

Leukaemia and childhood cancer in twins

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SUMMARY Data from the United Kingdom on childhood leukaemia and childhood cancer in twins and from the United States on leukaemia in twins are analysed by a new method. The method distinguishes determinants occurring before the stage of pregnancy corresponding with the cleavage of MZ pairs, from determinants occurring after this point. It derives estimates of the frequencies of each class of determinant. Different aetiological models are characterised by particular combinations of frequencies, and can thus be identified. The results of the analysis suggest that the major determinants of childhood leukaemia, and possibly of the solid cancers as well, operate before the time of cleavage. They operate either on the early zygote or its component germ cells. These early determinants are not, however, sufficient causes and require combination with postcleavage determinants, which subsequently occur in about a quarter of all children, before leukaemia can ensue.

The aetiology of childhood leukaemia remains obscure. A few cases can be attributed to medical diagnostic irradiation and a few are associated with Down's syndrome, but nothing certain is known about the great majority. Many of the other epidemiological associations of leukaemia are best interpreted in non-causal terms—for example, through selective survival. Thus the relatively recent emergence of the “preschool peak” may be attributed to the improved survival of children who would in earlier times have died from an infection before a diagnosis of leukaemia could be made.^{1 2} The seasonal variation of leukaemia onsets could also be due to passage at an earlier age through a seasonal filter of pre-emptively fatal hazards, such as pneumonia. The social class and urban/rural differences can be interpreted in a like manner.

The ambiguities of interpretation surrounding such findings demand new approaches if the aetiology of this disease is to be resolved. Our purpose is to introduce such an approach.

We intend to exploit a novel method of analysing twin pairs in which one or both of the children is affected.³ It can be applied to sets of twins undifferentiated with respect to their zygosity. Unlike traditional approaches to undifferentiated twin data, its primary purpose is not to distinguish directly between genetic and environmental determinants, but to establish the timings of the main causal events, irrespective of their nature. It may then be possible to draw conclusions regarding the nature of the determinants, but this is a separate process and not the *direct* purpose of the technical approach.

Materials and methods

The sex constitution of affected twin pairs, and the presence of absence of concordance, differentiates the pairs into seven different types namely, MM, FF, MM, FF, MF, MF, MF, . . . where the “bar” indicates “affected.” The measured frequencies of the seven different types of twins provide the basis of our approach. The exact population from which the twins are drawn is not usually known, so that our information is limited to the *relative* frequencies of the different twin pair types rather than their *absolute* frequencies.

An aetiological model can be resolved into three main elements. Firstly, sibships are regarded as “susceptible” or as “non-susceptible”; the matings can, or cannot, give rise to affected children. The postulate is reasonably general in that no limits are attached to the actual proportion of susceptible matings. The proportion can be small, in which case the risk to individual members of the susceptible sibships must be high; or, at the other extreme, all sibships can be regarded as susceptible, in which the risk to individuals within these sibships is reciprocally low. The postulate is thus a general and conceptual one, covers a full range of possibilities, and it does not prescribe or preempt particular proportional values.

Secondly, the population is characterised by a twinning rate and, within this, by a proportion (p) of MZ pairs. So long as we concern ourselves with *relative* frequencies, rather than *absolute* frequencies, the twinning rate itself is unimportant, and p is the crucial parameter. We further suppose that the value

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of p is the same in susceptible and in non-susceptible sibships.

Thirdly, it is supposed that there are two classes of determining events operating within susceptible sibships; one set is located before the embryonal stage corresponding with the cleavage of an MZ pair, whereas the other is located after this time. The first set corresponds with the first few hours after conception, or with the period during which the germ cells develop; the second corresponds with the remainder of the gestation period together with postnatal life.

The chief outcome of our analytical method is a determination of the frequencies of the precleavage and the postcleavage causes within the susceptible sibships. These events may be complex and ultimately resolvable into components, but this does not invalidate our global approach to the determination of their composite frequencies. The frequency of a complex event is the product of the frequencies of its necessary components. The precleavage and postcleavage causes, as well as any subcomponents, are conceived as *necessary* causes. Thus a value of 0.0, for either, is "forbidden" in that this would have precluded the occurrence of the disease. Likewise, universal susceptibility at either stage is associated with a value of 1.0. In the case of otitis media the only necessary precleavage event is the acquisition of the genetic material to ensure that the infant has ears! The precleavage event-frequency is therefore 1.0.

