Sex Chromatin and Chromosome Abnormalities among 10,412 Liveborn Babies

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Pantelakis, S. N., Chrysosotomidou, Olia-Maria, Alexiou, D., Valaes, T., and Doxiadis, S. A. (1970). *Archives of Disease in Childhood*, 45, 87. **Sex chromatin and chromosome abnormalities among 10,412 liveborn babies.** Among 10,412 liveborn infants surveyed in a large maternity hospital in a 2-year period, there were 13 cases (0.13%) of discrepancy between sex phenotype and sex chromatin (0.15% among male infants and 0.10% among female infants). There were 22 cases of trisomy-21 (incidence 0.21%), 3 cases of trisomy-18, and 1 case of trisomy-13-15. 9 cases presented multiple congenital abnormalities but the karyotype was normal.

Only the incidence of cases with Down's syndrome is higher than that reported in the literature. Maternal and paternal age, seasonal clustering, infectious diseases before or at the beginning of pregnancy, and x-ray exposure of parents, showed no correlation with Down's syndrome. Among the cases of Down's syndrome there was a significantly higher maternal and paternal mean age and a maternal history of infectious hepatitis was more frequent.

Since Moore and Barr (1955) described the method of determining the sex chromatin by examining the nuclei of the cells of the oral mucosa, it has been possible to screen large populations for sex chromosome abnormalities. During the past few years several authors have undertaken such sex chromatin surveys on newborn populations in order to establish the frequency of discrepancies between sex genotype and sex phenotype.

A range of discrepancy, perhaps related to differences of the ethnic groups studied, has been found from 0% in India (Naik and Shah, 1962) to 0.23% in Scotland (Maclean *et al.*, 1964) and Mexico (Márquez-Monter, Carnevale-López, and Kofman-Alfaro, 1968). Some authors (Robinson and Puck, 1965) have suggested that sex chromosome abnormalities due to non-disjunction are perhaps related to external factors. More studies are needed in order: (1) to elucidate the problem of the over-all frequency, (2) to look for any differences in the incidence between the two sexes, (3) to investigate the relation of the frequency in the ethnic group studied to external factors such as

epidemics of viral infections, use of drugs, or exposure to x-rays.

The present study concerns a neonatal survey of all liveborn babies born at the State and University Maternity Hospital 'Alexandra' during a two-year period (1.10.66–30.9.68). The current practice in Greece, and particularly in the area of Athens, is for unselected hospital delivery, and this is the case for over 90% of the deliveries.

Material and Methods

During the period of study each pregnant woman admitted for delivery was included in the survey. Details about her social class, age, previous medical history, and medical history of her husband were collected, on a questionnaire of about 80 coded questions. For each newborn child sex chromatin determination was performed blindly on the amnion membranes according to the method of Klinger (1957) as modified by J. H. Edwards (personal communication, 1964). The results were afterwards compared with the sex phenotype. All newborns were clinically examined by one of us. In cases of discrepancy or low Barr bodies count, buccal smears were examined for confirmation of the findings by the method of Moore and Barr (1955) with minor modifications. For all newborn infants

who either presented a discrepancy between sex chromatin and sex phenotype, or were found to have multiple congenital abnormalities or anomalies suggesting one of the known trisomic syndromes, a full clinical examination was done and karyotyping was performed by white blood cell culture according to the method of Moorhead *et al.* (1960) as modified by de Grouchy, Roubin, and Passage (1964).

Results

The method we have used for the evaluation of sex chromatin from amnion membranes gave satisfactory and reliable results (Pantelakis and Panagopoulos, 1968). Among 500 female newborns the mean percentage of nuclei with presence of Barr bodies was 42.5% (SD 10.4%) (Fig.).

FIG.—Percentage distribution of Barr body positive nuclei in amniotic membrane preparations from 500 female newborns.

Among the male infants scores were never above 2%. All cases with a score below 2 SD from the mean were considered as suspects for mosaicism, and the karyotype was examined.

During the two-year period of the investigation 13,823 women were admitted for delivery. The liveborn babies in whom sex chromatin was examined were 10,412 and they were not selected (Table I). In the material studied the rate of

TABLE I
Analysis of Material

Women delivered during period of survey	13,823
Stillbirths	3.2%
Multiple births	1.2%
Primiparae	38%
Multiparae	62%
Liveborn babies with sex chromatin determination	10,412 (78%)

stillbirths was 3.2% and that of multiple births 1.2%. 38% of the mothers were primiparae and 62% were multiparae. These frequencies were found to be essentially the same when considering either the total number of women admitted or the number of women whose babies had their sex chromatin examined.

