Chromosome studies in a neonatal population

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Summary: The results of chromosome studies on 6809 consecutive newborn infants are presented. One hundred and one (1.48%) were heterozygous for a marker chromosome, the significance of which is not at present clear. Twenty-two infants (0.32%) had a major chromosome abnormality. Only six of these infants (0.09%) had a clinically recognizable abnormal phenotype (Down's syndrome). The occult chromosome abnormalities included five sex chromosome abnormalities (one 47,XYY; two 47,XXY; two 47,XXX) and 11 balanced translocations. Seven of these were t(DqDq) and four were reciprocal translocations. The results of the present survey are combined with four other similar neonatal surveys in which a total of 23,328 newborns have been screened. Of these, 117 (0.5%; range 0.65-0.32%) had major chromosome abnormalities. The majority of these (72.7%) would not have been detected at birth without chromosome studies, an important fact in the context of prenatal diagnosis of chromosome disease and the early ascertainment of high-risk families.

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The frequency and effect of chromosome abnormalities in the general population are not yet well understood. The present study was designed to identify in a prospective fashion and subsequently to follow up all abnormalities and marker chromosomes identified in a consecutive neonatal population. We present here preliminary data on the chromosome findings in a Canadian population which is primarily of Anglo-Saxon or Scottish origin with considerable German, Ukrainian, and North American Indian components.

Methods

The survey covered 6829 consecutive liveborn infants delivered between February 1, 1970 and September 30, 1971 in the Women's Pavilion, Winnipeg General Hospital. This is the largest maternity unit in Winnipeg where annually between 3500 and 4000 deliveries take place. In Winnipeg, over 99% of all mothers are delivered in hospital and ascertainment is therefore virtually complete.

Cord blood (0.5 to 5 ml.) was collected at each delivery by "milking" the cord into a sterile, heparinized, wide-mouthed container. The blood samples were stored at 4°C. for up to 24 hours (48 hours at weekends) and collected each morning with the exception of Sundays. Standard 48-hour lymphocyte cultures were set up (72-hour cultures were established on Fridays).

Repeat cultures, using capillary blood samples collected on the third or fourth day post partum, were carried out on all children with a chromosome abnormality or marker chromosome, and at this time a blood sample was also collected from the mother and cultured. Capillary blood cultures were also established in the event of failure of the initial cord blood culture. Slides were made by standard air drying procedures. In three instances of neonatal death, no blood sample was obtained at birth and a postmortem blood or skin sample was cultured and analyzed.

The chromosomes of two cells from each infant were counted and fully analyzed by direct microscopy immediately after harvesting; if both cells had the same analysis, and this was either 46,XX or 46,XY, then the chromosome complement was considered normal. If both were abnormal, then a further three cells were analyzed. If all five cells had the same analysis, then this was considered the chromosome com-

plement. A further five cells were scored if any variation occurred in the initial five cells analyzed, and a decision was made on the basis of these 10 cells. All abnormalities or marker chromosomes were checked by a second observer who was not aware of the postulated abnormality. In the event of a disagreement, a third observer was consulted and his decision was considered final.

Table I Chromosome studies on newborn infants

Total livebirths in period	6829
males	3476
females	3353
Sex ratio (M/F)	1.04
Failed cultures	20 (8 ♂♂, 12 ♀♀)
Chromosomes studied	6809
Normal chromosomes	6686
Major chromosome abnormalities	22 (0.32%)
Marker chromosomes	101 (1.48%)

For the purposes of the present study a marker chromosome is defined as a chromosome which can be readily recognized from its homologous partner by two individual observers. A marker chromosome must be visible in at least 80% of the cells examined from a given individual to be considered significant, and must be sufficiently distinctive to be of use in family studies.

For each infant with a chromosome abnormality, two controls were selected based on sex, a normal chromosome complement, and time of birth. In a few cases, birth weight was also taken into consideration. The mothers of test and control infants were interviewed while still in hospital using a standardized recording form, and the control and test infants were examined clinically. Family studies are now in progress on all control infants and infants in whom a chromosome abnormality or marker chromosome was detected. These will be reported later.

