled with FITC-21-dUTP and Texas Red-5-dUTP (Vysis Inc., Dowers Grove, IL, USA), respectively (in reverse labeling reference DNA was labeled in FITC while test DNA in Texas Red) by the nick translation method. Approximately 400 ng (nanograms) of each DNA and 20 µg Cot1 DNA (Invitrogen Life technologies Inc.) were hybridized to normal metaphase chromosomes. Chromosome metaphase spreads were prepared according to standard protocols from phytohemagglutinin-stimulated peripheral blood lymphocytes from karyotypically normal

males. The slides were hybridized for 3 days in a humid chamber, washed and counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with Vectashield antifade solution (Vector Laboratories, Burlingame, CA, USA). CGH image capture was performed with a CytoVision (Applied Imaging, Sunderland, UK), interfaced with an Axioscope fluorescence microscope (Zeiss, Germany), and the images were analyzed on the CytoVision Genus 2.8 (Applied Imaging). At least 10 metaphases were analyzed with normal and reverse labeling.

RESULTS

Using a conventional G-banding karyotype analysis, a mosaic karyotype of 4 clones has been detected: 46, XY [1]/ 46, XY, r(21) [10]/ 46, XY, -21, +mar [30]/ 45, XY, -21 [9] (Fig. 3). The number of metaphases containing a particular karyotype is given in angle brackets; a total of 50 metaphases have been analysed. The biggest share came to a clone with marker chromosome without normal chromosome 21. CGH analysis revealed deletion in terminal regions of chromosome 21 46, XY, del(21)(q21.1;qter) (Fig. 4). The derivative chromosome had to be formed from abnormal chromosome 21. To confirm the initial CGH result, reverse DNA labeling was performed; it also showed deletion in chromosome 21.

CGH is a powerful tool in detecting chromosome abnormalities such as deletions or duplications, but in this case where the karyotype is mosaic this method is not so informative. It is clear that the marker chromosome is the derivative chromosome 21; it shows a deletion on the q arm, but the other clone in this karyotype is a ring chromosome. To form a ring chromosome two chromosome breaks on different chromosome arms are necessary, if the chromosome is intended to be transmitted to daughter cells during cell division. The human chromosome 21 is acrocentric; this means that the short arm of the chromosome consists of satellites with many repetitive sequences which gives cross-hybridization with other acrocentric chromosome satellite sequences, so it makes quite difficult to detect if there is a deletion in the short arm of chromosome 21 in this case. On the other hand, since there are no polypeptides coding the genes located in the short arms of the acrocentric chromosome, DNA sequence shortening in this chromosome region does not manifest on the phenotype, the same as for balanced Robertsonian translocation carriers.

For genetic prognosis, karyotype analysis has been performed to the proband's parents and sibs. The parents' as well as younger brother's karyotypes were found to be normal, but for the elder brother of the proband cytogenetic analysis revealed karyotype 47, XYY (Fig. 5).

