Analiza citogenetică și consilierea genetică la pacienții cu sindrom Down

Cytogenetic analysis and genetic counseling of patients with Down syndrome

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Abstract

Objective. *The aim of this study was to confirm the presence of Down syndrome (DS) using cytogenetic testing and to assess the inheritance using parental karyotype, as a basis for predicting the risk of recurrence and for genetic counseling.*

Study design. *A total of 114 cases suspected to have Down syndrome were included during a period of 18 months from the Department of Medical Genetics of IOMC "Prof Dr Alfred Rusescu". All this 114 cases suspected to have Down syndrome undergo clinical assessment. Samples were collected and cytogenetic analysis was performed in 96 patients.*

Results. *Chromosomal abnormalities were confirmed in all of the patients that were analyzed by cytogenetic techniques. Most of the children with DS were born to mothers under 35 years old and were the second born in the birth order. Though advanced maternal age is an established risk factor for DS, this study revealed an increased number of DS babies born to the young mothers.*

Conclusion. *Karyotypes collected from children and their parents, family history and parental ages had a crucial contribution in providing genetic counseling, prenatal diagnosis and estimating the risk for the next conception. Prenatal diagnosis should be offered to all mothers at risk: > 35 years or younger mothers, if the karyotype analysis indicates an altered chromosomal pattern. Prenatal cytogenetic analysis from chorionic villi or amniotic fluid could provide early and reliable diagnosis, and help the family to avoid the recurrence.*

Keywords: *Down syndrome, karyotype, trisomy, mosaicism, translocation, counseling*

Introduction

Down syndrome (DS) is a chromosomal abnormality characterized by the presence of an extracopy of genetic material on the 21 chromosome, either in whole (trisomy 21) or part (such as due to translocation), which causes delays in the way a child develops and often leads to mental retardation. While some kids with DS have no other health problems, others may experience a lot of medical issues that require extra care.

Despite the variability in Down syndrome, children with Down syndrome have widely recognized characteristic appearance. The head may be smaller than normal and abnormally shaped. Typical facial features include a flattened nose, protruding tongue and upward slanting eyes with epicanthal fold.[1] These change with age. The hands are short and broad with short fingers, and they often have a single crease in the palm. Normal growth and development is usually retarded.

The frequency of trisomy 21 in the population is 1 in 650 to 1,000 live births. Advanced maternal age remains the only well-documented risk factor for maternal meiotic nondisjunction. However, understanding of the basic mechanism behind the maternal age effect is lacking: with a maternal age of 35 years, the risk is 1 in 385 and increases over this age.[2]

Types of chromosomal alteration for Down syndrome

a. Trisomy 21 is the cause of approximately 95% of observed Down syndromes and is usually caused by meiotic nondisjunction in the gametes prior to conception, and all cells in the body are affected. With nondisjunction, a gamete (i.e., a sperm or egg cell) is

Table.1 Karyotype analysis

Karyotype	No.	%	Mean maternal age
1. Free trisomy 21 47,XY,+21 47,XX,+21	82 M :F=1.1 :1 43 39	*71.93%*	*33.43*
2. Translocation 46,XY,der(21;21)(q10;q10),+21 46,XX,der(21;21)(q10;q10),+21 46,XY,der(14;21)(q10;q10),+21 46,XX,der(14;21)(q10;q10),+21	11 *M:F=1.2:1* 3 3 3 2	*9.65%*	*26.54*
3. Mosaics mos47,XY,+21[36]/46,XY[14] mos47,XY,+21[34]/46,XY[16] mos46,XX,der(21;21)(q10;q10)[87]/46,XX,del21p[13]	3 M:F=2:1 1 1 1	*2.63%*	*34.66*
4. Wihout karyotype	**18** *M:F=1:1.25*	*1.79%*	*30.25*
TOTAL	114		

produced with an extra copy of chromosome 21; the gamete thus has 24 chromosomes. When combined with a normal gamete from the other parent, the embryo has 47 chromosomes, with three copies of chromosome 21.[3]

b. Mosaicism is the cause of 2–4% of the observed Down syndromes and can arise by postzygotic (mitotic) nondisjunction of a normal zygote or the postzygotic loss of a chromosome 21 from a trisomic zygote. In these cases people have a mixture of cells (cell lines): some cells have a normal set of chromosomes, and other cells have trisomy 21. [4]

c. Robertsonian translocation is the cause of 1–2% of observed cases of Down syndrome. In this case, the long arm of chromosome 21 is attached to another chromosome, often chromosome 14 or itself.

d. Duplication of a portion of chromosome 21 is very rare. This means that there are extra copies of some, but not all, of the genes on chromosome 21.

