*Am J Hum Genet* 32:477–483, 1980

# Evidence for Genetic Control of Nondisjunction in Man

OMAR S. ALFI,[1] ROHNU CHANG,[2] AND STANLEY P. AZEN[2]



SUMMARY

Data on factors associated with the occurrence of Down syndrome in a highly inbred population were evaluated to investigate the presence of a genetic control of nondisjunction in man. In Kuwait, close consanguinity occurs in 40% of marriages. In its main obstetric hospital, 20 trisomic Down babies out of 11,614 singleton births were delivered over a 12-month period. Chi-square analyses indicate the occurrence of Down syndrome to be linked to two independent factors: consanguinity of parents and maternal age. The relative risk is approximately four times greater for closely related than for nonrelated parents ($P < .005$); a possible explanation for this is the existence of a gene that induces mitotic nondisjunction in the homozygous fertilized ovum. An alternative explanation is the existence of an autosomal recessive gene which results in meiotic nondisjunction in the homozygous parents. Consanguinity is usually perpetuated in certain families, or sections of the population, and parents in highly inbred families have a higher probability to be homozygotes for that gene.

## INTRODUCTION

Since the identification of nondisjunction by Bridges in 1916 [1], various factors have been postulated or reported to influence that phenomenon. Effects of environmental factors, notably of viruses, radiation, and chemicals, have been demonstrated [2–3], and genetic factors influencing nondisjunction have been reported in *Drosophila* [4]

Received September 28, 1979; revised December 3, 1979.

This study was supported in part by project no. MCT-000422 from the Department of Health, Education, and Welfare, and by the Michael J. Connell Foundation.

[1] Division of Medical Genetics, Children's Hospital of Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90027.

[2] Department of Community and Family Medicine, University of Southern California School of Medicine, Los Angeles, CA 90033.

and in *maize* [5]. A genetic role in man has also been suspected by some workers [6–9], and disputed by others [10–11].

To investigate the existence of a genetic control of nondisjunction in man, we evaluated data on the occurrence of Down syndrome in a highly inbred population. Kuwait has one of the world's highest rates of consanguineous marriages [12–13]: close consanguinity (first cousins, first cousins once removed, and second cousins) occurs in approximately 50% of marriages among Kuwaitis (about 47% of the population), and in 40% of marriages among non-Kuwaiti residents [12].

Between January and December, 1970, a prospective study of all single births in the only obstetric hospital in the city of Kuwait was carried out. Data were collected on the frequency of major identifiable malformations and on associated maternal factors. This paper reports the frequency of Down syndrome in this population and evaluates the association of parental consanguinity and other factors with this disorder.

## MATERIALS AND METHODS

The study population consisted of 11,614 single births delivered in the Kuwait Obstetric Hospital during the period of January through December, 1970. All newborns were examined by one of three pediatricians within the first three days of life. Data were obtained on sex of infant, age and nationality of mother, parental consanguinity, number of pregnancies (gravidity), previous abortions, previous stillbirths, complications during pregnancy, and month of delivery. Relationships between Down syndrome and maternal factors were evaluated statistically using the Mantel-Haenszel chi-square test, the Mantel-Haenszel summary test [14], and a modification of the Woolf test for homogeneity [15].

## RESULTS

Twenty newborns with confirmed Down syndrome were identified, giving an overall occurrence of 1.7 per 1,000 single births (1:581). All 20 cases were of the trisomic type.

Maternal history factors and sex of infant stratified by outcome for Down syndrome are shown in table 1. Mantel-Haenszel chi-square analysis suggests that the risk for Down syndrome is likely to be linked to three factors: maternal age, consanguinity, and gravidity. When estimated without stratification by other factors, the relative risk for bearing infants with Down syndrome is about 10 times greater for mothers aged $\geq 40$ than those $< 40$ ($\chi^2_1 = 20.12$, $P < .001$); about four times greater for closely related parents ($\chi^2_1 = 9.32$, $P < .005$); and about three times greater for mothers having gravidity $> 3$ than for those having gravidity $\leq 3$ ($\chi^2_1 = 4.12$, $P < .05$). Comparing these factors pairwise shows that consanguinity is not significantly related to maternal age ($\chi^2_1 = 0.63$, not significant), but gravidity is significantly related to maternal age ($\chi^2_1 = 91.89$, $P < .001$) and to consanguinity ($\chi^2_1 = 11.40$, $P < .001$).

Table 2 presents the Mantel-Haenszel analysis of the occurrence of Down syndrome in relation to all combinations of pairs of these factors. Each factor (e.g., maternal age) was evaluated within strata defined by each of the other two factors (e.g., gravidity and consanguinity). This has the potential of evaluating the association of a given factor while removing the "effect" of each of the other factors.