Results

A satisfactory chromosome analysis was obtained on all but 20 (0.3%) of the 6829 infants studied, and of the remaining 6809 infants, 22 (0.32%) had a major chromo-

Table II
Details of 22 infants with abnormal chromosomes

No.	Karyotype	Sex chromatin	Gestation weeks	Birth weight g.	Parental age Mother	Parental age Father	Parental karyotype Mother	Parental karyotype Father	Comments
152	47, XXX	++	40	3962	44	46	. .	. .	Normal female
1151	47,XXX	++	42	3147	20	20	46, XX	. .	Normal female
3613	47,XYY	. .	42	3509	19	26	46, XX	. .	Normal male
5420	47,XXY	+	38	3005	29	33	46, XX	. .	Normal male
6196	47, XXY	+	42	3487	18	21	46, XX	. .	Micrognathia, otherwise normal male
99	46, XX, t(Bp+; Dq−)	. .	40	3000	31	37	t(Bp+; Dq−)	46, XY	Normal female
1170	46, XY, t(Cq−; Dq+)	. .	39	3279	23	25	46, XX	. .	Normal male
1540	46, XY, t(Cq−; Cq+)	. .	39	2948	18	20	46, XX	. .	
5661	46, XX, t(Cq+; Fq−)	. .	40	2637	24	24	46,XX	t(Cq+; Fq−)	Normal female
709	45, XX, D−, D−, t(DqDq)+	. .	40	3629	32	41	46, XX	. .	Normal female
1070	45, XY, D−, D−, t(DqDq)+	. .	40	3657	39	40	46, XX	t(DqDq)	Normal male 1 sib 46, XY 2 sibs t(DqDq)
2016	45, XX, D−, D−, t(DqDq)+	. .	41	3714	27	31	46, XX	t(DqDq)	Normal female
2513	45, XX, D−, D−, t(DqDq)+	. .	38	2580	24	27	t(DqDq)	. .	Normal female
3347	45, XX, D−, D−, t(DqDq)+	. .	40	3345	32	37	t(DqDq)	46, XY	Normal female 1 sib t(DqDq)
5543	45, XX, D−, D−, t(DqDq)+	. .	40	3969	25	26	46, XX	. .	Normal female
5912	45, XX, D−, D−, t(DqDq)+	. .	38	3090	27	30	t(DqDq)	46, XY	Normal female
1919	47, XX, G+	. .	40	3170	28	35	46, XX 16q+	46, XY	Down's syndrome
2498	47, XY, G+	. .	40	1956	20	27	46, XX	46, XY	Down's syndrome
3732	47, XX, G+	. .	36	2240	47	. .	46, XX	. .	Down's syndrome, twin pregnancy, co-twin macerated and stillborn
3292	47, XY, G+	. .	37	2778	36	35	46, XX	. .	Down's syndrome Sib died with congenital malformations, intersexuality, 46, XX karyotype
3514*	47, XY, G+	. .	36	2066	45	44	46, XX	. .	Down's syndrome, duodenal atresia, malrotation of gut
6136	47, XX, G+	. .	39	3902	32	34	46, XX	. .	Down's syndrome

*Neonatal death . . Not done + Chromatin positive ++ Chromatin double positive −Chromatin negative

some abnormality and 101 (1.48%) carried a marker chromosome (Table I). No data can be obtained from this type of study about the frequency of chromosome mosaicism (mixoploidy) in the population.

Major chromosome abnormalities—Some data on the 22 infants with a major chromosome abnormality are summarized in Table II. Five infants had sex chromosome abnormalities. Of these two males were 47,XXY (0.6/1000 ♂ births), and one was 47,XYY (0.3/1000 ♂ births); two female infants were 47,XXX (0.6/1000 live ♀ births). Six babies (three ♂♂, three ♀♀) had G trisomy (1.0/1000 live births) and clinical Down's syndrome. The mother of one G trisomic infant had a marker 16 (16q+). The remaining 11 babies (1.6/1000 births) all carried balanced translocations, seven (1.0/1000) of which were balanced t(DqDq), and four (0.6/1000) were balanced reciprocal translocations which involved other chromosomes. Of the seven with t(DqDq), an identical translocation was carried by three mothers and two fathers, while in two cases the mother had a normal karyotype and the father has not yet been studied. Of four reciprocal translocations, the mother was heterozygous in one family and the father in another while studies in the other two families are not yet complete.

