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No Evidence for Genomic Imprinting in Liver-Born Down Syndrome Patients

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Abstract. Despite numerous studies, the clinical heterogeneity of Down syndrome has no explanation. We have attempted to investigate the role of genomic imprinting in the phenotype of liveborn Down syndrome patients. Hundred fifty eight patients were investigated for parental origin of the extra chromosome 21 with standard cytogenetic analyses and with DNA plymorphic markers. The extra chromosome 21 was of paternal origin in 8 cases and of maternal origin in 150 cases.

The phenotype of Down svndrome patients in whom the nondisjunction was of maternal origin, was not different from the phenotype of Down syndrome patients in whom the nondisjunction was of paternal origin.

We conclude that imprinting may probably not play a role in the heterogeneity of Down syndrome phenotype.

Key words: Genomic imprinting, Down syndrome, Chromosomal nondisjunction

Research in mice and humans has demonstrated that identical genes may be marked differently during maternal versus paternal germ development. This phenomenon, termed genomic imprinting, has important implications for childhood cancers, numerous disorders associated with mental retardation and chromosomal disease [1].

Despite numerous studies, the clinical heterogeneity of Down syndrome DS has no explanation. Genomic imprinting may play a role in this heterogeneity. To test this hypothesis, we compared the clinical features of live-born DS patients who received an extra chromosome 21 from their father to-those who received an extra chromosome 21 from their mother.

PATIENTS AND METHODS

The subjects for this study were consecutive births of known outcome recorded in our registry of congenital malformations [2]. Patients were seen in our specialist clinic in the Medical Genetics Department in Strasbourg.

A control child was selected for each child with DS. The control subject was a normal child of the same sex born immediately after the case in the same maternity hospital and matched for maternal age with the case.

Chromosomes were studied for every case. The parental origin of the extra chromosome 21 was determined by traditional cytogenetic methods using QFQ banding and DNA polymorphisms as markers.

Cytogenetic analysis

Cytogenetic analyses to determine the origin of nondisjunction were performed in the families of children with DS. Chromosomes were prepared from peripheral blood cultures of patients with DS and from their parents using standard techniques.

Polymorphic DNA markers

High-molecular-weight DNA was isolated from peripheral blood leukocytes. A number of DNA polymorphisms mapping on the long arm of chromosome 21 were scored after PCR amplification.

The following DNA polymorphic markers were used: D21S215, D21S167 and PKFL (phosphofructokinase gene).

The parental origin of the supernumerary chromosome 21, and therefore the parental origin of nondisjunction, was determined after scoring the polymorphic alleles in the parents, the proband, and siblings if available. No attempt was made to establish the meiotic stage of the nondisjunction (first versus second meiotic division), since none of the DNA polymorphisms used mark the centromere of chromosome 21.

The study group consisted of 158 DS patients (89 males and 69 females between 6 months and 21 years of age), all white.

Phenotype of the DS patients

The following parameters were recorded for each child with DS: age, sex, body height (cm), body weight (kg), head circumference (OFC, cm), age at evaluation, microcephaly, brachycephaly, flat occiput, hypotonia, lax ligaments, poor suck at birth, delayed milestones, short stature, failure to thrive, dementia, flat facies, up-slanted palpebral fissures, short palpebral fissures, epicanthic folds, increased intercanthal width, brushfield spots, flat nasal bridge, abnormal dentition, macroglossia, high palate, open mouth, cupped or low-set ears, small ears, short neck, alopecia, broad hands, brachydactyly, fifth-finger clinodactyly, wide space between first and second toes, third interdigital loops, hypothenar ulnar loop, bilateral distal axial triradius (t"), single transverse palmar crease, hallucal tibial arch, palmar interdigital II pattern, finger combinations, heart anomaly, gut anomaly, ophthalmic problems, hearing loss.

Statistics

According to Farkas et al. [3], measurements within 1 SD of the mean are regarded as optimal. Measurements 2 SD below the mean are considered the smallest normal value

("normal-small" or "borderline-small"), and those 2 SD above the mean, the largest normal measurement ("normal-large" or "borderline-large"). Subnormal describes measurements smaller than the mean – 2 SD, and supernormal those larger than the mean + 2 SD. Data were compared by applying the test with Yates' correction and Student's t test.

