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Down Syndrome: Parental Origin, Recombination, and Maternal Age

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The aims of the present study were to assess (1) the parental origin of trisomy 21 and the stage in which nondisjunction occurs and (2) the relationship between altered genetic recombination and maternal age as risk factors for trisomy 21. The study included 102 cases with Down syndrome from the Croatian population. Genotyping analyses were performed by polymerase chain reaction using 11 short tandem repeat markers along chromosome 21q. The vast majority of trisomy 21 was of maternal origin (93%), followed by paternal (5%) and mitotic origin (2%). The frequencies of maternal meiotic I (MI) and meiotic II errors were 86% and 14%, respectively. The highest proportion of cases with zero recombination was observed among those with maternal MI derived trisomy 21. A higher proportion of telomeric exchanges were presented in cases with maternal MI errors and cases with young mothers, although these findings were not statistically significant. The present study is the first report examining parental origin and altered genetic recombination as a risk factor for trisomy 21 in a Croatian population. The results support that trisomy 21 has a universal genetic etiology across different human populations.

Introduction

Trisomy 21 or Down syndrome (DS) is one of the most common chromosomal abnormalities. The majority of full trisomy 21 is caused by chromosomal nondisjunction occurring during maternal meiotic division (~90%). Errors occur more frequently in the first maternal meiotic division than the second (73% vs. 25%) (Antonarakis, 1991; Antonarakis *et al.*, 1992; Yoon *et al.*, 1996; Hassold and Sherman, 2000; Freeman *et al.*, 2007; Ghosh *et al.*, 2010).

In addition to the well-established effect of maternal age on bearing a child with trisomy 21 (Hassold and Chiu, 1985; Sherman *et al.*, 2005; Allen *et al.*, 2009), altered genetic recombination has also been identified as a risk factor (Lamb *et al.*, 1996, 2005; Sherman *et al.*, 2006). Warren *et al.* (1987) were the first to report reduced levels of chromosome 21 recombination in meioses leading to trisomy 21. Although the achiasmate bivalents rarely occur in normal female meiosis, about 45% of trisomy 21 cases are derived from maternal meiotic I (MI) nondisjunction without recombination (Lamb *et al.*, 1997). In addition, the location of recombinant events influences the ability of homologs to segregate during meiotic division. In cases of maternal MI-derived trisomy 21, the majority of recombination events occurred at the telomere of 21q, whereas exchanges occurring among meiotic II (MII) cases of trisomy 21 clustered at the pericentromeric region (Lamb *et al.*, 1997). Recently, an association between maternal age and altered recombination was observed in a U.S. population (Lamb *et al.*, 2005; Oliver *et al.*, 2008). These studies indicated that achiasmate meiosis and single telomeric exchange impose a risk for MI nondisjunction that is independent of the maternal age risk. On the other hand, the analysis of MII errors showed that the presence of a single exchange within the pericentromeric region of 21q is associated with maternal age-related risk factors. A study by Ghosh *et al.* (2009), on an Indian population, confirmed these results and suggested that the genetic etiology underlying the occurrence of trisomy 21 may be similar across human populations.

The aims of the present study were to assess (1) the parental origin and stage that nondisjunction occurs in trisomy 21 in a population from Croatia, geographically located between Central and South-eastern Europe, and (2) the relationship between altered genetic recombination and maternal age as risk factors for trisomy 21.

Methods

In collaboration with DS associations from larger cities in Croatia (Rijeka, Zagreb, Pula, Zadar, Split, Karlovac,

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Table 1. The Parental Origin and Stage of Nondisjunction Error Resulting in Trisomy 21 According to Parental Age

Parental origin and stage of error	*No. of cases (%)*	*Percentage of parental origin*	*Mean maternal age (years ± SD)*	*Mean paternal age (years ± SD)*
Maternal		93		
Meiosis I	82 (86)		31.06 ± 6.69	34.52 ± 7.11
Meiosis II	13 (14)		33.84 ± 4.93	35.11 ± 4.31
Paternal		5		
Meiosis I	4 (80)		23 ± 5.65	25.60 ± 3.71
Meiosis II	1 (20)		27 ± 0.0	29 ± 0.0
Mitotic	2	2	23.50 ± 2.12	26 ± 0.0
Total cases	102			

SD, standard deviation.