Marker chromosomes—Marker chromosomes or chromosome variants include (Table III): (a) Enlarged short arms or satellites of the D and G chromosomes; these must be at least twice as large as the next largest satellites or short arm of any chromosome in the group concerned. (b) Deleted short arms; absence of the short arm of an acrocentric chromosome. (c) Large or small Y chromosome; this is considered outside the range of normal variation when it is about the length of or larger than the smallest chromosome in the E group or if it is only about half as long as the longest G group chromosome. (d) Other marker chromosomes include: heteromorphism of pair No. 1, pair No. 16, or satellites on chromosome 17.

D group—Eighteen infants were heterozygous for enlarged satellites or short arms of one D group chromosome (Ds+ or Dp+). Three infants were heterozygous for a deletion of the short arm of one D chromosome, while one infant had both a Dp+ and a Dp−. The Dp+ was inherited from the mother, the Dp— from the father.

G group—Twenty-seven infants were heterozygous for enlarged short arms or satellites of one G group chromosome. No G group chromosomes with deleted short arms were observed.

Other autosomal markers—Three infants were heterozygous for an elongated secondary constriction of No. 1 (1h+). In one family, the father, paternal grandfather, and one sib were also heterozygous (46,XY,1h+); the remaining two families have not been studied. Five infants were heterozygous for a 16q+ which was transmitted in one instance from the mother and in one from the father; three families remain to be studied. Three infants had satellited E group chromosomes, all were No. 17 (17ps). In one family the mother and two sibs were also heterozygous for the marker; the two other families have not yet been studied. Two unrelated infants were mosaic for an aberrant No. 2 chromosome. In each case approximately 75% of cells had three chromosomes resembling No. 1 and only one resembling No. 2. This is most simply interpreted as a pericentric inversion of a No. 2. The remaining cells were normal. The chromosome complement was 46,XX/46,XX,2-,?inv(2p+q−)+.

Y chromosome—Thirty-two male infants had a Y chromosome as large as, or larger than a No. 18 and seven males had a Y chromosome less than half the length of the G group autosomes.

Clinical observations

The six infants with G-trisomy all had the classical features of Down's syndrome. No clinical abnormalities were found in any of the remaining 16 infants with major chromosome abnormalities, except micrognathia and horizontal palpebral fissures in one infant who had a 47,XXY karyotype.

In the family of one of the infants with Down's syndrome (3292), the previous child, born one year earlier, died three hours after birth and had multiple congenital anomalies, virilized external genitalia, normal appearing tubes and ovaries and a chromosome complement of 46,XX.

Discussion

The incidence of major chromosome abnormalities in Winnipeg is 3.2/1000 live births which is significantly lower than the pooled frequencies of major chromosome abnormalities observed in four other populations (Table IV)[1-5] ($\chi^2 = 6.07$, $0.025>P>0.01$) and can be accounted for primarily by a deficiency of sex chromosome abnormalities among male babies in the Winnipeg population ($\chi^2 = 8.19$, $0.005>P>0.001$). The frequency of all other types of major chromosome abnormalities is similar in all five populations studied. The frequency of Down's syndrome is lower but not significantly so, than the generally accepted figure of 1.6/1000 births.[6] The frequency of balanced t(DqDq) heterozygotes is similar in all the populations studied and approximates to 1:1000 live births; it is the commonest single type of chromosome rearrangement in the population. Balanced reciprocal translocations have been recorded in two other populations with an almost identical frequency to that found in the population under study.

The significance of the low frequency of non-mixoploid sex chromosome abnormalities among male infants in Winnipeg is not clear. It seems unlikely to be due to a sampling error as, if this were so, it might be expected that differences would occur between other categories of abnormality as well. The lower overall maternal age distribution of this population cannot account for these differences. If this were the explanation, then a lower

Table III Chromosome variants in the population

Variant	Number	Frequency per 1000 births
Yq+*	32	9.2
Gp+ or Gs+	27	4.0
Dp+ or Ds+	18	2.6
Yq−*	7	2.0
16q+	5	0.7
Dp−	3	0.4
Ih+	3	0.4
Dp+, Dp−	1	0.1
17ps	3	0.4
?inv(p+q−)**	2	0.2

*Frequencies related to male births only
**46, XX/46, XX, 2−, ?inv(2p+q−)+

frequency of 47,XXY, 47,XXX and 47,XX or XY,G+ infants would be expected, while the frequency of 47,XYY infants might be expected to be comparable to that found in other neonatal populations. This is not so. Other etiological factors at present under study include paternal age, racial distribution, virus infections and radiation levels to which this population has been exposed. If the reason for this low frequency of male infants with sex chromosome abnormalities can be determined, it may give a clue as to the cause of certain types of chromosome abnormality in more general terms.