The over-all incidence of discrepancy between sex phenotype and sex chromatin as well as the incidence separately in male and female newborns is shown in Table II. As can be seen in the

TABLE II
Incidence of Sex Chromatin Abnormalities Among 10,412 Liveborn Babies

Phenotype	No. of Infants	No. of Abnormal Cases	%
Male	5382	8	0.15
Female	5030	5	0.10
Total	10,412	13	0.13

Table there were 8 cases of discrepancy among the 5382 phenotypical male newborns screened (an incidence of 0.15%) and 5 cases among the 5030 phenotypical females (an incidence of 0.10%).

In Table III are given the details of the chromosomal constitution of the 13 cases which have

TABLE III
Chromosomal Constitution of 13 Cases of Sex Chromatin Discrepancy

Syndromes	No. of Cases	Incidence %
Klinefelter XXY	6	0.11
Mosaics XXY/XY	2	0.04
Turner XO	—	—
Mosaics XO/XX	2	0.04
Superfemale XXX	3	0.06
Total	13	0.13

considered as abnormal on sex chromatin screening because they were found to present: (a) a discrepancy between phenotype and sex chromatin, (b) a Barr body count less than 2 SD from the mean for our method (mean $42.5\% \pm 10.4\%$), (c) two or more chromatin masses. Thus, among the male newborns there were 6 cases of XXY Klinefelter's syndrome and 2 cases of XXY/XY mosaics. Among the female newborns there were 2 cases of XO/XX mosaics and 3 cases of XXX.

In Table IV are presented the social class and

TABLE IV
Social Class and Maternal and Paternal Age of Cases with Sex Chromatin Abnormalities

Social Class	Total No. Examined	Abnormal Cases	Mean Maternal Age (yr.)		Mean Paternal Age (yr.)	
			Normal	Abnormal	Normal	Abnormal
1	396	—	28 8/12	—	36	—
2	1,353	—	28 2/12	—	34	—
3	8,663	13	27 5/12	29 8/12	32 3/12	35 2/12
Total	10,412	13	27 6/12	29 8/12	32 7/12	35 2/12

the maternal and paternal mean ages of the whole material in comparison to those of the 13 cases with sex chromosome abnormalities. For practical reasons it was decided to distinguish the women studied in three main social classes according to education, occupation, and standard of living. As can be seen from this Table the great majority of these women (83%) belong to the lower social class. The mean maternal and paternal age of the abnormal cases was a little higher than that of the total but not significantly so.

In Table V is shown the incidence of phenotypically abnormal babies with autosomal trisomies. There were 22 cases of trisomy-21,

3 cases of trisomy-18, and 1 case of trisomy-13-15. 9 cases with multiple congenital abnormalities were found to have normal karyotypes.

In Table VI are presented the social class and the maternal and paternal mean age of these cases. 18 cases of Down's syndrome were born to women belonging to the 3rd social class, while 4 infants were born to women belonging to the 1st and 2nd social class. The mean maternal and paternal ages of the newborns with Down's syndrome were significantly higher than the maternal and paternal mean ages of the normal newborns.

The analysis of factors such as infectious diseases or x-ray exposure immediately before or at the beginning of pregnancy showed no relation between these factors and the occurrence of sex chromatin discrepancy or Down's syndrome.

A history of maternal infectious hepatitis (as diagnosed at the time of the disease by the attending doctor) before the present pregnancy was more frequent among the mothers of babies with Down's syndrome. As cases with Down's syndrome were born to mothers of an older age and the chances of having had infectious hepatitis were higher, we present in Table VII a more detailed analysis of the incidence of infectious hepatitis in our material in different age-groups. As it is shown in this Table the incidence of infectious hepatitis in the maternal history increases from 3.35% among

TABLE V

Incidence of Autosomal Trisomies and Multiple Congenital Abnormalities among 10,412 Liveborn Newborns

Syndrome	No. of Cases	Incidence (%)
Trisomy-21 ..	22	0.21
Trisomy-18 ..	3	0.03
Trisomy-13-15 ..	1	0.01
Multiple congenital abnormalities with normal karyotype	9	0.09

