JoDD
Journal on Developmental Disabilities
Le journal sur les handicaps du developpment
Volume 14, Number 2, 2008

A First Cytogenetic Study of Down Syndrome in Sudan

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Keywords

Down syndrome,
genetic counselling,
cytogenetic analysis,
molecular cytogenetic analysis,
Sudan

Abstract

In this study, we report the first series of cases of Down syndrome (DS) cytogenetically analyzed in Sudan. Five children with clinical features of DS underwent cytogenetic and molecular cytogenetic analyses. Cytogenetic analysis of parents was also performed for counselling purposes. All children showed karyotypes consistent with DS. One child showed a Robertsonian translocation that was not present in either of her parents. The other cases showed classical trisomy 21. Molecular cytogenetic analysis confirmed the diagnosis in one case. Cytogenetic analysis of suspected DS is of value to objectively confirm the diagnosis and to provide a basis for genetic counselling.

Down syndrome (DS) affects approximately 1 in 650-1000 live-born children world-wide (Hook, 1982) and is the most common known genetic cause of intellectual disability (Ghani, Maniar, Khilji, Azi, & Khurshid, 1995). The risk of having a live-born child with DS at maternal age 30 is 1:1,000 and at maternal age 40 is 9:1,000 (Hook, 1982; Hook, Cross, & Schreinemachers, 1983). There also are reports that advancing paternal age, especially when the maternal age is greater than 40 years, and increased age of the maternal grandmother may increase the risk of DS (Fisch, Hyun, Golden, Hensle, Olsson, & Liberson, 2003; Malini & Ramachandra, 2006). A highly significant change in the survival of individuals with DS has occurred during the last two generations, with life expectancy estimates increasing from 12 to nearly 60 years of age (Bittles, Bower, Hussain, & Glasson, in press). (See also Lovering & Percy, 2007.)

Besides intellectual disability, individuals with DS often have major congenital problems—"inherited problems"—such as those of the heart (atrio/ ventricular septal defect) and gastrointestinal tract (duodenal stenosis/atresia, abnormal narrowing or

contraction of a duct or canal); imperforate anus; Hirschsprung disease, congenital absence of the parasympathetic nerve ganglia in the anorectum or proximal rectum) (Lovering & Percy, 2007; Wallace & Dalton, 2006). Ninety percent of all people with DS who are seen clinically have a significant hearing loss, usually of the conductive type due to a defect in the sound-conducting apparatus, that is, of the external auditory or middle ear (Mazzoni, Ackley, & Nash, 1994). Individuals with DS also develop neuropathologic hallmarks of Alzheimer disease at a much earlier age than individuals without trisomy 21 (Prasher, Percy, Jozsvai, Lovering & Berg, 2007; Wisniewski, Wisniewski, & Wen, 1985). Moreover, there is an increased incidence of leukemia (both ALL and AML) and leukemoid reactions (Fong & Brodeur 1987; Lovering & Percy, 2007; Robinson, 1992) in individuals with DS. Estimates of the relative risk for hematological malignancies are 10 to 20 times higher than in the normal population; in particular, acute megakaryocytic leukemia occurs 200 to 400 times more frequently in DS patients than in the chromosomally normal population (Zipursky, Peeters, & Poon, 1987). (See also Zwaan, Reinhardt, Hitzler, & Vyas, 2008.)

Most individuals (95%) with trisomy 21 have 3 separate copies of this chromosome (classical trisomy 21). In approximately 4% of such people, one extra copy of the chromosome is translocated to another acrocentric chromosome, most often chromosomes 14 or 21 (Mikkelsen, 1977, Thuline & Pueschel, 1982). In 1-4% of cases with classical trisomy 21, there is recognizable mosaicism with parallel trisomic and normal cell lines (Mikkelsen, 1977). (See also Lovering & Percy, 2007.)

Warren et al. (1987) and Sherman et al. (1991) have described an association between trisomy 21 and reduced recombination at meiosis. A significant proportion (at least 30%) of maternal meiosis I nondisjunction of chromosome 21 is associated with failure to recombine (Lovering & Percy, 2007). The mechanism behind recombination failure (asynapsis or abnormalities during or after synapsis) is as yet unclear. All de novo translocation 14;21 trisomies studied have originated from rearrangement of heterologous chromosomes in the maternal germ line (Petersen et al., 1991; Shaffer, Jackson-Cook, Stasiowski, Spence, & Brown, 1992). For de novo translocation 21;21, the situation is different. In most cases, the underlying mechanism is formation of an isochromosome from homologous chromatids rather than the result of a Robertsonian translocation between heterologous chromatids. The vast majority of the 21q21q rearrangements occur de novo; of the remaining, about half are of paternal and half of maternal origin. In the few studied cases of true Robertsonian translocation 21;21, the rearrangement of heterologous chromosomes was in the maternal germ line.