In those strata where there were no cases of Down syndrome, the relative-risk ratio could not be calculated. For those cases, the Mantel-Haenszel chi-square test revealed

TABLE 1

SEX OF INFANT AND MATERNAL HISTORY FACTORS STRATIFIED BY OUTCOME OF DOWN SYNDROME

| | DOWN SYNDROME | | STUDY POPULATION | MANTEL-HAENSZEL CHI-SQUARE TEST |
|---|---|---|---|---|
| | No. | (per 1,000)* | No.† | *P* VALUE |
| Sex of infant: | | | | |
| Male | 12 | (2.0) | 5,981 | NS‡ |
| Female | 8 | (1.4) | 5,612 | |
| Age of mother: | | | | |
| < 40 | 16 | (1.4) | 11,326 | < .001 |
| ≥ 40 | 4 | (14.7) | 268 | |
| Nationality of mother: | | | | |
| Urban Kuwaitis | 5 | (1.6) | 3,079 | |
| Bedouin Kuwaitis§ | 6 | (3.0) | 1,979 | NS |
| Others | 9 | (1.4) | 6,536 | |
| Consanguinity: | | | | |
| Closely related‖ | 14 | (3.5) | 3,989 | < .005 |
| Not closely related | 6 | (0.8) | 7,436 | |
| Gravidity: | | | | |
| ≤ 3 | 5 | (0.9) | 5,821 | < .05 |
| > 3 | 15 | (2.6) | 5,773 | |
| Previous abortions: | | | | |
| No | 17 | (1.8) | 9,408 | NS |
| Yes | 3 | (1.4) | 2,186 | |
| Previous stillbirths: | | | | |
| No | 19 | (1.8) | 10,632 | NS# |
| Yes | 0 | (0.0) | 117 | |
| Complications of pregnancy**: | | | | |
| No | 18 | (1.8) | 10,116 | NS |
| Yes | 2 | (1.3) | 1,478 | |
| Month of delivery: | | | | |
| March–June†† | 10 | (2.6) | 3,762 | NS |
| July–February | 10 | (1.3) | 7,832 | |

* Frequency per 1,000 single births.
† No. = 11,614; totals less than this no. are due to missing data.
‡ NS = not significant.
§ Bedouin: mostly desert nomads.
‖ Closely related consanguinity includes first cousins, first cousins once removed, and second cousins.
# Using Fisher exact test.
** Complications include hydramnios, toxemia, diabetes, antepartum hemorrhage, and placental insufficiency.
†† Infants conceived during hottest period in Kuwait (July–October).

that maternal age was significantly associated with Down syndrome for women with gravidity > 3 ($\chi^2_1 = 12.17$, $P < .001$) and that gravidity was not significantly associated with Down syndrome for women aged < 40 ($\chi^2_1 = 1.84$, not significant).

In those strata where the relative-risk ratios could be calculated for each stratum, the Woolf test for homogeneity showed that these ratios were not significantly different in both strata, and a common-risk ratio was calculated. For these cases, the Mantel-Haenszel summary test showed that maternal age was significantly associated with Down syndrome irrespective of consanguinity ($\chi^2_1 = 19.29$, $P < .001$). Similarly, consanguinity was significantly associated with Down syndrome irrespective of maternal age ($\chi^2_1 = 9.03$, $P < .005$) and gravidity ($\chi^2_1 = 8.81$, $P < .005$). Gravidity, on

TABLE 2

ANALYSIS OF DOWN SYNDROME IN RELATION TO MATERNAL AGE, CONSANGUINITY, AND GRAVIDITY

| Factor | Stratified by | Strata | Relative risk | Risk ratio | Mantel-Haenszel summary test *P* value |
|---|---|---|---|---|---|
| Maternal age ..... | Gravidity | ≤ 3 | No data | ... | ... |
| | | > 3 | 7.6* | | |
| | Consanguinity | Not closely related | 8.7 | 10.1 | < .001 |
| | | Closely related† | 10.6 | | |
| Consanguinity ..... | Maternal age | < 40 | 4.1 | 4.3 | < .005 |
| | | ≥ 40 | 5.0 | | |
| | Gravidity | ≤ 3 | 8.0 | 4.1 | < .005 |
| | | > 3 | 3.5 | | |
| Gravidity ..... | Maternal age | < 40 | 2.3‡ | ... | ... |
| | | ≥ 40 | No data | | |
| | Consanguinity | Not closely related | 5.3 | 2.8 | Not significant |
| | | Closely related | 2.3 | | |

* $P < .001$ using the Mantel-Haenszel $\chi^2$ test.
† Closely related includes first cousins, first cousins once removed, and second cousins.
‡ Not significant using the Mantel-Haenszel $\chi^2$ test.

the other hand, appeared not to be associated with Down syndrome when evaluated in conjunction with consanguinity ($\chi^2_1 = 3.74$, not significant).