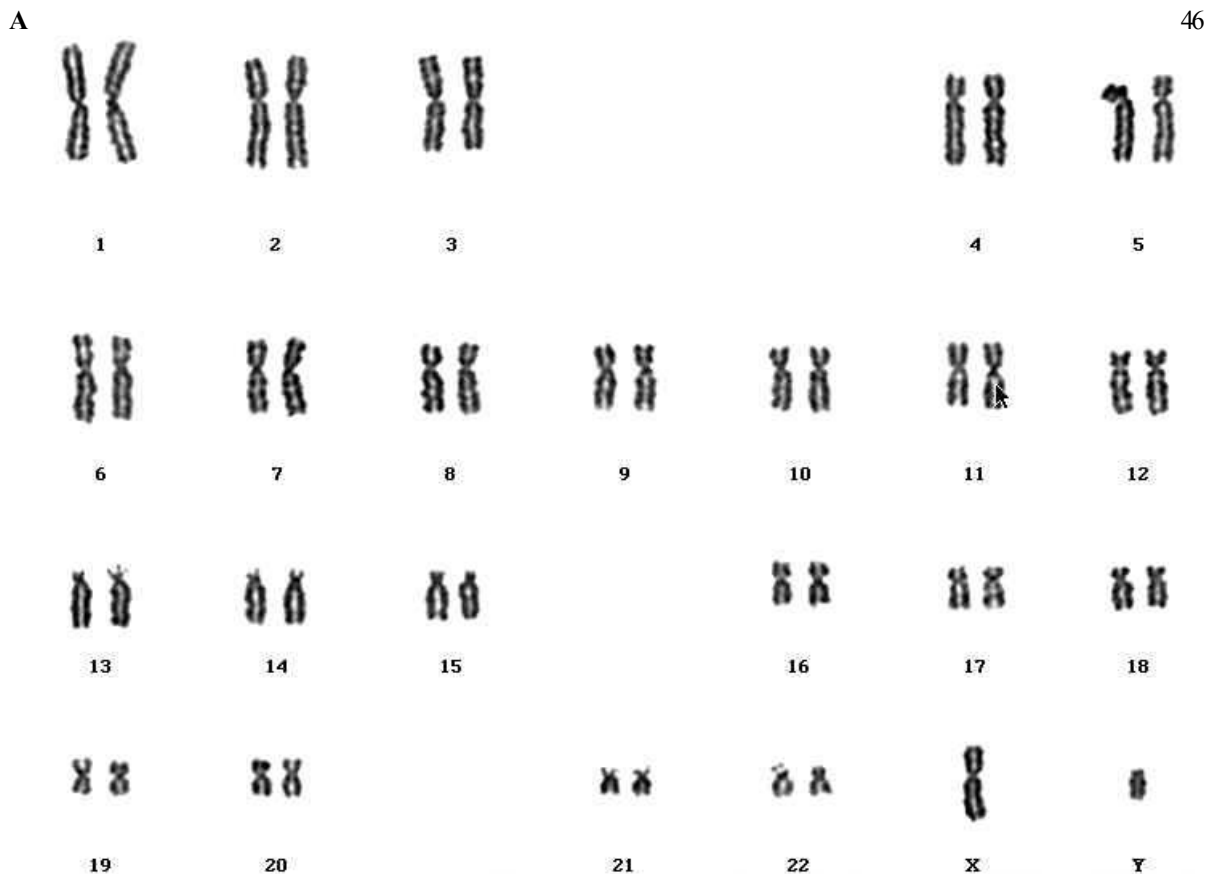


Fig. 3. G-banded karyotype of the proband (chromosome magnification $\times 1000$): A – clone with normal male karyotype

