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RESEARCH ARTICLE

Prevalence of mosaicism for trisomy 21 and cytogenetic variant analysis in patients with clinical diagnosis of Down syndrome: a 24-year review (1986-2010) at the Servicio de Genética, Hospital General de México "Dr. Eduardo Liceaga"

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ABSTRACT

Background. Down syndrome is the principal genetic cause of learning disabilities, with an incidence of 1/650 live births. Diagnosis is confirmed by karyotyping. Chromosomal variants are important for genetic counseling. We determined the prevalence of cytogenetic variants in patients with clinically diagnosed Down syndrome in the Hospital General de México Dr. Eduardo Liceaga and discussed its relevance according to maternal age.

Methods. We reviewed karyotype data of patients with clinically diagnosed Down syndrome from January 1986 to December 2010 and obtained maternal and patient ages.

Results. From 581 patients analyzed, 71 (12.22%) had normal karyotype. In 510 patients we confirmed that 445 (87.3%) had a regular trisomy, 22 (6.3%) were the product of a Robertsonian translocation and mosaicism was found in 43 (8.4%) patients. Maternal age was higher in patients with regular trisomy (median: 30 years) and mosaicism (median: 29 years) than in those with translocations (median: 20 years).

Conclusions. Characterization of chromosomal aberrations in Mexican Down syndrome patients allows us to offer appropriate genetic counseling and to establish the prevalence of mosaicism in our population, which was higher than the reported data. Cytogenetic analysis for detection of mosaicism is important in patients with scarce clinical data of Down syndrome or with learning disabilities of unknown origin.

Key words: Down syndrome, trisomy 21, mosaicism, chromosomal aberrations.

INTRODUCTION

Down syndrome is the most common genetic cause of intellectual disability in the population and is due to a gene dosage effect of the presence of an additional chromosome 21[1] or a partial trisomy, mainly in the 21q22 region.[2] Patients have a characteristic phenotype that includes brachycephaly, flat facies, upward slanting palpebral fissures, epicanthus, low-set round ears with abnormal folds, epicanthus and unique transverse palmar crease, among others.[3] They may also present with heart disease (40–50%) and duodenal atresia, in addition to an increased risk of acute myeloid leukemia, Hirschsprung's disease and Alzheimer's disease (especially after their fourth decade).[1] In Mexico, the incidence of Down syndrome is 1/650 newborns.[3] Diagnosis is based on clinical features. However, it is advisable to confirm the diagnosis by karyotype in order to determine the cytogenetic variant and to provide appropriate genetic counseling. Down syndrome presents five cytogenetic variants:

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- Regular trisomy 21 (T21)—with karyotype 47,XX,+21 or 47,XY,+21, present in ~95% of the cases[2,4]
- Robertsonian translocations (rob)—involves the rearrangement of chromosome 21 with another acrocentric chromosome (group D or G)[2,4] (46,XX or 46,XY, rob(D or G;21)(q10; q10),+21
- Isochromosomes of the long arm of chromosome 21—with chromosomal complement 46,XX or XY,+21,i(21)(q10). Along with rob, they present in ~4% of Down syndrome patients.[2,4]
- Mosaicism—presence of two or more different cell lines in the same individual. In this case, one line with T21 and another normal line, represented by the formula 47,XX or XY,+21/46,XX or XY and corresponds to 1–3% of all cases[2,4]
- Partial trisomy of the region 21q22.3—with karyotype 46,XX or 46,XY, dup(21)(q22.3) (observed in <1% of the cases)[2,4]

T21 is frequently the result of nondisjunction in maternal meiosis (~90%). Most occur in meiosis I (MI). Errors of meiosis II (MII) only constitute 20% of maternal errors.[2] Trisomies of paternal origin are less common (3% in MI and 5% in MII),[5] and in ~4% of the cases the additional chromosome is the result of a postzygotic error.[6]

It is important to mention that the percentage of mosaicism may be underdiagnosed because the number of cells analyzed generally is insufficient to detect cell lines in low proportion. Mosaicism in Down syndrome may originate from a normal zygote or as a result of a trisomy due to postzygotic nondisjunction or trisomic rescue, respectively.[7]

This study was conducted to retrospectively analyze cytogenetic data of patients from the Hospital General de México Dr. Eduardo Liceaga (HGM) with suspected diagnosis of Down syndrome and/or confirmed karyotype and to determine the prevalence of cytogenetic variants in this population with Down syndrome in order to perform an analysis in relation to maternal age. It is worth noting that there are few reports of the prevalence of mosaicism in Down syndrome in the Mexican population.