Material and method

The present study was undertaken in referred Down syndrome cases. 114 patients attending Genetics Department of IOMC "Prof Dr Alfred Rusescu" were included in the present study. Detailed information was collected from the patients and their families from different regions of the country.

Chromosomal analysis was performed using standard protocols. Heparinized whole blood samples were set up in nutrient media. The cultures were stimulated with phytohaemagglutinin and incubated for 72h at 37°C. The cultures were arrested with colchicine and treated with KCl. The cultures were fixed with fixative (methanol : acetic acid = 3:1). The chromosomes were prepared on slides and were subjected to GTG-banding. The slides were treated with trypsin in buffer and stained with Giemsa stain.

At least 30-well spread and banded metaphases were analyzed under microscope in each case and 5 cells karyotyped. In cases of mosaicism, 50 metaphases were scored. Parents of children having robertsonian translocation were investigated to rule out the origin of altered chromosome.

The karyotypes were interpreted according to the recommendations of ISCN 2005 (an International System for Human Cytogenetic Nomenclature).

Results and discussion

The retrospective study included 114 children in the age range of 6 days to 10 years (at the time of examination). They were referred between the periods January 2007 – June 2008 to Genetics Department to confirm the clinical diagnosis of Down syndrome.

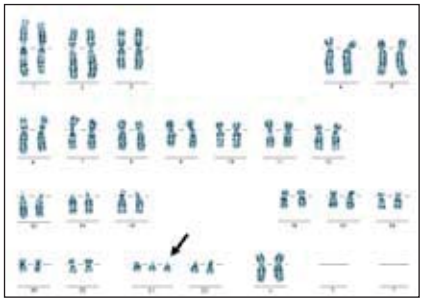

Fig.1 Karyotype of a girl with Down syndrome – Trisomy 21

The birth frequency of DS babies in younger mother (<35 years) was 67.89 % compared to older mother (>35 years) with 32.11 %.

Cytogenetic analysis of 96 cases of Down syndrome is presented in Table 1. There were 82 cases of free trisomy (71.93%), 11 cases with translocation (9.65%) and 3 cases

had mosaicism (2.63 %). Male to female ratio in patients of DS with free trisomy 21 was 1.1:1 and that of translocation was 1.2:1. Translocation in Down syndrome is usually of Robertsonian type with the fusion of chromosome 21 to D or G group chromosomes. The forms are t(21;21) and t(14;21).

Table.2 Effect of maternal age on Down syndrome

Maternal age	Free trisomy		Translocation		Mosaicism		Cases without karyotype	Total
	No.	%	No.	%	No.	%		
< 35 years	53	55.21	10	10.42	2	1.04	10	74
> 35 years	27	28.13	1	1.04	1	2.08	6	36
? age *	2	2.08	0	0	0	0	2	4
TOTAL 96 cases with karyotype	82		11		3		18	114

** Information not available because of the abandon of the child*

Birth order of Down syndrome revealed a high frequence of second borns (41,23%) followed by first borns (34,21%) and 24,56% are from families with three and more children. Only one case is from twins.

Familial inheritance in robertsonian translocation was seen only in one case, whereas the remaining is arisen as de novo. One case with t(14;21) (fig.2) showed maternal inheritance. The mother being a carrier of balanced chromosomal rearrangement (fig.3), the risk of recurrence of birth of a child with chromosomal imbalance is increased. Counseling for the risk of recurrence of DS children is necessary once the carrier for such balanced chromosomal rearrangement is identified.