Even though the majority of cases of DS can be identified based on clinical findings, cytogenetic analysis is essential to confirm the diagnosis. Because translocation-associated DS is connected to an elevated risk of recurrence at later-age pregnancies, chromosomal analysis is also often recommended to provide information for genetic counselling to such families. Although there is some controversy about genetic counselling in its universal application, it is important to provide information for the family about the possibility of having another child with the same disability. Cytogenetics is therefore performed as part of the routine work-up on DS patients in most modern medical institutions. However, cytogenetic technology is still not available in many low-income countries. In this study, we report the first cytogenetic series of patients with DS from Sudan.

Method

Three suspected cases of DS were referred to our medical clinic for cytogenetic investigations. Another two patients were referred because of dysmorphology without a clear clinical suspicion of DS according to the referring physicians. However, by our evaluation, all five patients showed clinical features associated with DS including up-slanting eyes, flat nasal bridge and single transverse palmar crease. Parents' age varied between 22 and 60 years (Table 1). Except for patient 3, who had a first cousin with DS, there was no history of DS in any of the families. All parents were asked to leave blood samples at the family's first consultation, to be analyzed if indicated by the cytogenetic findings in the proband.

All cultures were harvested after 72 hours. Colcemid (0.1 ml; 10µl/ml) was added 30 minutes before harvesting. The cultured cells were then centrifuged at 1000 rpm for 10 minutes, the supernatant was removed and the remaining cells were re-suspended by adding 6-7 ml of hypotonic solution (KCl 0.075 M), and left at room temperature for 15 minutes before being centrifuged at 1000 rpm for 10 minutes. The supernatant was then removed, the pellet was re-suspended in 5ml fresh fixative (4:1 methanol: acetic acid). The fixation was repeated three times using fresh fixative (3:1 methanol: acetic acid) each time. The preparations were centrifuged at 1000 rpm for 10 minutes before removing the supernatant. The cells were re-suspended in approximately 0.5-1 ml fresh fixative (3:1 methanol: acetic acid). One or 2 drops of the suspension was placed on a clean dry glass slide. The quality and spreading of chromosomes were checked through a phase contrast microscope. In cases where spreading was suboptimal, 20-40 µl of concentrated acetic acid was added to the cell suspension and a new preparation was made after 5 min. The slides with good spreading and quality of chromosomes were kept in an oven at 60 C° overnight, whereas slides for fluorescent *in situ* hybridization (FISH) were kept at -20 C^{o}. The slides were washed with buffer solution (2x SSC) in water bath at 60 C° for 2-3 hours. After that, the buffer solution was poured off and the slides were washed repeatedly under tap water, air-dried for 1 hour and then stained with Wright's stain. A minimum of 12 metaphase cells were analyzed under the light microscope from each case. The clonality criteria and the karyotypic descriptions were according to the ISCN recommendations (Mitelman, 1995). FISH analysis with the LSI 21 single-copy probe (Vysis Inc; Downers Grove, IL) was performed at Lund University Hospital, Sweden, according to standard procedures (Gisselsson, 2001).