SUBJECTS AND METHODS

Data were collected from patients with the clinical diagnosis of Down syndrome who attended the HGM Genetics Service during the period from 1986 to 2010. Results were recorded for cytogenetic analysis, gender, age and clinical data in regard to the purpose and to maternal age at the time of the birth. We calculated measures of central tendency for patients' and mothers' ages. For karyotyping, samples were processed and banded by conventional cytogenetic techniques. Twenty to 100 metaphases were analyzed for each case, depending on the indication and the quality of the analyzed material.

RESULTS

During the 24-year study period, there were 581 patients reported with the clinical diagnosis of Down syndrome and according to results of karyotyping; 71 patients presented normal karyotype (Table 1). The predominant chromosomal abnormality was T21 followed by mosaicism and rob (Figure 1). In the majority of the cases of mosaicism, the trisomic cell line was the most abundant. The average ratio of the abnormal line was 72% (range: 2–98%). Only two patients showed intellectual disability and limited data of Down syndrome with the trisomic line corresponding to <10% (2–8%). In a few cases, due to translocation, it was possible to study the parents, and most corresponded to *de novo* rearrangements. With respect to the age of the patients at the time of consultation, no significant variations were observed (Table 2). Maternal age was higher in patients with T21 and mosaicism than in patients with rob translocation (Figure 2).

DISCUSSION

A maternal meiotic nondisjunction occurs in ~90% of cases of Down syndrome, which correlates with the most frequent cytogenetic variant being T21 (445 patients) (Table 1). Another mechanism for this aneuploidy is that one of the parents, especially the mother, exhibits mosaicism. It is estimated that 3% of the couples who have a child with T21 present mosaicism[8] and that 1–2% of the general adult population presents autosomal mosaicism.[9] In Mexico, it has been reported that 2.36% of the parents of children with Down syndrome are mosaics with one line with T21 in a very low proportion (2.7–4.3%).[10] Recent studies detected mosaicism in 4.5% of the parents with a child with T21 and in up to 36% of the parents with recurrence of Down syndrome.[2] In apparently normal fe-

male fetuses, Hultén et al. found germinal mosaicism for trisomy 21, suggesting that the effect of maternal age is caused by the differential selection of these cells during fetal and postnatal development until ovulation. A high degree of germinal mosaicism may explain the existence of young women who have recurrence of children with Down syndrome.[4] The risk of recurrence for T21 in a family with an affected child is 1 to 2% and can increase with maternal age.[3]

With respect to rob translocation (Table 1, Figure 1), the proportion that we found was in accordance with the literature. However, it was not possible to establish the prevalence of each type of translocation. The most common translocation in Down syndrome is rob (14;21), followed by rob (21;21) or i(21q).[11,12] When a patient with Down syndrome has one of these variants, it is necessary to conduct karyotyping of the parents to identify if one is a carrier and to establish an appropriate risk for recurrence. Most translocations occur *de novo*. The reported frequency of carriers is 5 to 20% and, usually, the mother is the carrier.[12-14] In our study, although in a few cases it was possible to study the parents cytogenetically, the results agree with the above points. The risk of recurrence is <1% if the translocation is *de novo*.[12] In the case of it being inherited, the empirical risk for rob (D,21) when the mother is a carrier is ~10 to 20% and when it is the father the risk is ~2–5%. For a carrier of a rob (21,21), the risk for recurrence is 100%.[15]

The prevalence of mosaicism in this study was higher than that reported in the literature[2-4] but is consistent with other epidemiological studies in patients with Down syndrome (Table 3).[7,12-14,16-22] Down syndrome patients with mosaicism with a trisomic cell line in a proportion >90% are diagnosed as regular trisomy because, unless analyzing >50 cells, the normal line is not detected. On the contrary, if the trisomic line is in a proportion <10%, the diagnosis often goes unnoticed.[7,23] By analyzing 25 metaphases, lines in >25% of proportion are detected.[23] Thus, it is considered that the reported frequency of mosaicism in cases of Down syndrome (1–3%) is lower than the true frequency.[7] This study quantified, on average, 38 cells per case (range 20–100 cells).