RESULTS

Only the families in which cytogenetic and molecular studies were concordant were taken into consideration. The origin of the extra chromosome 21 could be determined in 158 patients with DS: it was paternal in only 8 cases. There was no case of translocation in our material. The results of the study of the clinical phenotype of DS patients in whom the nondisjunction was of maternal origin (DS mat) compared to those in whom the nondisjunction was of paternal origin (DS pat) are presented in Tables 1-3. There was no

Table 1 - Age and sex of children with DS

	DS mat (n = 150)	DS pat (n = 8k)
Age, years	0.5-14	0.5-13
Sex		
Male	84	5
Female	65	3

Table 2 - Measurement of children with DS

	Subnormal			Mean			Supernormal		
	Severe	Moderate	Mild	-2 SD	±1 SD	+2 SD	Mild	Moderate	Severe
Head circumference									
DS mat	1	58	42	37	12				
DS pat		4	2	2					
Height									
DS mat	1	5	74	39	29	2			
DS pat			5	2	1				
Weight									
DS mat			3	21	32	48	24	12	10
DS pat					3	3	1	1	

Values are numbers of patients.

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Table 3 - Phenotypes of children with DS

	DS mat (n = 150)	DS pat (n = 8)
Age at evaluation, years	0.5-21	0.5-18
Microcephaly	101	6
Brachycephaly	134	7
Flat occiput	138	8
Hypotonia	129	7
Lax ligaments	128	6
Poor suck at birth	129	6
Delayed milestones	150	8
Short stature	119	7
Failure to thrive	34	2
Dementia	2	0
Flat facies	129	7
Up-slanted palpebral fissures	116	6
Short palpebral fissures	140	8
Epicanthic fold	141	8
Increased intercanthal widht	124	7
Brushfield spots	130	7
Flat nasal bridge	142	8
Dentition abnormal	139	8
Macroglossia	144	8
High palate	1	0
Open mouth	126	7
Cupped or low-set ears	143	8
Small ears	143	8
Short neck	146	8
Heart anomaly	69	4
Gut anomaly	8	0
Alopecia	16	1
Broad hands	92	5
Brachydactyly	128	7
Fifth-finger clinodactyly	92	5
Wide space between first and second toes	114	6
Hypothenar ulnar loop		
Bilateral	58	3
Unilateral	27	2
Bilateral distal axial triradius (t")	108	6

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Table 3 - continued

	DS mat (n = 150)	DS pat (n = 8)
Single transverse palmar crease		
Left	46	4
Right	44	4
Hallucal tibial arch		
Unilateral	21	2
Bilateral	68	5
Palmar interdigital II pattern		
Left	13	1
Right	23	2
Finger combinations		
All ulnar loops	44	3
Radial loop IV or V	23	2
Ophthalmic problems		
Refractive error (myopia)	116	6
Strabismus	78	4
Cataract	5	0
Hearing loss	112	6
Conductive tube placement	51	3

Values (except for age) are numbers.

statistically significant difference between the two groups of DS children for the parameters studied. Therefore, our data support the conclusion that genomic imprinting probably does not occur in live-birth DS patients.

DISCUSSION

Genomic imprinting appears to be a form of regulation [4]. It has been shown in the mouse that chromosome segments appear to have a major differential effect on growth, behavior, and survival [5-7]. In the mouse, when uniparental disomies are produced by translocation, it is not clear whether the major phenotypic effect is a result of, the duplication or the deficiency. In humans, uniparental disomy has now been documented in several conditions. It is to be expected that relevant examples will be found in conditions other than Angelman syndrome and Wiedemann-Beckwith syndrome [8].

Despite numerous studies, the origin of the clinical heterogeneity of DS is still unknown. Genetic imprinting may play a role in the expression of DS. If this is true, then

the sex of the transmitting parent should predict the expression of the associated symptoms. The purpose of the present study was to investigate this possibility.

Our study-demonstrated that trisomy 21 is not another example of genomic imprinting. This conclusion is supported by the study of Blouin et al. [9] who described a healthy male with 45, dup(21q) who was ascertained through his trisomy 21 offspring. No phenotypic abnormalities were noted in the physical exam. For the nine informative chromosome 21 markers studied, there was no maternal allele contribution to the genotype of the proband: in addition, there was always reduction to homozygosity of a paternal allele which indicated that there was paternal uniparental isodisomy for chromosome 21. Uniparental disomy can reveal imprinting effects, as in this phenomenon both members of a chromosome pair are inherited from the same parent. Therefore, the authors concluded that paternal uniparental isodisomy for chromosome 21 is not associated with abnormal phenotypes and that there are probably no imprinted genes on chromosome 21.