Čakovec, and Osijek), 116 blood samples were collected from DS subjects. Both the mother and father were available in 76 cases. The blood samples only from the mother were obtained in 40 cases. All participants were of the same ethnicity (Caucasian). The karyotypes of the parents were confirmed as normal. The mean ages of the mothers and fathers calculated at the time of birth of a child with DS were 31.00 ± 6.5 and 34.12 ± 6.8 years, respectively. All DS cases were free trisomy 21. The Ethical Committee of the School of Medicine, University of Rijeka, approved the study. All participants provided written informed consent prior to participation in the genetic analysis.

DNA analysis

Genotyping analyses were performed using 11 short tandem repeat (STR) markers spanning from the centromere to the telomere of chromosome 21q. STR markers divided 21q into three intervals. The proximal interval included the following markers: D21S258, D21S120, D21S1414, D21S1432, and D21S11; the medial interval included D21S1435, D21S226, D21S1270, and IFNAR; and the distal interval included D21S1412 and D21S1411 markers. Markers were selected from the Ensembl Genome Browser database (www.ensembl.org/index.html). The STR markers D21S120, D21S1414, D21S1432, D21S11, D21S1435, D21S1412, and D21S1411 were amplified one by one with polymerase chain reaction (PCR) as described elsewhere (Gómez *et al.*, 2000). PCR products were separated by electrophoresis, which was carried out in Spreadex gels (EL 300, 600, 800, 1200) at 55°C, 120 V, for 2–6 h, depending on the size of the PCR products. Gels were stained with Syber Green fluorescent dye and distained with appropriate buffer according to the manufacturer's recommendations (Elchrom Scientific). Products were analyzed using the digital dosage analysis software, Kodak 1D. The STR markers D21S258, D21S120, D21S11, D21S1435, D21S226, D21S1270, IFNAR, and D21S1411 were amplified together in two single-assay quantitative fluorescent PCRs. The reaction products were subsequently separated on an ABI 3130 genetic analyzer and analyzed with GeneMapper software (Pavlinić *et al.*, 2008). The detection of parental origin and stage of nondisjunction (meiotic/mitotic) and the analysis of recombination events were done as previously described (Freeman *et al.*, 2007).

Statistical analysis

Statistical analyses were performed by the chi-squared test of independence, nonparametric tests for correlations, and simple linear regressions using Statistical software package for Windows (2001; Stat soft, Inc.). Results were considered statistically significant at $p < 0.05$.

Results

The parental origin of trisomy 21

The parental origin was successfully determined in 75 of 76 complete families and in 27 of 40 cases for which we had samples from the mother and child only. Table 1 shows the frequencies of the parental origin of trisomy 21 and the stage

Table 2. The Frequency of Recombination Events Along Chromosome 21 During Maternal Meiosis Among Mothers of Different Age Groups

Stage of error	*Maternal age group*	*Mean maternal age (years ± SD)*	*Sample size*	*No. (frequency) of subjects with:*		
				0 observed recombination	*1 observed recombination*	*2 observed recombinations*
MI	Young (≤ 28)	25.00 ± 3.65	32	25 (0.78)	6 (0.19)	1 (0.03)
	Middle (29–34)	31.60 ± 2.07	24	19 (0.79)	4 (0.17)	1 (0.04)
	Older (≥ 35)	37.75 ± 1.70	26	21 (0.80)	5 (0.20)	0
MII	Young (≤ 28)	28.33 ± 0.57	3		2 (0.66)	1 (0.33)
	Middle (29–34)	32.00 ± 2.00	5		3 (0.60)	2 (0.40)
	Older (≥ 35)	39.25 ± 3.30	5		4 (0.80)	1 (0.20)

MI, first meiotic division.
MII, second meiotic division.