Most of the cases of mosaicism found in this study (Table 1) and other studies (Table 3) present only one trisomic and one normal line. Although the proportions are not known, it is expected that there is a predominance of the trisomic line. Gonzalez et al. studied 100 Mexican patients with Down syndrome. They analyzed 100 metaphases and found mosaicism in 39 patients, 13 with 97% of the trisomic line, in 35 the line with T21were in a proportion >90%, and in only four with <50%.[7] In our study, only two of 43 patients had mosaic trisomic line in a proportion <10% and presented intellectual disabilities with some features of Down syndrome. In the other patients,

Table 1. Cytogenetic variants detected in 510 patients with diagnosis of confirmed Down syndrome

Cytogenetic variant	*No. of cases*
47,XX,+21	214
47,XY,+21	229
47,XX,+21/46,XX	25
47,XY,+21/46,XY	17
46,rob(D;21),+21	15
46,+21,rob(21;21)(q10;q10)	6
46,XX,+21,rob(21;21)/46,XXa	1
47,XX,del(5)(p?),+21b	1
47,XY,t(8;14)(q22;q32),+21/46,XY,t(8;14)(q22;q32)c	1
48,XX,+21,+mar/47,XX,+21d	1
Total	510

rob, robertsonian translocation; [a-d], nomenclature for individual patients; del, deletion; t, translocation; mar, additional marker.

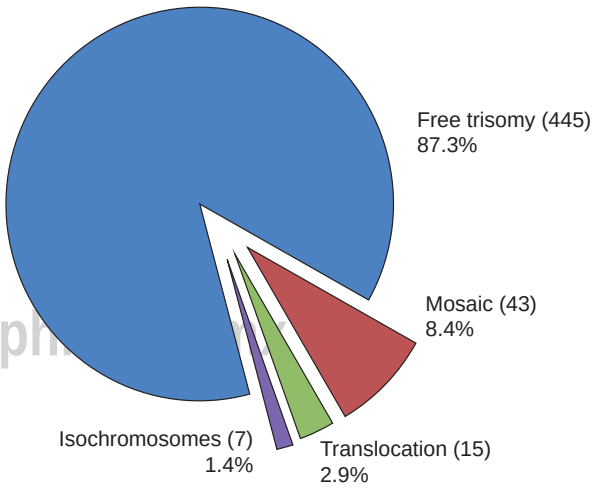

Figure 1. Proportion of cytogenetic variants found in patients diagnosed with Down syndrome.

Table 2. Maternal age at birth and age of patient at the time of consultation

	Maternal age					*Age of patient*				
Variant	*No. of patients*	*Average (years)*	*Median (years)*	*Mode (years)*	*Interval (years)*	*No. of patients*	*Average (years)*	*Median (months)*	*Mode (months)*	*Interval (years)*
General	338	29.8 ± 8.51	29	23	13-51	483	3.25 ± 7.52	3	RN	0-52
T21	295	30.3 ± 8.39	30	23	13-51	424	2.41 ± 6.19	3	RN	0-52
Mosaicism	26	30.1 ± 9.47	29	22	15-50	38	6.14 ± 11.28	3	2	0-43
Rob	17	22.5 ± 5.49	20	20	16-36	21	4.65 ± 9.15	1	RN	0-28

No, number; T21, trisomy 21 regular; rob, robertsonian translocation; NB, newborn.

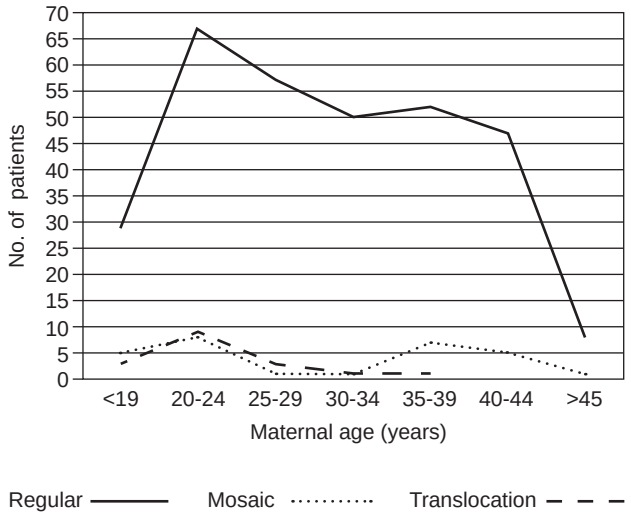

Figure 2. Incidence of Down syndrome according to maternal age and type of cytogenetic alteration.

the proportion of the T21 cell line was equal to that of normal (50%) or higher. It has been established that several individuals diagnosed as typical Down syndrome are mosaics with a small proportion of normal cells. In cultured cells, it has also been shown that some individuals with minimal Down syndrome features, and even without them, may be low-degree mosaics with variations in proportions in other tissues.[19,24]

When mosaicism is suspected or detected, it is recommended to look for the trisomic line, in at least two tissue samples. It has been observed that the number of abnormal cells in the oral mucosa is significantly related to IQ (cells derived from ectoderm). In contrast, cardiac defects correlate with the proportion found in lymphocytes because both tissues are derived from the mesoderm.[25] Because this study was retrospective in nature, the presence of mosaicism in other tissues was not verified, although it is considered to be included in future studies.