Henderson et al. [10] carried out a systematic search for uniparental disomy in tissues from 23 cases of early embryonic failure, using variable number of tandem repeat analysis. Two cases of maternal uniparental heterodisomy were identified. In one case, maternal uniparental heterodisomy for chromosome 21 was the only chromosomal abnormality found. In the other case, trisomy for chromosomes 7 and 9 was also present. The authors postulated that there may be developmentally important genes on human chromosome 21 which are imprinted such that both parental copies are essential for normal embryogenesis. However, only one of these two cases had trisomy 21 only. Moreover, these two conceptions failed to reach term. Therefore, even if there are imprinted genes on chromosome 21, these genes are essential for embryonic development.

Niikawa and Kajii [11] studied four children with diploid/trisomic mosaic DS using Q and R banding heteromorphisms as markers. Three mosaic subjects started as a trisomic zygote followed by the loss of a chromosome 21 at an early mitotic division. The diploid cell lines in these mosaics consisted of uniparental chromosomes 21, one of maternal origin, the other of paternal origin; one was a girl and the other a boy. Unfortunately, no information on the phenotype of these children was given except that they were DS.

Petersen et al. [12] studied two independent cases of chromosome 21 abnormalities in whom there were abnormal karyotypes at birth but blood cells with normal karyotype predominated later in life, and the cells with abnormalities disappeared. Uniparental disomy was observed in the normal cells in these individuals. These two patients were reported at birth to have mosaic karyotypes with respect to chromosome 21. The two chromosomes in the blood cells were identical and derived from the same parent, resulting in a compensatory isodisomy. Neither of the two patients showed any alleviation of the physical or mental disabilities associated with their original chromosomal abnormality.

Imprinting of areas of the mouse chromosome corresponding to human chromosome 21 have not been described [5, 7].

In our study, the extra chromosome 21 was of paternal origin in only 8 out of 158 DS cases. This proportion is in agreement with the study of Antonarakis et al. [13] regarding the origin of the extra chromosome 21 in DS children. Because in our study only 8 trisomy 21 children inherited the extra chromosome 21 from their father, more studies are needed to definitively answer the question: is there genomic imprinting in live-born DS patients?

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REFERENCES

1. Wilson GN, Hall JG, de la Cruz F: Genomic imprinting: Summary of an NICHD conference. Am J Med Genet 1993; 46: 675-680.
2. Stoll C, Roth MP: The northeastern France birth defects monitoring system; in: Marois P (ed) Prevention of Physical and Mental Congenital Defects New York, Liss 1985; part. B. pp. 157-162.
3. Farkas LG, Posnick JC, Hrczko T: Anthropometry of the head and face in 95 Down syndrome patients; in Epstern (es): Morphogenesis of Down Syndrome. New York Wiley-Liss, 1991, pp. 53-97.
4. Hall JG: Genomic imprinting: Review and relevance to human diseases. Am J Hum Genet 1990; 46: 857-873.
5. Cattanach BM: Chromosome imprinting in the mouse. Mouse Newslett 1988; 82: 93.
6. Cattanach BM: Imprinting of distal chromosomes 2 and lack of imprinting with distal chromosome 8. Mouse Newslett 1989; 83: 161-162.
7. Kirk KM, Searle AG: Phenotypic consequences of chromosome imbalance in the mouse; in Daniel A (ed): The Cytogenetics of Mammalian Autosomal Rearrangements. New York, Liss, 1988, pp. 739-768.
8. Engel E: Uniparental disomy revisited: The first twelve years. Am J Hum Genet 1993; 46: 670-674.
9. Blouin JL, Avramopoulos D, Pangalos C, Antonarakis SE: Normal phenotype with paternal uniparental isodisomy for chromosome 21. Am J Hum Genet 1993; 53: 1074-1078.
10. Henderson DJ, Sherman LS, Loughna SC, Bennet PR, Moore GE: Early embryonic failure associated with uniparental disomy for human chromosome 21. Hum Mol Genet 1994; 3: 1371-1376.
11. Niikawa Kajii T: The origin of mosaic Down syndrome: Four cases with chromosome markers. Am J Hum Genet 1984; 36: 123-130.
12. Petersen MB, Bartsch O, Adelsberger PA, Mikkelsen M, Scwinger E, Antonarakis SE: Uniparental isodisomy due to duplication of chromosome 21 occuring in somatic cells monosomic for chromosome 21. Genomics 1992; 13: 269-274.
13. Antonarakis SE and the Down Syndrone Collaborative Group: Parental origin of extra 21. N Engl J Med 1991; 324: 872-876.

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