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SHORT PAPERS

Heritability of cranium bifidum and spina bifida in the golden hamster

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SUMMARY

The heritability (h^2) and frequency of the neural tube closure defects, cranium bifidum (CB) and spina bifida (SB), have been estimated for a population of 9-day-old hamster embryos through half-sibling analysis. The average frequency of the total affected embryos per litter is approximately 17 % while the pooled estimate for h^2 based on between sires and between dams within sires components was 4 %. This value points to the importance of environmental factors in contributing to the variance in defect frequencies observed within this population.

1. INTRODUCTION

Two congenital defects of the central nervous system (CNS), anencephaly and spina bifida cystica (ASB), have been estimated to occur in human populations with a frequency ranging from 1/1000 births in New York State (Janerich, 1973) to an incidence that exceeds 10/1000 births in parts of Ireland and South Wales (Elwood, 1972; Laurence, Carter & David, 1968). This is an underestimate of their true occurrence since their prevalence in induced and spontaneous abortuses is much higher (Bell & Gosden, 1978; Creasy & Alberman, 1976; Roberts & Lloyd, 1973). Nishimura (1970) has demonstrated that as many as 90 % of all neural tube malformations are lost early in pregnancy. Both defects are the result of a failure of the neural tube to close in a specific location: anencephaly occurring in the cranial region and spina bifida occurring in the spinal region of the CNS.

Current attempts to interpret the human data with regard to recurrence risks in relatives of affected individuals and the data implicating environmental variables such as vitamin deficiencies (Smithells *et al.* 1976) point to a genetic and environmental aetiology for these defects (Carter, 1976; Fraser, 1976). This multifactorial/threshold model of CNS birth defect production assumes that the underlying genetic system is polygenic. The proportion of the total phenotypic variance

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of a trait that is due to the additive genetic variance is referred to as the heritability (h^2) of that trait (Cavalli-Sforza & Bodmer, 1971). Strictly speaking, this is the only portion of the variation associated with a genotype that can be inherited from a parent. The expected liability of an individual to a condition is a function of the heritability for liability for that trait.

On the basis of techniques that compare recurrence rates of ASB in relatives of affected individuals to the recurrence rates in a randomly selected population (Smith, 1970, 1971; Edwards, 1969; Falconer, 1965), h^2 of liability estimates of ASB ranging from 70 to 100% have appeared in the literature (Cavalli-Sforza & Bodmer, 1971; Carter & Evans, 1973). These estimates are subject to a large number of reservations. Chief among these concerns the assumption that genotype and environment are uncorrelated. If a correlation exists, it would tend to inflate any estimate of h^2. Increased recurrence risks in relatives of affected individuals might just as easily be due to their shared environmental background as their shared genes.

For this reason, an animal model was sought with a spontaneous rate of occurrence of ASB on the same order of magnitude as the human population. It was then hoped that the problem of correlated environments could be eliminated. Although one cannot compare h^2 estimates from one population to another, estimates of h^2 in mammalian populations other than man, add to our knowledge of the genetic heterogenity underlying these defects.

Closure of the neural tube is complete by $8\frac{1}{4}$–$8\frac{1}{2}$ days of gestation in the golden hamster (Marin-Padilla, 1970; Boyer, 1953). As a developmental defect cranium bifidum (CB) is represented by a stage of encephaloschisis in a 9-day hamster embryo but progresses to the condition known as exencephaly or anencephaly in the human fetus. Spina bifida (SB) appears, at the same time in development, as an opening in the neural tube posterior to the cranial area. By sacrificing females at 9 days of gestation, it is possible to estimate the spontaneous rate of occurrence of these CNS defects. The heritability of CB/SB can be estimated through half-sibling analysis. In this way the hypothesis that the total variance in frequency of CB/SB has a large genetic component associated with it can be tested.

2. MATERIALS AND METHODS

A homogeneous group of mature virgin female and male golden hamsters (*Mesocricetus auratus*, Charles River Lakeview, Massachusetts, LAK : LVG Syrians) were obtained and maintained under standard lighting conditions (Charles River Lakeview) on Wayne Lab-Blox and water *ad libidum*. This outbred strain has been commercially reared in the Lakeview hamster colony since 1949 and has produced in excess of $\frac{1}{4}$ million animals.

In a preliminary study to establish baseline frequencies for the production of CB/SB, 50 females in oestrus were selected and randomly mated to one of 20 males. The length of the copulation period was recorded, usually averaging 20 min, and embryo age 0 taken as the middle of this observed period. Females were then

individually caged and handled only at the time of sacrifice. Females were sacrificed with ether exactly 9 days following the timed matings. At that time the uterus was removed, each gestation sac was opened and litter size including resorptions recorded. The embryos were then evaluated and preserved in 10% buffered neutral formalin. A total of 44 litters were recovered in this manner for a total of 510 describable embryos.