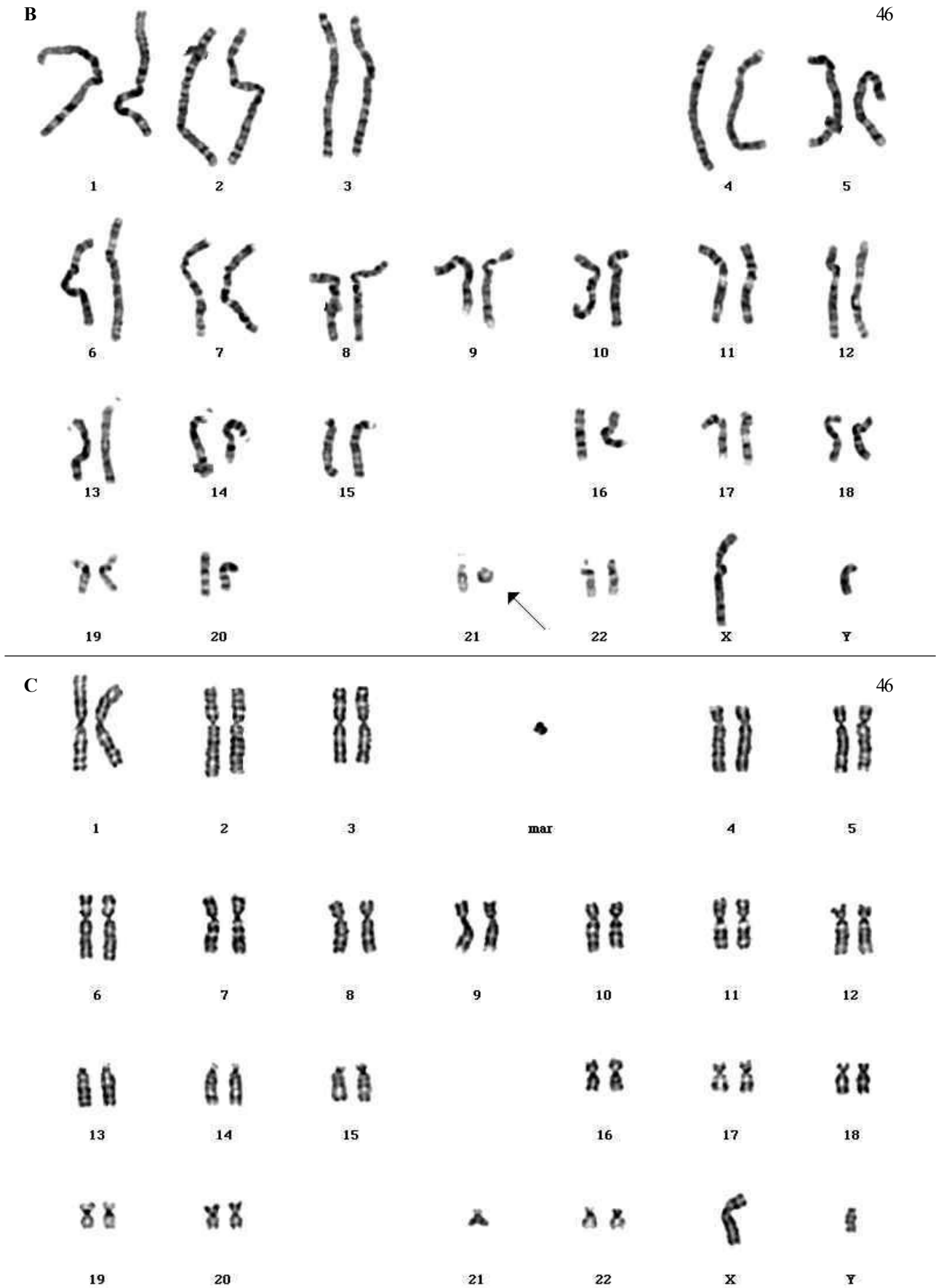


Fig. 3 (continued). G-banded karyotype of the proband: B – clone with ring chromosome 21 (indicated with arrow); C – clone without normal chromosome 21 and with marker chromosome