Of the atypical variants identified (Table 1, patients [a–d]), the first patient ([a]) presented a rob (21;21) or i(21q) in mosaic with a normal line. It is inferred to be due to a postzygotic error; however, it is required to conduct molecular and cytogenetic testing, to rule out chimerism and establish the origin of the structural aberration. Patient ([b]), in addition to the T21, presented partial monosomy of the 5p so that his phenotype was modified with clinical features of the cri du chat syndrome (cat's cry). Patient ([c]), with a constitutive balanced reciprocal translocation t(8;14)(q32;q22), was mosaic for T21. The apparently balanced rearrangement had no effect on phenotype; however, it is important to karyotype the parents to rule out either one as a carrier. The last patient ([d]), in addition to the T21, presented mosaicism for an additional chromosome marker. Cytogenetic characterization must be performed because the presence of this additional marker can modify the phenotype and have implications for genetic counseling. Other studies have also reported additional chromosomal alterations to T21.[11,13]

Patients' ages at the time of diagnosis showed a range from birth to 52 years of age (median 3 months) and no significant difference was observed. Most patients are referred to genetic counseling by different specialists—primarily neonatologists and pediatricians—because of their phenotype. In maternal ages, there was a significant difference between the cytogenetic variants. Maternal age of patients with T21 (median: 30 years) and mosaicism (median: 29 years) was higher than that of rob (median: 20 years) (Table 2). The literature reports a similar pattern with >35 years of age for T21 and <25 years of age for rob. Maternal age

Table 3. Proportion of cytogenetic variants in patients with Down syndrome from various epidemiological studies

Reference	*González*[7]	*Sheth*[12]	*Thomas*[13]	*Chandra*[14]	*Astete*[16]	*Jyothy*[17]	*Staples*[18]	*Devlin*[19]	*Azman*[20]	*Catović*[21]	*Wang*[22]	*HGM*
Country and year of publication	México 1998	India 2007	India 1992	India 2010	Chile 1991	India 2000	Australia 1991	Inglaterra 2004	Singapore 2007	Bosnia 2005	China 2010	México 2013
Patients studied	99	382	365	1016	243	1001	635	208	149	155	86	510
Cells analyzed	100	50-100	--	--	--	--	--	--	--	--	--	20-100
T21 (%)	60.6	86.9	86.6	84.2	92.6	87.9	93.9	94.7	94.6	89.7	93.02	87.3
rob (%)	0	9.2	7.7	5	3.3	4.4	4.1	1.4	0.7	5.8	3.49	4.3
Mosaic (%)	39.4	3.9	5.8	10.8	4.1	7.7	2	3.8	4.7	4.5	3.49	8.4
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100

HGM, Hospital General Mexico; T21, regular trisomy 21; rob, robertsonian translocation.

reported for T21 is higher than that observed in our data, which may be explained according to reproductive behaviors.[11,14,16,18,26] Down syndrome due to rob translocation is usually due to a *de novo* structural abnormality or segregation of this alteration in healthy carriers and not to errors in chromosome disjunction; therefore, it is not associated with the effect of advanced maternal age.[11,14]

Figure 2 shows that the number of cases of advanced maternal age were higher in T21 than in the other alterations. There are two peaks in T21 and mosaicism: the first corresponds to the average age of reproduction and the second indicates the increase in cases due to maternal age, which is consistent with the literature.[11,16,17] Because mosaicism can result from postzygotic nondisjunction or trisomic rescue, there may also be an effect due to maternal age; therefore, the behavior is similar to T21.[3,5] In the case of rob, there is only one peak corresponding to the average reproductive age. As for patients with Down syndrome phenotype and normal karyotype, it would be desirable to perform molecular studies such as FISH with probe for the 21q22 region in different tissues in order to rule out the low proportion of mosaicism or cryptic rearrangements.

In this paper, a review of the cytogenetic data allowed us to characterize the prevalence of different types of cytogenetic abnormalities in Down syndrome in our population. This study, to date, includes the largest number of Mexican patients studied. Data obtained are of utmost importance to calculate risks for recurrence and to provide appropriate genetic counseling. Because the prevalence of mosaicism was higher than that reported in the literature, it should emphasize the importance of appropriate studies in individuals with few Down syndrome traits or with intellectual disabilities of unknown origin.

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