By sacrificing at 9 days, the CNS defects can be observed before the more severely affected embryos die *in utero* and undergo resorption. Our experience as well as that of previous investigators (Elis & DiPaolo, 1967), indicates that prolonging the time of observation results in a significant decrease in the frequency of the defects observed and a significant increase in the frequency of resorptions. Recovery of embryos beyond 12 days of gestation yields only a small fraction of the number of defects that can be recovered at 9 days.

The heritability for the liability of CB/SB in the golden hamster was estimated according to a method developed by Robertson & Lerner (1949) for threshold traits. A hierarchical design of experiment was employed in which ten identified males were each mated to seven females, for an initial total of 70 females utilized, and their litters scored for the frequency of CB/SB.

Litters produced from the same sire are half-sibs and have approximately $\frac{1}{4}$ of their genes in common. Intuitively, if half-sibs are more alike with regard to the trait than randomly drawn individuals from a population maintained under the same environmental conditions, a genetic component for the trait is suggested. A total of 59 litters and 644 describable embryos were recovered from this second study.

3. RESULTS

Initial estimates of the frequency of these neural tube closure defects in 9-day-old hamster embryos can be found in Table 1. Approximately 17% of all embryos were affected with CB and/or SB. Of these, approximately 7% exhibited CB alone, 7% exhibited SB alone and 3% exhibited both defects. Although higher than some of the frequencies reported in human spontaneous and induced abortions, the reported frequencies are probably an underestimate of the true occurrence of these defects.

Because there appears to be a strong association between the defects CB and SB with regard to their pattern of embryonic development and their rate of occurrence together (expected rate of occurrence of both defects together if independent of one another = 0·005), the second study deals only with the total abnormal frequency, not distinguishing between the two defects or the defect combination.

The heritability, as calculated from the sire component is as follows (Robertson & Lerner, 1949):

$$h^2 = \frac{1}{rn_0}\left\{\frac{\sum_{i=1}^{n}\frac{a_i^2}{n_i} - \frac{(\Sigma a_i)^2}{\Sigma n_i}}{\bar{P}(1-\bar{P})} - (N-1)\right\},$$

Table 1. *Frequency of spontaneously occurring neural tube closure defects in 9-day-old hamster embryos*

Number of pregnant females	44
Number of describable embryos*	510
Litter size	11·59 ± 0·42†
Frequency of embryos per litter with the lone defect, cranium bifidium (CB)	0·070 ± 0·014
Frequency of embryos per litter with the lone defect, spina bifida (SB)	0·072 ± 0·013
Frequency of embryos per litter with the defect combination, CB and SB	0·032 ± 0·009
Frequency of embryos per litter with any of the above defects or defect combinations	0·174 ± 0·022

* This number reflects the 5·5% of embryos lost due to resorption and the 5% lost due to handling.

† Mean ± standard error of the mean.

where a_i = number of affected offspring produced by the ith sire, n_i = total number of offspring produced by the ith sire, N = number of sires = 10, r = the average genetic relationship between the members of a sire family $\simeq 0{\cdot}290$, $\overline{P}$ = mean frequency of affected individuals = 0·164, $n_0 = \sum n_i - (\sum n_i^2 / \sum n_i) - (N-1)$.

And the standard error of estimate of h^2 is σ_t / r
where

$$\sigma_t = \frac{[1+(n-1)t]\,(1-t)\sqrt{2}}{\sqrt{[n(n-1)(N-2)]}},$$

t = the phenotypic correlation between the members of the sire family and equals rh^2 and n = average number of offspring per sire = 64·4.

This estimate of h^2 for CB/SB derived from our second study (Table 2) was 0·068 or approximately 7% of the total variance, with standard error 0·060. In the analysis between dams within sires, where the average number of dams per sire was 5·9, the average number of offspring per dam was 10·9 and $r = 0{\cdot}25$, h^2 and the standard error were estimated as $0{\cdot}012 \pm 0{\cdot}070$. These two estimates did not differ significantly and they were therefore pooled, with weighting by the reciprocals of their variances, to give a final h^2 estimate of $0{\cdot}044 \pm 0{\cdot}064$. Transformation to the probit scale yields an estimate of the heritability of liability of only 0·099, a much lower estimate than is commonly found with the same human congenital malformations, and perhaps indicating that our outbred hamster population is less variable, genetically, than is the human population.

