SHORT COMMUNICATION

Phenotypic Heterogeneity and Parental Origin of Extra Chromosome 21 in Down Syndrome

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Received: November 23, 2008; Initial review: December 23, 2008; Accepted: February 19, 2009.

We compared the frequency of phenotypic features of 40 children with Down syndrome between individuals with a maternally or paternally derived extra chromosome 21, using quantitative FISH for comparing heteromorphisms of the nucleolar organizing region. Parental origin was determined in 90% of families. Hypotonia and craniofacial abnormalities were present in 90% or more individuals, irrespective of parental origin of chromosome 21. Congenital heart defects were more frequent in cases with a maternally derived extra chromosome 21. Imprinted gene(s) may contribute to the development of congenital heart defects in Down syndrome.

Keywords: *Congenital heart disease, Down syndrome, Fluorescent in-situ hybridization, Genomic imprinting, Trisomy 21.*

Published online: May 10, 2009. PII : S097475590800683-2

Eighty features are described in Down syndrome (DS)(1). However, not all features are observed in an individual with DS. Congenital heart defects (CHD) and duodenal stenosis are present in only 50% and 7% of cases, respectively(2). Relationship between extra genes or gene products on chromosome 21 and craniofacial abnormalities, heart defects, mental retardation and dermatoglyphics has contributed to the construction of a phenotypic map within the Down syndrome critical region (DSCR)(3,4). The mechanism for phenotypic variability has yet not been understood.

Genomic imprinting – a phenomenon where expression of a gene or a set of genes is determined by the parent of origin, is a fascinating hypothesis. Features in DS may vary with the parent of origin of extra chromosome 21. This study aimed at comparing phenotypic features of cases with maternal versus paternal origin of extra chromosome 21.

METHODS

The study was performed over 10 months after approval of the Institutional Ethics Committee. We prospectively enrolled 61 patients with Down syndrome. The inclusion criteria were free trisomy 21 confirmed by karyotype and availability of both biological parents. Blood count, radiographs, ECG, 2D echocardiogram and color doppler, thyroid function tests (serum T3, T4 and TSH), audiometery or BERA, developmental quotient, and ophthalmologic consultation were obtained for all subjects.

Nucleolar organizing region (NOR) heteromorphisms were studied using quantitative FISH (Q-FISH) by digitally capturing images and measuring the fluorescent signal intensity. Probes used were FlourX labeled probe (green fluorescence) hybridizing to the NOR of all acrocentric chromosomes including chromosome 21 and Cy3 labeled probe (red fluorescence) hybridizing only to the chromosome 21 specific

21q21.2 region. The probes were synthesized using standard protocols involving nick translation. The child's and the parents' fluorescent NOR signals of chromosome 21 were quantitated for width, length and fluorescence ratio using Imstar software (France) and compared. Parent of origin was assigned on the basis of a match with one parent.

Physical features, congenital anomalies and other defects were compared between cases with a maternally derived extra chromosome 21 and those with a paternally derived extra chromosome by applying Fisher's test.

RESULTS

Free trisomy 21 was detected in 40 cases (21 males, 19 females). The mean age was 22.1 ± 27.1 months (range: 45 days to 12 years). The mean maternal age and paternal age at delivery was 27 ± 5.6 years (range, 21 to 40 years) and 32.7 ± 7.2 years (range, 24 to 55 years), respectively. Parental origin of chromosome 21 could be determined in 36 families (90%). The non-dysjunction error was maternal in 77.8% [23 patients (82.1%) with 1st meiotic nondysjunction and 5 (17.9%) with 2nd meiotic nondysjunction] and paternal in 22.2% [1st meiotic error in 6 cases (75%) and 2nd meiotic error in 2 (25%)]. Maternal 1st meiotic nondysjunction accounted for 63.9% of the total cases.