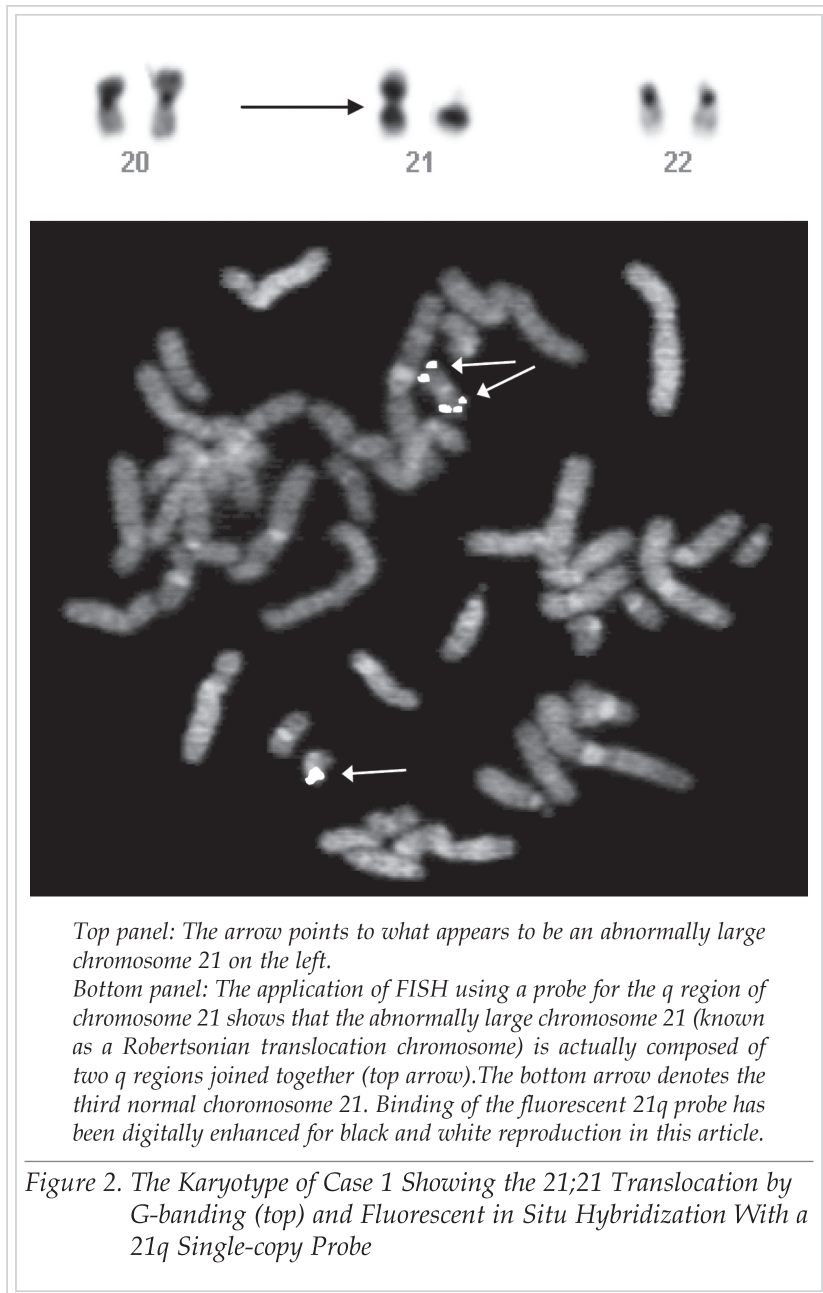
The method was approved by the Research Ethics Board of the University of Khartoum and consent forms were signed by the patients.

Table 1. Characteristics of Five DS Cases

Case No	*Age*	*Sex*	*Paternal age (years)*	*Maternal age (years)*	*Karyotype*
1	10 months	F	31	22	46,XX,der(21;21)(q10;q10),+21[22]
2	6 years	F	40	38	47,XX+21[10]
3	2 months	F	45	35	47,XY+21[11]
4	1 year	M	60	unknown	47,XY+21[11]
5	7 months	F	30	23	47,XX+21[11]

Results

All five patients showed karyotypes consistent with Down syndrome. Four cases showed classical trisomy 21. The fifth case showed a Robertsonian 21;21 rearrangement by chromosome banding which was confirmed by FISH (Fig. 2). FISH was also applied to corroborate trisomy 21 in patient 4, as chromosome banding was suboptimal in this case. The parents of case 1 with the 21;21 translocation both showed normal karyotypes.



Top panel: The arrow points to what appears to be an abnormally large chromosome 21 on the left.
Bottom panel: The application of FISH using a probe for the q region of chromosome 21 shows that the abnormally large chromosome 21 (known as a Robertsonian translocation chromosome) is actually composed of two q regions joined together (top arrow).The bottom arrow denotes the third normal choromosome 21. Binding of the fluorescent 21q probe has been digitally enhanced for black and white reproduction in this article.