4. DISCUSSION

Neural tube closure defects have been referred to as the polio of our time, affecting 11 000 new individuals yearly in the United States alone. If minimization

Table 2. *Embryos in separate litters sired by each of the ten males in Study 2*

Male	No. defective/total describable embryos							Totals
1	0/12	2/13	2/15	0/13	1/14	0/13	0/10	5/90
2	0/11	0/7	2/12	3/15	0/5	3/13	2/13	10/76
3	2/18	3/10	1/12	2/12	2/6	0/10	—	10/68
4	3/12	3/13	0/7	1/13	0/5	4/12	—	11/62
5	4/8	2/10	1/8	0/7	1/13	1/12	—	9/58
6	2/6	2/5	2/14	0/13	1/13	4/14	—	11/65
7	0/13	4/14	5/18	4/11	3/14	1/6	—	17/76
8	4/10	0/7	5/11	3/8	2/16	—	—	14/52
9	3/14	1/3	0/6	0/13	1/10	—	—	5/46
10	2/14	6/13	2/8	0/6	4/10	—	—	14/51
Total								106/644

of the occurrence of these birth defects is to be achieved, the aetiology of these defects must be determined and contributing factors identified. We have estimated the relative genetic contribution to the total variance observed in the spontaneous occurrence of CB/SB, using the hamster model, to be only 4·4%. This either points to the overwhelming importance of the environmental component in contributing to the variance in CB/SB frequencies in this model, or, perhaps, to a genetic component related to the susceptibility to teratogens. In either case, it becomes important to identify those environmental variables that affect development in a beneficial as well as a deleterious manner. The hamster model has previously provided a mechanism by which suspected teratogens could be rapidly evaluated (Ferm, 1965). We are currently using it to (*a*) estimate the heritability for the susceptibility to various teratogens in the production of CB/SB and (*b*) to lower the frequency of these defects with pre-conceptual supplementation of various nutrients.

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REFERENCES

Bell, J. E. & Gosden, C. M. (1978). Central nervous system abnormalities – contrasting patterns in early and late pregnancy. *Clinical Genetics* **13**, 387.

Boyer, C. C. (1953). Chronology of development for the golden hamster. *Journal of Morphology* **92**, 1.

Carter, C. O. (1976). Genetics of common single malformations. *British Medical Bulletin* **32**, 21.

Carter, C. O. & Evans, K. (1973). Spina bifida and anencephaly in Greater London. *Journal of Medical Genetics* **10**, 209.

Cavalli-Sforza, L. L. & Bodmer, W. F. (1971). *The Genetics of Human Populations*. San Francisco: W. H. Freeman and Co.

Creasy, M. R. & Alberman, E. D. (1976). Congenital malformations of the central nervous system in spontaneous abortions. *Journal of Medical Genetics* **13**, 9.

EDWARDS, J. H. (1969). Familial predisposition in man. *British Medical Bulletin* **25**, 58.

ELIS, J. & DIPAOLO, A. (1967). Aflatoxin B_1 induction of malformations. *Archives of Pathology* **83**, 53.

ELWOOD, J. H. (1972). Major CNS malformations notified in Northern Ireland 1964–8. *Developmental Medicine and Child Neurology* **14**, 731.

FALCONER, D. S. (1965). The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Annals of Human Genetics* **29**, 51.

FERM, V. (1965). The rapid detection of teratogenic activity. *Laboratory Investigations* **14**, 1500.

FRASER, F. C. (1976). The multifactorial/threshold concept uses and misuses. *Teratology* **14**, 267.

JANERICH, D. T. (1973). Epidemic waves in the prevalence of anencephaly and spina bifida in New York State. *Teratology* **8**, 253.

LAURENCE, K. M., CARTER, C. O. & DAVID, P. A. (1968). Major CNS malformations in South Wales. I. Incidence, local variations and geographical factors. *British Journal of Preventive and Social Medicine* **22**, 146.

MARIN-PADILLA, M. (1970). The closure of the neural tube in the Golden Hamster. *Teratology* **3**, 39.

NISHIMURA, H. (1970). Incidence of malformations in abortions. *Proceedings of the 3rd International Conference on Congenital Malformations* (ed. F. Clark-Fraser and V. A. McKusick). Amsterdam: Excerpta Medica.

ROBERTS, C. J. & LLOYD, S. (1973). Area differences in spontaneous abortion rates in South Wales and their relation to neural tube defect incidence. *British Medical Journal* **4**, 20.

ROBERTSON, A. & LERNER, I. M. (1949). The heritability of all-or-none traits: viability of poultry. *Genetics* **34**, 395.

SMITH, C. (1970). Heritability of liability and concordance in monozygous twins. *Annals of Human Genetics* **23**, 85.

SMITH, C. (1971). Recurrence risks for multifactorial inheritance. *American Journal of Human Genetics* **23**, 578.

SMITHELLS, R. W., SHEPPARD, S. & SCHORAH, C. J. (1976). Vitamin deficiencies and neural tube defects. *Archives of Disease in Childhood* **51**, 944.