Frequency of microcephaly, flat occiput, flat facial profile, epicanthic folds, oblique palpebral fissures, flat nasal bridge, open mouth, short neck, curved fifth finger and hypotonia did not differ significantly with respect to parental origin of chromosome 21. However, the proportion of children with CHD, high arched palate and short fingers [94.7% (18/19 cases), 85.7% (24/28 cases) and 71.4% (20/28 cases), respectively] was significantly greater with maternally derived extra chromosome 21 as compared to those of paternal origin [16.7% (1/6 cases), 50% (4/8 cases) and 25% (2/8 cases), respectively] (P=0.0006, 0.053 and 0.03, respectively). Peripheral hypoplasia of the iris was more common when the extra chromosome was paternally derived [37.5% (3/8 cases) versus 3.6% (1/28 cases) of maternal origin, P=0.02].

DISCUSSION

Despite insights into the molecular mechanisms of DS, there is no convincing explanation for the phenotypic heterogeneity. Given the role of genomic imprinting in disorders such as Prader-Willi and Angelman syndromes, it seemed an intriguing hypothesis for the heterogeneity in DS. Imprinted genes have been identified on mouse chromosome 16 which is the human homologue of chromosome 21. Henderson, *et al*.(5) have postulated the presence of developmentally vital imprinted genes on chromosome 21. No imprinted genes were identified on chromosome 21 previously and the role of imprinting in DS was rejected(6,7). Recently, Luedi, *et al*.(8) predicted the SIM2 gene as a candidate imprinted gene localizing within the DSCR with expression of the paternal allele(8). Overexpression of this gene is implicated in the pathogenesis of mental retardation in DS. This development prompts re-examination of the role of imprinting in the pathogenesis of a subset of features in DS.

CHD is the commonest malformation in DS, though it seldom occurs in mosaic DS(2,9). Previous studies examining the relationship between parental origin of chromosome 21 and heart defects, failed to show an association, at least with atrioventricular septal defects (AVSD)(10). This contrasts with the present study where a significantly higher proportion of cases with a maternally derived extra chromosome 21 had CHD, suggesting that one or more imprinted genes on chromosome 21 may influence the likelihood of developing CHD in DS.

It is now evident that trisomy of genes in the heart defects critical region are required for development of cardiac defects. This region contains 64 known or predicted genes. Some of these genes are expressed in the fetal heart(11,12). It is possible that one or more genes in the heart defects critical region could be imprinted and that two copies of the maternally derived genes could tilt the balance in favor of development of CHD. This study also shows that despite the heterogeneity, the craniofacial appearance and neurological abnormalities are almost universal in DS and could be the direct consequence of non-specific mechanisms of the trisomic state(7).

WHAT THIS STUDY ADDS?

- Heart defects are more common in Down syndrome individuals with a maternally derived extra chromosome 21.

Lack of access to highly accurate technology using polymorphic DNA markers to establish parental origin with a success rate of 96% was a relative disadvantage of the present study. QFQ banding and silver NOR staining to study heteromorphisms is subjective and identifies only 54% of cases(13,14). Q-FISH used in the present study yields an efficiency of 80% to determine chromosome 21 non-dysjunction and demonstrated a detection rate of 90% in the present study(15).

The number of patients was also less to infer a definite role for parent of origin in pathogenesis of CHD in DS. It is also possible that the more frequent origin of the extra chromosome 21 from the mother could be a confounding factor explaining the observations in the present study(7). Nevertheless the study provides a basis to examine this phenomenon further.

ACKNOWLEDGMENT

Mrs Seema Korgaonkar, Research Associate, Cytogenetics Division, Institute of Immuno-hematology, Mumbai, for cytogenetic preparation and analysis. Dr ME Yeolekar, Dean, Seth GS Medical College and KEM Hospital, Mumbai for granting permission to publish the paper.

Contributors: MM designed and supervised the study, interpreted the data and revised the manuscript and will act as guarantor for the paper. TC collected and analyzed the data and prepared the draft of the manuscript. VB provided inputs for the methodology and interpreted the tests.

Funding: None.

Competing interests: None stated.

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