Table 3. Positional Distribution of Single Recombination Events for Meiotic I and Meiotic II Derived Trisomy 21 Among Mothers of Different Age Groups

Stage of error	*Maternal age group*	*Sample size*	*Mean maternal age (years ± SD)*	*No. of cases with one recombination stratified by chromosomal intervals*		
				Proximal	*Medial*	*Distal*
MI	Young (<35)	10	27.75 ± 4.51	0	3	7
	Older (≥35)	5	37.75 ± 1.70	0	3	2
MII	Young (<35)	5	31.22 ± 2.90	5	0	0
	Older (≥35)	4	39.75 ± 2.75	4	0	0

of nondisjunction according to parental age. The mean maternal age was not statistically significantly different between maternal MI- and MII-derived cases of trisomy 21 ($p = 0.153$).

Analysis of recombination events

Frequency of recombination and maternal age. Table 2 shows the frequencies of recombination events along chromosome 21 during maternal meiosis among mothers of different age groups. The greatest proportion of zero recombination (79%) was observed in the group of cases with MI-derived trisomy 21. The frequency of this achiasmate meiosis was not statistically significantly different among the three different maternal age groups (young, middle, and old age groups; $p = 0.803$).

Location of recombination and maternal age. Table 3 shows the location of recombination in MI- and MII-derived trisomy 21 cases between two different maternal age groups. Regression analysis performed on MI cases did not show a statistically significant relationship between location of recombination and maternal age ($t = -1.072$; $p = 0.303$).

Discussion

Here we present, for the first time, the parental origin of regular trisomy 21 in a Croatian population. The vast majority of trisomy 21 was of maternal origin (93%), followed by paternal (5%) and mitotic origin (2%). Our findings confirm the model for DS origin found in other populations (Antonarakis, 1991; Gómez *et al.*, 2000; Petersen and Mikkelsen, 2000; Machatkova *et al.*, 2005; Freeman *et al.*, 2007; Ramírez *et al.*, 2007; Ghosh *et al.*, 2010). The obtained frequencies of maternal MI-derived (86%) and MII-derived (14%) trisomy 21 were different from the study reported by Freeman *et al.* (2007), but similar to the studies on Mediterranean and Eastern Europe populations (Gómez *et al.*, 2000; Machatkova *et al.*, 2005). The discrepancy was probably due to both the small sample size and maternal age distribution covered by the study. As the sample size increases, the results are more similar to that obtained by Freeman *et al.* (2007), which included an impressive number of cases. Allen *et al.* (2009) found that the ratio of maternal MI- to MII-derived trisomy 21 cases was less in the youngest (<15) and the oldest (40–50) maternal age groups compared with that in the other maternal age groups. For example, the MI-to-MII ratio in the 25–29 and 30–40 years age groups were 3.5 and 4.7, respectively. Further, our study confirmed the well-established phenomenon of advanced maternal age as a risk factor for DS, because elevated maternal age was confined to maternally derived trisomy 21 and was associated with both maternal MI and MII errors (Antonarakis *et al.*, 1992; Yoon *et al.*, 1996; Lamb *et al.*, 2005; Sherman *et al.*, 2006; Oliver *et al.*, 2008; Allen *et al.*, 2009).

It has been postulated that among maternal MI-derived trisomy 21 cases, the vast majority of nondisjunction is associated with either a lack of an exchange or a telomeric exchange, and that this pattern influences the risk for nondisjunction irrespective of maternal age (Lamb *et al.*, 2005; Oliver *et al.*, 2008). In contrast, among maternal MII errors the pericentromeric exchanges were enriched among older women and were an age-dependent factor (Oliver *et al.*, 2008; Ghosh *et al.*, 2009). We also found that the highest proportion of zero recombination occurred in cases with maternal MI-derived trisomy 21. No statistically significant difference was observed in the frequency of these cases among different maternal age groups, supporting the theory of an age-independent risk factor. Although we had a small sample for analyzing the effect of the chiasmata position along 21q on the susceptibility for nondisjunction, a higher proportion of telomeric exchanges were present in cases of MI-derived trisomy 21 with younger mothers. The results support that trisomy 21 has a universal genetic etiology across different human populations.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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