Maternal age at conception is an important factor in genetic counseling. Maternal ages of children with Down syndrome at the time of conception were available for only 110 cases out of 114 cases studied. It is obvious from the present data that 55,21 % free trisomy Down syndrome children were born to mother's <35 years age. In case of translocation 10,42 % were born to mother <35 years age. The mean maternal age of free trisomy DS cases was estimated to be 33.43 years and for translocation was 26.54 years.

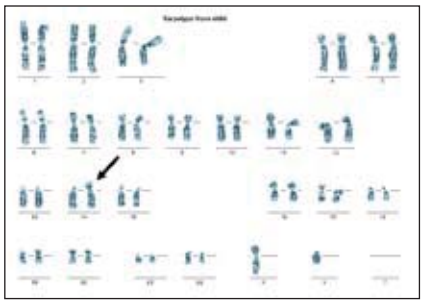

Fig.2. Karyotype of DS child with t(14;21)

Our cases report also associated malformations of Down syndrome. Among them, heart defects have occurred more frequently. Individuals with Down syndrome had specific major congenital heart malformations: atrioventricular canal, ventricular septal defect, atrial septal defect, patent ductus arteriosus, and of the gastrointestinal tract, such as duodenal stenosis. Other associated anomalies: cleft palate, congenital hypothyroidism, genitalia abnormalities (cryptorchidism, epispadias, hypospadias, hypertrophic clitoris, ophthalmic disorders (nystagmus, strabismus, asimetric palpebral fissures), orthopedic problems.

Chromosomal investigations, family history, pedigree analysis, parental ages and parental karyotypes are essential factors in offering genetic counseling, estimating the risk for the next conception.[5]

✘ Some women who have had a trisomy 21 conception may have a small increased risk for other aneuploidies.

✘ De novo Robertsonian translocation – risk is low, but recurrence has been reported

✘ If mother carries Robertsonian translocation involving 21, e.g. rob(14q21q), risk is 10-15%

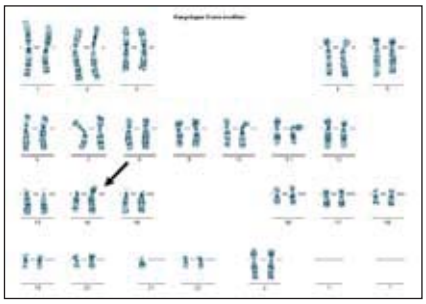

Fig.3. Karyotype of the mother carrier with t(14;21)

✘ If father carries Robertsonian translocation involving 21, e.g. rob(14q21q), risk is <1%

✘ If parent carries rob(21q21q) translocation, risk approaches 100%

✘ If a woman with Down syndrome, 47,XX,+21, becomes pregnant, the risk of +21 in the offspring is ~50% (fertility in man with +21 is exceptionally rare).

Prenatal Screening and Diagnosis

There are two types of prenatal tests available to detect Down syndrome in a fetus: screening tests and diagnostic tests.

✘ Screening tests estimate the risk that a fetus has DS. They are noninvasive and include:

- nuchal translucency testing
- the double and triple test
- detailed ultrasounds

✘ Diagnostic tests can tell whether the fetus actually has the condition.

Because they are performed in intrauterine period, they are associated with a risk of miscarriage and other complications. For this reason, they are generally recommended only for women age 35 or older, those with a family history of genetic defects, or those who have had an abnormal result on a screening test.[6]

Diagnostic tests include: chorionic villus sampling (CVS), amniocentesis or percutaneous umbilical cord blood sampling (PUBS).

Conclusions

Our study based on a medium group showed the importance of chromosomal analysis for reffered Down syndrome cases. Most of the DS children were born to mother's < 35years of age and were second born in the birth order. Though advanced maternal age is an established risk factor for DS, this study shown increased number of DS babies born to the young mothers.

Parental karyotypes are also essential for all patients with a translocation, to be sure that the rearrangement was not inherited.

Cytogenetic techniques are very important for the identification of the genetic variant of Down syndrome. The information obtained provides basis for determining the risks of recurrence and for genetic counseling.

Prenatal diagnosis must be offered to all mothers at risk: women >35years or younger mothers, if the karyotype analysis indicates an altered chromosomal pattern. Prenatal cytogenetic analysis from chorionic villi or amniotic fluid can provide early and accurate diagnosis and help the family to avoid the recurrence.

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