Table 3 shows that when compared with other groups of Kuwaitis, the Bedouin Kuwaitis, having the highest occurrence of Down syndrome (3.0 per 1,000), also have the highest proportion of close consanguinity (501.4 + 15.3 = 516.7 per 1,000), and mothers aged ≥ 40 (14.3 + 15.3 = 29.6 per 1,000). The last column of table 3 contains estimated Down rates specific for each combination of maternal age and parental consanguinity groups and shows that mothers aged ≥ 40 and/or closely related in marriage have a higher risk of bearing infants with Down syndrome than do mothers age < 40 and not closely related in marriage. Accordingly, comparison of the expected and observed frequencies of Down syndrome for each nationality shows reasonable agreement ($\chi^2_2 = 0.22$, not significant).

## DISCUSSION

Our results show an overall occurrence of 1:581 for Down syndrome in the study population. There were no significant relationships between the occurrence of Down syndrome and the sex of the newborn, the ethnic background of parents, the number of miscarriages or stillbirths, the presence of specific pregnancy complications, or the month of delivery. Although gravidity was initially shown to be significantly associated with Down syndrome (table 1), stratification by maternal age or parental consanguinity (table 2) shows that gravidity is likely to be associated with one or both of these factors.

Combined clinical and epidemiological research has repeatedly demonstrated the increased risk for Down syndrome with increasing maternal age. Our study also shows that maternal age is significantly associated with Down syndrome (table 1), and stratification by gravidity or consanguinity still reveals this significant relationship (table 2).

TABLE 3

EXPECTED VS. OBSERVED FREQUENCY OF DOWN SYNDROME FOR DIFFERENT NATIONALITIES

| | | FREQUENCY/1,000 | | | |
|---|---|---|---|---|---|
| | | Urban | Bedouin* | Others | Down rate |
| Maternal age: | Consanguinity: | | | | |
| < 40 | Not closely related | 647.4 | 469.0 | 776.6 | .000687 |
| < 40 | Closely related† | 333.0 | 501.4 | 200.0 | .002818 |
| ≥ 40 | Not closely related | 12.9 | 14.3 | 18.6 | .005988 |
| ≥ 40 | Closely related | 6.7 | 15.3 | 4.8 | .030000 |
| Expected frequency of Down syndrome (per 1,000) | | 1.7 | 2.3 | 1.4 | ... |
| Observed frequency of Down syndrome (per 1,000) | | 1.6 | 3.0 | 1.4 | ... |

* Bedouin: mostly desert nomads.

† Closely related includes first cousins, first cousins once removed, and second cousins.

Consanguinity is also shown to be significantly associated with Down syndrome (table 1), and stratification by gravidity or maternal age shows consanguinity still strongly associated with the occurrence of Down syndrome (table 2). Consequently, the occurrence of Down syndrome appears to be directly related to two factors: maternal age and parental consanguinity.

To verify these findings, the expected frequency of Down syndrome for each ethnic group was calculated for combinations of maternal age and consanguinity groups (table 3). There was no significant difference between the observed and the expected frequencies.

These findings suggest several hypotheses. One is the existence of a gene that results in mitotic nondisjunction in the homozygotes. The presence of such a gene was postulated in several studies of families with multiple aneuploidies in various members [6, 7, 9]. When both parents are heterozygous, the homozygous fertilized ovum may undergo mitotic nondisjunction (for chromosome 21), either in the first or in an early subsequent division. With loss of the monosomic 21 cell, the zygote develops into a complete trisomic or mosaic trisomic. In this hypothesis, a Down patient would be considered homozygous for that nondisjunction gene. Dahlberg's equation [17] was applied to test that hypothesis. In that equation: $k = c\,(1 + 15q)/[16\,(q + F)]$, where $k$ is the frequency of first-cousin matings among parents of homozygotes; $c$ ( = .278), the frequency of first-cousin marriages in the population; $q$ ( = .041), the gene frequency; and $F$ ( = .019), the coefficient of inbreeding. On the assumption that Down patients represent the homozygous recessives, the frequency of occurrence of Down syndrome was considered = $q^2$, and the gene frequency = $q$. Expected values of $k$ were compared with observed values of $k$ for: (1) the study population; (2) for mothers < 35, and (3) for mothers < 25.