Only one other study, that from New Haven,[2] records the frequency of chromosome variants but a comparison between the present study and the New Haven study is meaningless because of the different criteria used. At present the significance of these markers is not clear. They have no direct clinical effect and it is hoped that family studies will determine whether they have any indirect effect on reproductive fitness, morbidity or nondisjunction as has sometimes been suggested.[7] The use of fluorescence microscopy and other special staining methods is at present in progress to identify each chromosome abnormality or variant. When combined with the collection of epidemiological data these methods may provide information as to whether certain variants are more harmful than others. Determination of the significance of inherited chromosome variants is important in its relation to genetic counselling.

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Résumé

Etude chromosomique chez une population de nouveau-nés

Les auteurs donnent les résultats d'une étude chromosomique effectuée sur 6809 nouveau-nés consécutifs. On comptait parmi eux 101 sujets (1.48%) qui étaient hétérozygotes pour un chromosome particulier (marqueur). La signification de cette particularité n'est pas encore claire. Chez 22 nouveau-nés (0.32%), on notait une aberration chromosomique majeure. Six de ces 22 nouveau-nés (0.1%) avaient un phénotype anormal cliniquement identifiable (syndrome de Down). Parmi les anomalies chromosomiques occultes figuraient cinq anomalies des chromosomes sexuels (un était 47,XYY, deux 47,XXY et deux 46,XXX) et 11 étaient porteurs de translocations équilibrées. Sept de ces 11 sujets avaient une translocation t(DqDq) et quatre présentaient des translocations réciproques. Les résultats de la présente étude statistique ont été amalgamés à ceux provenant de quatre études similaires du nouveau-né, au cours desquelles on avait étudié 23,328 sujets. Parmi ceux-ci, 117 presentaient des aberrations chromosomiques majeures (0.5% avec des valeurs extrêmes de 0.65% et de 0.32%). Dans la majorité de ces cas (72.7%), on n'aurait pas pu découvrir ces anomalies dès la naissance sans avoir procédé à l'étude des chromosomes.

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Table IV Summary of neonatal chromosome surveys

	Boston[3]††	Edinburgh[4]†	New Haven[2]††	London Ontario[1]††	Winnipeg†	Total
Trisomics						
G+	1 (0.7)	13 (1.5)	3 (0.7)	—	6 (0.9)	23 (1.0)
D+	—	—	1 (0.2)	1 (0.3)	—	2 (0.1)
E+	—	2 (0.2)	1 (0.2)	—	—	3 (0.1)
Translocations						
Robertsonian	2 (1.4)	9 (1.0)	3 (0.7)	2 (1.0)	7 (1.0)	23 (1.3)
Reciprocal	—	6 (0.7)	3 (0.7)	—	4 (0.6)	13 (0.6)
Other structural rearrangements	1 (0.7)	4 (0.7)	—	—	—	5 (0.2)
Sex chromosomes*						
47, XYY	3 (2.2)	11 (1.7)	3 (1.4)	4 (3.8)	1 (0.3)	22 (1.6)
47, XXY	2 (1.4)	7 (1.1)	4 (1.8)	1 (0.9)	2 (0.6)	16 (1.1)
47, XXX	—	3 (1.3)	3 (1.4)	—	2 (0.6)	8 (0.9)
45, X	—	—	1 (0.5)	—	—	1 (0.1)
46, XX (Male)**	—	1 (0.4)	—	—	—	1 (0.1)
Total	9 (6.5)	56 (6.4)	22 (5.1)	8 (3.8)	22 (3.2)	117 (5.0)
Total births	1384	8701	4353	2081	6809	23,328
Male	1384	6336	2176	1066	3468	14,430
Female	—	2365	2177	1015	3341	8898

( ) = Frequency per 1000 Births
* = Related to male and female births respectively
** = Related to female births
† = Data to September 30th, 1971
†† = Data to November, 1970