TABLE VI

Social Class and Maternal and Paternal Age in Cases with Down's Syndrome

Social Class	Total Examined and Percentage	Down's Syndrome	Other Trisomies	Multiple Congenital Abnormalities	Maternal Age (yr.) (mean and range)		Paternal Age (yr.) (mean and range)	
					Normals	Down's	Normals	Down's
1st	396 (3.8%)	2	—	1	28 8/12 (17-44)	29, 31	36 (23-60)	39, 41
2nd	1353 (12.9%)	2	—	—	28 2/12 (16-41)	27, 35	34 (18-65)	32, 42
3rd	8663 (83.3%)	18	4	8	27 5/12 (14-50)	35 (24-50)	32 3/12 (17-68)	38 (28-60)
Total	10,412	22	4	9	27 6/12	34 1/12	32 7/12	38 1/12

TABLE VII
*History of Infectious Hepatitis in Mothers of
Different Age-groups*

Age-group (yr.)	No. of Cases	No. with Positive History of Infectious Hepatitis	Incidence (%)
-24	3455	116	3.35
25-29	3075	122	3.96
30-34	2500	146	5.84
35 and over	1248	77	6.17
Total	10,278	461	4.48

women up to 24 years old to 6.17% among women aged 35 years and more.

In Table VIII the incidence of a history of infectious hepatitis among mothers over 30 years with normal babies is compared with that of mothers who gave birth to infants with Down's syndrome. As it appears from this Table a history of maternal infectious hepatitis was commoner in the group with Down's syndrome. The numbers of the cases with this condition are small for definite conclusions; statistical analysis, however, showed the difference between the incidence in the two groups to be significant ($\chi^2 = 6.45$ and $p < 0.025$).

TABLE VIII
*Incidence of Infectious Hepatitis in Maternal History
of Newborns with Down's Syndrome and of Normal
Newborns Born to Mothers Older than 30 years*

	History of Maternal Infectious Hepatitis		
	Absent	Present	Total
Normal newborns ..	3748	223 (5.9%)	3.971
Newborns with Down's syndrome ..	18	4 (18%)	22
Total	3766	227	3.993

$\chi^2 = 6.45$; $p < 0.025$.

Discussion

In surveys where nuclear sexing is used as screening method for the detection of sex chromosome anomalies those affecting the Y chromosome will not be detected. Thus a case of XYY will not present a discrepancy between sex chromatin and sex phenotype. The frequency of this abnormality is assumed to be 1:1000 in the liveborn male population (Court Brown, 1968).

The material usually examined for sex chromatin determination is buccal mucosa. This examina-

tion, however, can be performed in all other tissues. During the first 2 days of life it is known that the percentage of nuclei with presence of Barr bodies in buccal smears in female newborns can be below 20% (Smith *et al.*, 1962; Taylor, 1963; Frasier, Crudo, and Farrell, 1964). On the other hand, the collection of buccal smears from all newborns during the first 48 hours after birth presents technical difficulties.

We believe that the advantages of studying the sex chromatin on amnion membranes in the neonatal period can be summarized as follows: (a) the collection of placenta in the delivery room is simple, does not require specialized personnel, and does not disturb the neonate; (b) the examination can be performed in stillbirths or neonates dying a few hours after delivery; (c) the score of nuclei with Barr bodies present among the female newborns is high enough to make readings easy and to indicate suspects for mosaicism; (d) it is easy to examine as many specimens from the amnion membranes as are needed to obtain a satisfactory preparation.

The frequency of sex chromatin discrepancies in the newborn population in different countries reported in the literature is presented in Table IX.

TABLE IX
*Incidence of Sex Chromatin Anomalies in Newborns
as Reported in Various Parts of World and in Present
Study*

Authors	Country	Total No. of Subjects	Abnormalities (%)
Naik and Shah (1962) ..	India	3890	0
Wiesli (1962) ..	Switzerland	3029	0.03
Moore (1959) ..	Canada	3715	0.13
Bergemann (1961)	Switzerland	3728	0.13
Maclean, Harnden, and Court Brown (1961)	Scotland	6000	0.23
Maclean <i>et al.</i> (1964) ..	Scotland	14,725	0.16
Robinson and Puck (1967)	United States	6900	0.17
Taylor and Moore (1967)	England	9688	0.20
Márquez-Monter <i>et al.</i> (1968)	Mexico	3000	0.23
Present study ..	Greece	10,412	0.13

With the exception of the studies in India (Naik and Shah, 1962) and in Switzerland (Wiesli, 1962) where very low figures have been reported, the findings of the other investigators vary between 0.13% and 0.23%.

In fact, if one takes into consideration only the

surveys with more than 6000 cases, the variation is even smaller. The similarity of the findings shows that probably there are no important variations related to ethnic or geographic differences. The findings in India by Naik and Shah (1962) require confirmation.