Table 4 shows significant differences ($P < .01$) between the expected and observed values of $k$ for the total study population, and for mothers < 35 years. For mothers < 25 years, the expected and observed values of $k$ are comparable. This suggests that in the younger mother, the occurrence of Down syndrome may be genetically determined,

TABLE 4

EXPECTED VS. OBSERVED FREQUENCY OF DOWN SYNDROME IN FIRST-COUSIN MATINGS

| | Expected $k^*$ in % | Observed $k$ in % |
|---|---|---|
| Study population ($c$ = .278, $q$ = .041) | 47 | 65 |
| Mothers < 35 years ($c$ = .284, $q$ = .041) | 48 | 63 |
| Mothers < 25 years ($c$ = .294, $q$ = .037) | 51 | 50 |

* Calculated using Dahlberg's equation [17]: $k = c(1 - 15q)/[16(q + F)]$.

while in advanced maternal age, additional factors play a role. However, two problems arise from this assumption. The first is that if most of the occurrences in the younger mothers are genetically determined as a result of the proposed recessive gene, the recurrence risk would be expected to be close to 1:4, a situation that is not corroborated by statistics. In addition, younger mothers of Down babies in our study did not have an increased frequency of miscarriages or stillbirths. Second, the observed values for $k$ in older mothers are higher than the expected $k$, suggesting that advanced maternal age preferentially increases Down occurrence more in consanguineous than in nonconsanguineous matings, a phenomenon that is difficult to explain with the proposed hypothesis.

An alternative hypothesis is that an autosomal recessive gene results in meiotic nondisjunction in homozygous parents. Gowen [4] has identified a recessive gene that interferes with crossing over and reduction in the homozygous female *Drosophila*. It is known that preference for consanguineous marriages is not a uniform attitude but, rather, occurs in certain populations. Further, within these populations, it is perpetuated mostly within certain families or in sections of the population. Parents who are closely consanguineous have a higher probability of being themselves the offspring of consanguineous matings. Accordingly, the increased occurrence of Down syndrome among consanguineous matings, in our study, may be the result of a high probability for the parents, rather than for the Down newborns, to be the homozygote for the gene.

Our results show an increased occurrence of Down syndrome in offspring of consanguineous marriages, implying that trisomic Down syndrome is etiologically heterogeneous, and that in a subgroup of the trisomic Down patients, the nondisjunction may be genetically determined.

ACKNOWLEDGMENTS

We wish to thank G. N. Donnell, MD, and R. E. Falk, MD, for their assistance.

REFERENCES

1. BRIDGES CB: Nondisjunction as a proof of the chromosome theory of heredity. *Genetics* 52:107–163, 1916
2. GRIFFITHS AJF, DELANGE AM: *p*-Fluorophenylalanine increases meiotic nondisjunction in a *Neurospora* test system. *Mutat Res* 64:345–354, 1977
3. KAPLAN AR: Leukocyte chemistry and morphology correlated with chromosome anomalies. *Ann NY Acad Sci* 155:657–663, 1968
4. GOWEN JW: Meiosis as a genetic character in *Drosophila melanogaster*. *J Exp Zool* 65:83–106, 1933

5. BEADLE GW: A gene for sticky chromosomes in *Zea mays*. *Z ind Abstam Vererbungsl* 63:195–217, 1932
6. ABBO G, ZELLWEGER H, CUANY R: Satellite association (SA) in familial mosaicism. *Helv Paediatr Acta* 21:293–299, 1966
7. HSU LYF, HIRSCHHORN K, GOLDSTEIN A, BARCINSKI MA: Familial chromosomal mosaicism, genetic aspects. *Ann Hum Genet* 33:343–349, 1970
8. PENROSE LS: Mongolism. *Br Med Bull* 17:184–189, 1961
9. THERMAN E, PATAU K, SMITH DW, DEMARS RI: D-trisomy and XO gonadal dysgenesis in two sisters. *Am J Hum Genet* 13:193–204, 1961
10. MATSUNAGA E: Down's syndrome and maternal inbreeding. *Acta Genet Med Gamellol (Roma)* 15:224–229, 1966
11. KWITEROVICH PO JR, CROSS HE, MCKUSICK VA: Mongolism in an inbred population. *Bull Johns Hopkins Hosp* 113:268–275, 1966
12. EL-ALFI OS, SHAKER Y, SHAATH R, ABDUL SALAM T: Congenital malformations in Kuwait. *J Kwt Med Assoc* 2:99–108, 1968
13. EL-ALFI OS, BAHIG AH, ABDUL SALAM T: Birth weights in Kuwait, and their relation to consanguinity and to birth order. *J Kwt Med Assoc* 3:227–231, 1969
14. MANTEL N, HAENSZEL W: Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 33:719–748, 1959
15. WOOLF CM: in *Principles of Biometry*. New York, Van Nostrand, 1968, pp 239–244
16. AFIFI AA, AZEN SP: in *Statistical Analysis (A Computer Oriented Approach)*, 2nd ed. New York, Academic Press, 1979, pp 97–98
17. STERN C: in *Principles of Human Genetics*, 2nd ed. San Francisco and London, WH Freeman, 1960, pp 376–379