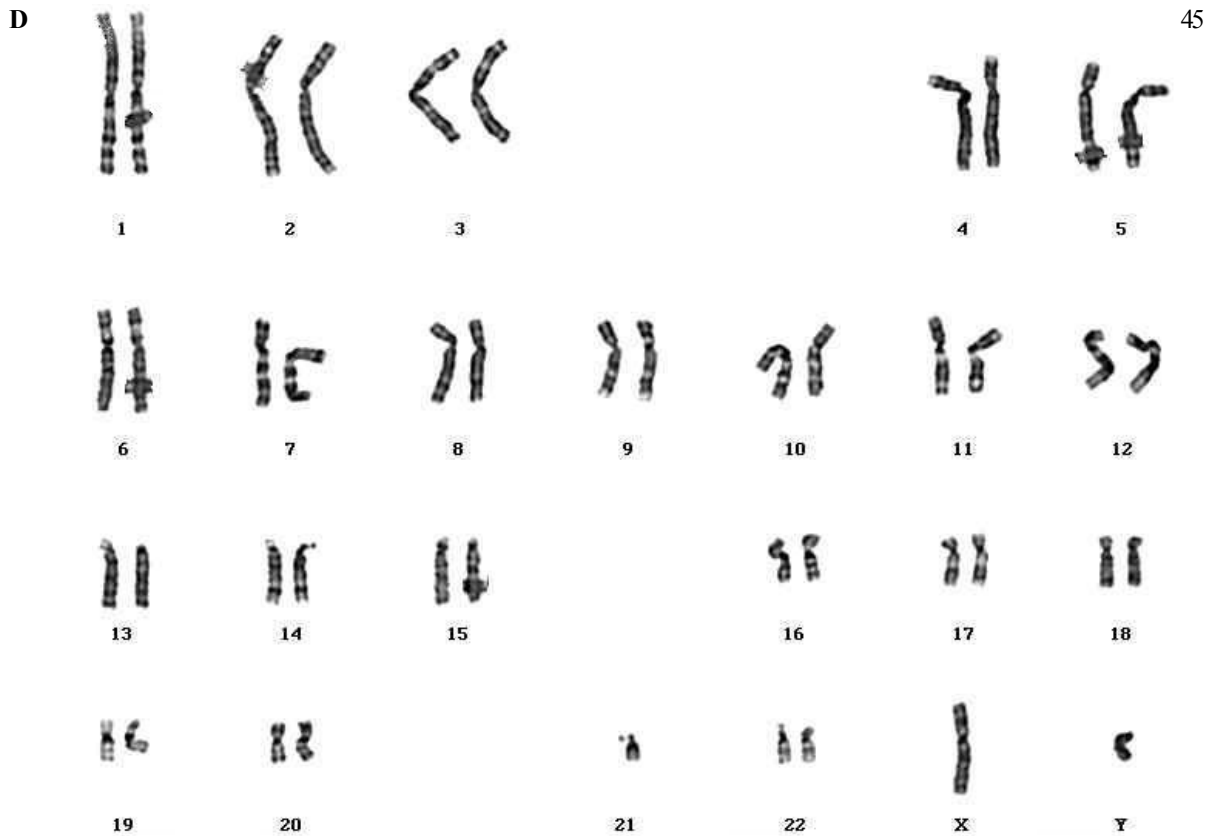


Fig. 3 (continued). G-banded karyotype of the proband: D – clone with monosomy chromosome 21