During the two years of our investigation we found 13 cases of sex chromosome abnormalities. All of them were clinically normal and they would never have been detected at this early age if sex chromatin examination was not performed. Among the 13 there was not a single case of Turner's syndrome. The low incidence of Turner's syndrome among liveborn babies agrees with the published reports (Moore, 1959; Maclean *et al.*, 1964), and is in contrast with the other anomalies of the X chromosome in females, such as mosaics XO/XX and XXX females. This fact may mean that the embryo with XO constitution is more vulnerable and more apt to end in an abortion. Studies on material of abortions and stillbirths have indeed shown a much higher incidence of XO aberrations (Carr, 1965; Maclean *et al.*, 1964; Szulman, 1965).

Kohn, Winter, and Mellman (1968) reviewing the literature on trisomy-X mention that only 12 newborn infants with an XXX constitution have been reported. This can perhaps be explained by the absence of characteristic phenotype in the newborn period. Diagnosis of isolated trisomy-X in the newborn period can only be made during neonatal surveys with routine sex chromatin examination. The present study adds three more asymptomatic cases; it will be extremely interesting to watch the future development of these children as well as those with Klinefelter's syndrome (XXY) and with mosaicism.

Robinson and Puck (1967) tentatively concluded that the incidence of sex chromosome abnormalities was related to the socio-economic group studied and moreover presented a seasonal clustering. They did not find the same factors to play any role in the occurrence of Down's syndrome. Frøland (1967), analysing the dates of birth of 80 patients with sex chromosome abnormalities, found a distribution not significantly different from that expected. The 13 cases with sex chromosome anomalies of the present study did not show any clear-cut seasonal clustering.

The present study does not help to elucidate the relation between socio-economic condition and incidence of sex chromosome abnormalities. 83% of the material studied belonged to the lower social class, and no comparison with a higher social class was therefore possible.

The analysis of other factors such as maternal and paternal age, infectious diseases immediately before or during pregnancy, and x-ray exposure did not show any correlation with the occurrence of sex chromosome abnormalities.

Among the 10,412 newborns studied there were 3 cases with trisomy-18 (0.03%) and 1 case with trisomy-13 (0.01%). Taylor (1968) reviewing the literature concerning a total of about 115,000 newborns reported an incidence of 0.02% for trisomy-18 and of 0.01% for trisomy-13. Our findings cannot be considered to differ significantly.

In the cases with Down's syndrome the most important single factor different from the normals was the advanced maternal age, a fact well established by all authors who studied this condition.

Pleydell in 1957 reported data suggesting a clustering of cases of Down's syndrome in space and time. Similar findings were reported later by Stoller and Collmann (1966). As Leck (1966) points out none of the above-mentioned reports is entirely conclusive: the data did not prove with certainty that the clustering in space and time exceeded that which might be expected by chance.

In the present investigation the births of babies with Down's syndrome were evenly distributed in the two-year period of the survey, as was also found by Leck (1966) and Robinson and Puck (1967).

The incidence of Down's syndrome in the present survey is 0.21%, higher than the incidence of 0.15% reported by Penrose (1963), of 0.16% by Leck (1966), of 0.11% by Robinson and Puck (1967), and of others. In our attempt to explain this higher incidence we have analysed different factors in the maternal and paternal health history, such as important infectious diseases before or at the beginning of pregnancy, x-ray exposure etc. From this analysis the only difference that we have been able to detect in the parental history of the cases with mongolism was a higher incidence of infectious hepatitis in the history of the mothers, 3 times higher than in the normal female population of a comparable age (aged 30 years and more). Though the number of cases with Down's syndrome was small, the difference was statistically significant.

Stoller and Collmann (1966) found an apparent relation between infectious hepatitis and Down's syndrome, but others (Stark and Mantel, 1966; Leck, 1966; and Ceccarelli and Torbidoni, 1967) have not confirmed these findings.

Unlike these other studies we have not tried to relate infectious hepatitis at the beginning of pregnancy with the birth 9 months later of a child with Down's syndrome, but we found that mothers

who have had clinical infectious hepatitis before their pregnancy have a 3 times higher risk of having a child with Down's syndrome than mothers of a comparable age without a history of infectious hepatitis.

We believe that the problem of a possible relation between viral infections and birth of children with chromosomal abnormalities such as trisomy-21 cannot yet be considered as solved, and more extensive studies are needed.

If, however, a positive relation of infectious hepatitis and birth of cases with Down's syndrome does exist, as our study tends to show, then the higher incidence of Down's syndrome of the present study could perhaps be due to differences in the incidence of infectious hepatitis between the ethnic groups studied.

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