Figure 2. The Karyotype of Case 1 Showing the 21;21 Translocation by G-banding (top) and Fluorescent in Situ Hybridization With a 21q Single-copy Probe

Discussion

The cytogenetic findings showed that four cases carried an extra free copy of chromosome 21, while case 1 showed a 21;21 translocation together with one normal chromosome 21 homologue. In all patients except case 4, chromosome preparations could be successfully produced at the laboratory in Sudan. In case 4, the chromosome banding quality was suboptimal and the tentative finding of trisomy 21 by G-banding had to be confirmed by FISH analysis at the Swedish collaborating department. FISH was also used to corroborate the 21;21 translocation in case 1.

Clinically, all five cases showed common findings associated with DS, such as hypotonia, gap between first and second toe, clinodactyly of the fifth finger, oblique eye fissures, flat nasal bridge and short broad hands. Only one patient showed congenital heart disease, while another showed imperforated anus, and a third patient showed hearing loss. All patients had psychomotor delay. In two of the cases, the referring physicians had not clearly stated a suspicion of DS.

After cytogenetic diagnosis, genetic counselling was offered to and accepted

by all families except the family of case 4. Since a familial Robertsonian translocation was not found in any of the five cases, the recurrence risk was approximately 1:200 for the families where maternal age was <35 years, and equal to the age-specific population risk for families where maternal age was >35 years [22].

Cytogenetic analysis contributes to diagnosis of DS also in low-income countries where physicians can be presumed to have acquired a high level of diagnostic skill in the absence of laboratory services. Cytogenetic data also provide a basis for the genetic counselling often requested by families into which DS children are born. This study shows that close collaboration with another, more experienced, cytogenetic laboratory may be important when establishing cytogenetic services in low-income countries in order to initially resolve technical issues.

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Glossary

Chromatid. Either of the two daughter strands of a replicated chromosome that are joined by a single centromere and separate during cell division to become individual chromosomes.

Chromosomes. The rod-shaped structures (found in most cells) that contain genetic information, or blueprints. In humans, each cell typically has 46 chromosomes, or 23 pairs; 22 of the pairs are called autosomes whereas the 23rd pair contains the sex chromosomes.

Fluorescent in situ hybridization (FISH). A cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which they share a high degree of sequence similarity.

Homologous chromatids. Chromatids of homologous chromosomes (i.e., those that are identical to one another in terms of the genetic loci they contain and that have the same number).

Heterologous chromatids. Chromatids of non-homologous chromosomes (i.e., those that are not identical to one another in terms of the genetic loci they contain and that have different numbers or designations).

Isochromosome. A chromosome in which one of its arms has been lost and replaced with an exact copy of the other arm.

Karyotype. A picture of the chromosomes from one cell, with pairs lined up in order according to size.

Leukemia. Any of various diseases of the bone marrow in which unusual proliferation of white blood cells occurs.

Megakaryocytic leukemia. Leukemia characterized by proliferation of megakaryocytes, a giant cell in the bone marrow that is the precursor of platelets.

Meiosis. The type of cell division that leads to the production of sperm and ova (egg cells).

Mitosis. The type of cell division that leads to the production of most of the cells of the body.

Mosaic Down syndrome. A form of Down syndrome (seen in about 3% of cases) in which a person typically has 2 different types of cells, one with 46 chromosomes and the other with 47, including an additional chromosome 21 (typical trisomy 21).

Nondisjunction. A condition in which there is a failure of paired chromosomes to disjoin (separate) during cell division so that both chromosomes go to one daughter cell and none to the other. Nondisjunction causes errors in chromosome number such as trisomy 21.

Recombination. The process by which a strand of genetic material (usually DNA; but can also be RNA) is broken and then joined to a different molecule of genetic material. In eukaryotes recombination commonly occurs between paired chromosomes during meiosis. This process leads to offspring having different combinations of genes from their parents.

Translocation Down syndrome. A type of Down syndrome (seen in about 4% of cases) that occurs when part of a chromosome 21 attaches to another chromosome. For these cases, there will be three doses of genetic information for part of the chromosome 21. Such translocations are sometimes called "Robertsonian" translocations. They are named after the American insect geneticist W. R. B. Robertson, who first described a Robertsonian translocation in grasshoppers in 1916.

Trisomy 21. In each cell of the body there is an extra chromosome 21, with a total of 47 rather than 46 chromosomes. About 95% of individuals with Down syndrome have trisomy 21.

Acknowledgement

We are grateful to the Alf Hanssons minnesfond, the ASTRA-Zeneca traveling fund, the Lund Medical Society, and the Royal Physiographic Society of Lund for providing funding for this study.