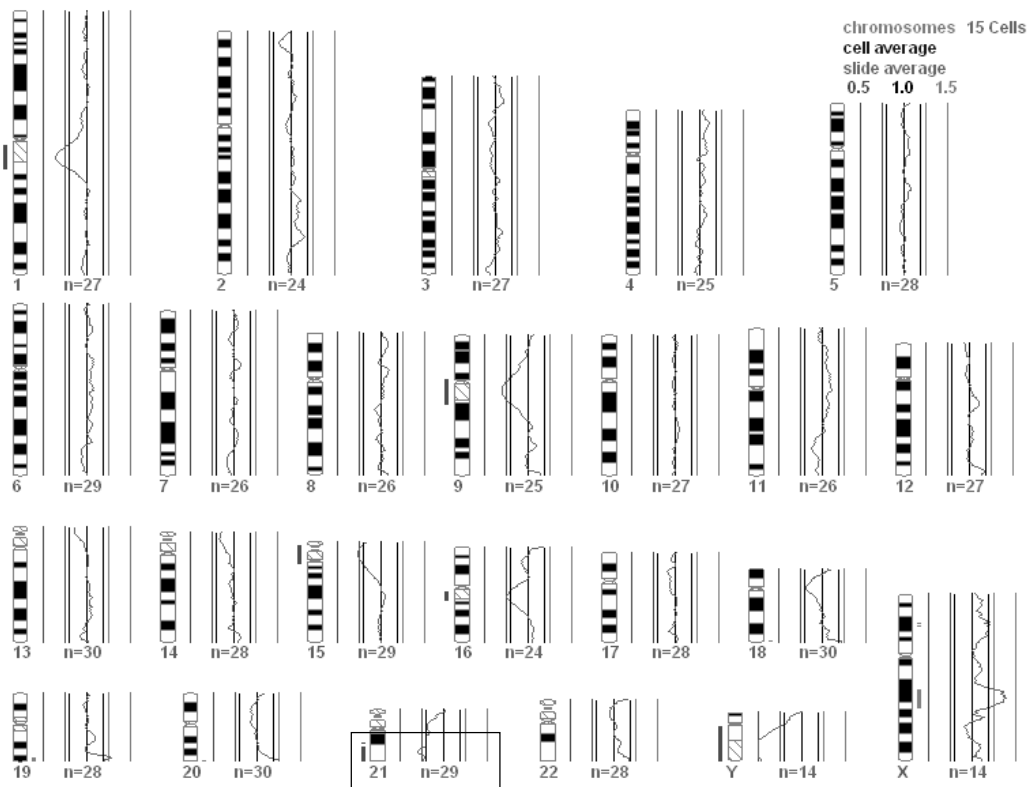


Fig. 4. CGH profile of the proband with abnormal chromosome 21. Abnormal chromosome is in rectangular. Black line near chromosome ideogram indicates lost chromosome part. Indications of other chromosome rearrangements are unreliable because they are localized either in the centromere regions of autosomes with high amount of repetitive sequences or in sex chromosomes, which are highly polymorphic

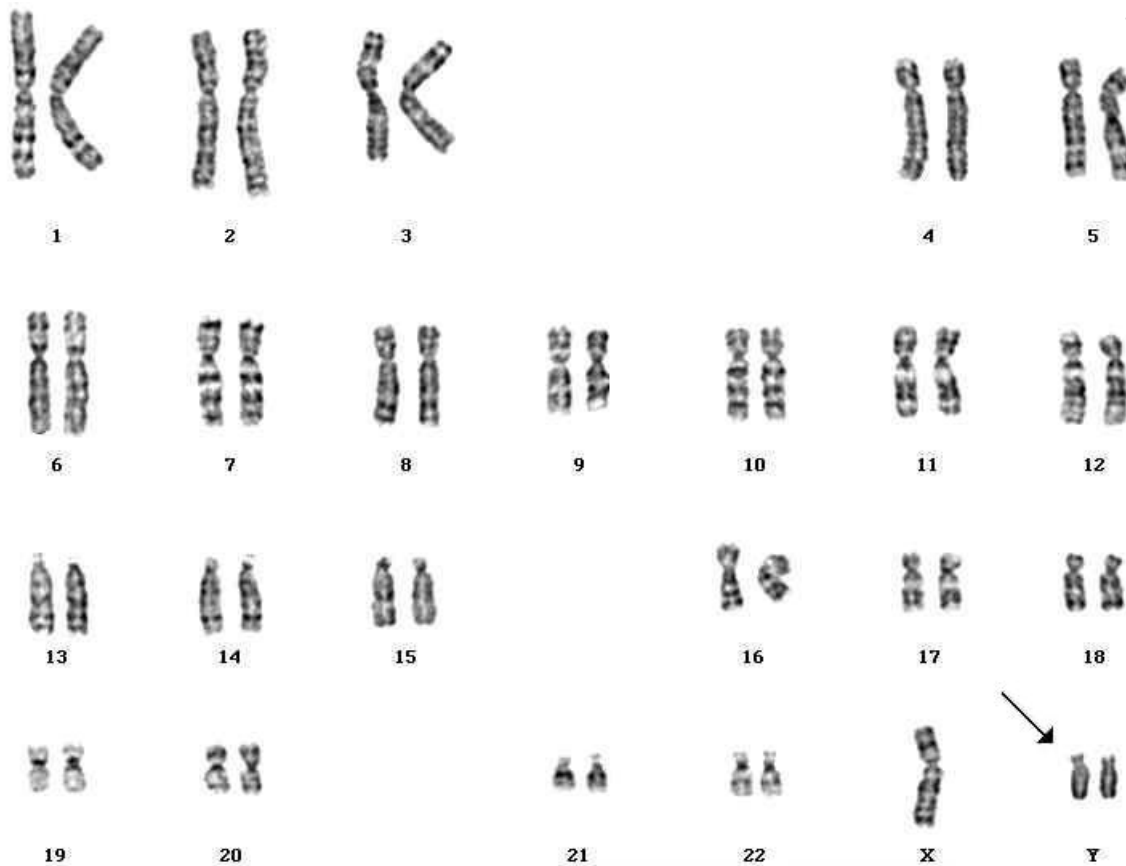


Fig. 5. 47, XYY. G-banded karyotype of the brother of the proband. Two Y chromosomes are indicated with arrow (chromosome magnification $\times 1000$)

DISCUSSION

It is known that in multifactorial genetic diseases which are of CLP type, the biggest share of genetic impact comes to several genes, but along with them morphogenesis could be controlled by many more genes (18). These genes are called “genetic background”, and they may participate in facial morphogenesis not directly. It could be about 20 such genes in different chromosome regions (6, 7). They alone make a very small impact on orofacial development, and their impact is usually left unnoticed in normal conditions, but in some particular content their influence could be easily spotted. One of such conditions could be the lack of a gene product in case of partial chromosome region monosomy. Moreover, the altered amount of gene product can be either itself directly involved into the development processes or be of regulatory type and enhance or suppress the genes’ activity by the effect of position or in other regions of the genome. Such complexity of gene interaction makes it very difficult to determine all the genes participating in orofacial morphogenesis.

Chromosome 21 has been involved in cytogenetic rearrangements in our case. Chromosome 21 trisomy (Down syndrome) is the most common chromosome rearrangement; full monosomy of chromosome 21 is rare (19, 20),

while partial monosomy 21 due to different chromosome structure abnormalities is more common (21–23). The mosaic karyotype consisting of 4 clones is also a rare cytogenetic finding. CLP has not been specified as a common feature either in trisomy 21 cases or in patients with a deletion of the distal part of chromosome 21, although different orofacial anomalies, such as a large/carp-shaped mouth, are often mentioned. The deleted region detected in our case is large, which is consistent with previous data on such abnormalities and could be involving several dozens of genes. There is a possibility that at least one gene located in the distal part of chromosome 21 has at least a minor impact on the clefting process. Unfortunately, a possibility should not be rejected that carbamazepine used during pregnancy could cause an independent phenotypic feature such as CLP.

Different chromosome rearrangements in the same family are a rare finding, but there are no indications that these findings could be associated in our case. The karyotype abnormality detected in the proband’s brother might be a coincidence or it might be determined by the environment – residence, work place, food, life habits, etc. This case illustrates that a comprehensive evaluation of families with chromosomal abnormalities is strongly recommended, because the recurrence risk of chromosomal abnormalities in siblings is increased.

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PACIENTAS SU SINDROMINIŲ LŪPOS IR GOMURIO NESUAUGIMU, MOZAIKINIAIS KARIOTIPO POKYČIAIS IR KARIOTIPO POKYŲ TURINČIU BROLIU

Santrauka

Įvadas. Lūpos ir gomurio nesuaugimas yra viena dažniausių įgimtų raidos anomalijų. Šio defekto atsiradimo priežastys gali būti tiek genetinės, tiek ir nulemtos aplinkos veiksmų. Daugeliu tyrimų šioje srityje yra nustatyta keletas genų, kurie gali nulemti lūpos ir gomurio nesuaugimą, tačiau visi tokie genai dar nėra nustatyti.

Metodai. DNR išskyrimas bei chromosomų preparatai buvo paruošti pagal standartinę metodiką. Pacientų kariotipas buvo

nustatytas analizuojant metafazines chromosomas, nudažytas G dažymo būdu. Lyginamoji genomine hibridizacija buvo atlikta pagal anksčiau aprašytą metodiką.

Rezultatai. Pacientui, turinčiam sindrominį lūpos ir gomurio nesuaugimą, buvo nustatytas mozaikinis kariotipas iš 4 ląstelių klonų. Pakitimai buvo susiję su 21-os chromosomos struktūros bei skaičiaus persitvarkymais. Probando broliui buvo nustatytas lytinių chromosomų skaičiaus pakitimas.

Išvados. Lūpos ir gomurio nesuaugimą lemiantys genai gali būti išsidėstę 21-os chromosomos q21-qter srityje. Geno poveikis gali išryškėti sumažėjus geno produkto dozei. Chromosomos sritis yra didelė, todėl joje gali būti išsidėstęs daugiau nei vienas genas.

Raktažodžiai: lūpos ir gomurio nesuaugimas, lyginamoji genomine hibridizacija, chromosomų pakitimai, mozaikinis kariotipas.