It is necessary, in general, to admit that event-frequencies might differ between boys and girls. We therefore represent the precleavage event and postcleavage event risk-frequencies in boys by the parameters a and b , and those in girls by c and d . It has been shown that the five parameters $p, a, b, c,$ and d determine the relative frequencies of the seven twin pair types. Conversely, the measured frequencies of the twin pair types can be used to determine the values of the parameters. A full analysis has been presented elsewhere,³ but a summary of the algebraic relationships is given in table 1, assuming $m = f = \frac{1}{2}$ and $z = \frac{1}{2}q$ in the notation of table 4 of our previous paper. Best-fit solutions for the parameters, within their absolute ranges $\rightarrow 0.0$ to 1.0 , or within an arbitrary range of (say) 0.001 to 0.999 , may be determined iteratively through maximising the likelihood-function, or through minimising chi-square; the latter also provides a basis for judging the degree of discrepancy between the observed and the "best" calculated distributions.

Data base

Three sets of data are examined in the present report. The first consists of leukaemia data from the Oxford

Survey of Childhood Cancer (OSCC) relating to the United Kingdom between 1953 and 1979. The second set consists of the OSCC data relating to solid cancers. The third consists of twins with leukaemia identified in the United States as reported by MacMahon and Levy.⁴

Before proceeding to the analysis of these data it may be useful to illustrate some of the biological mechanisms through which the twin pair frequencies can be determined and, conversely, the ways in which the estimated values of the parameters can subsequently be interpreted.

Firstly, let us consider a metabolic disorder determined by the homozygous state for an autosomal recessive gene of frequency $0.01 (= g)$. The frequency of susceptible doubly-heterozygous matings is about $0.0004 (= [2g(1-g)]^2)$. Within these families the frequency of the precleavage determining events—that is, the frequency of occurrence of homozygosity—is 0.25 , and we attach this value to the parameters a and c .

If we suppose the homozygous state to be fully penetrant then parameters b and d are set at 1.0 . For a United Kingdom population we would set p at 0.38 . We now have all the necessary parameters for calculating the expected relative frequencies for the seven types of twin pair as set out in table 1.

Table 1 Twin types and population frequencies

Pair type	Frequency
$\bar{M}\bar{M}$	$ab^2(p + za)/2$
$\bar{F}\bar{F}$	$cd^2(p + zc)/2$
$M\bar{M}$	$ab(p(1 - b) + z(1 - ab))$
$\bar{F}F$	$cd(p(1 - d) + z(1 - cd))$
$M\bar{F}$	$z a b c d$
$\bar{M}F$	$z a b (1 - cd)$
$M\bar{F}$	$z c d (1 - ab)$

Where p = the proportion of MZ pairs in the population from which the affected twins were drawn, and $z = (1 - p)/2$.

- a = Frequency of necessary precleavage determining events in boys.
- b = Frequency of necessary postcleavage determining events in boys.
- c = Frequency of necessary precleavage determining events in girls.
- d = Frequency of necessary postcleavage determining events in girls.

The "bar"—for example, \bar{M} —indicates "affected."

Conversely, given the actual twin pair type frequencies, we can find the values of $p, a, b, c,$ and d ; or, more practically, we can fix $p = 0.38$ and limit our investigation to the remaining four parameters. If we then find $a = c = 0.25$ and $b = d = 1.0$ we have the basis of an inference concerning the likely mode of determination. Furthermore, we can relate the incidence in affected families—that is, $(ab + cd)/2$ —to the measured incidence in the population (as obtained from other sources) and come to an estimate of the frequency of susceptible families within the population.

A second illustrative example is Down's syndrome, where the determinants of the karyotypic abnormality are located before the point of cleavage. In view of the low rate of recurrence within sibships and the high known penetrance of the abnormal karyotype (so that $b = d = 1.0$), the values for a and c are likely to be low. It has been shown that the theoretical twin pair frequencies derived from such values display a close match with observed values. It has also been shown that the incidence within susceptible sibships is close to the total population incidence; therefore, that susceptibility applies virtually to the whole population (table 2; final row).

Finally, consider fractured tibia. The determinants may be regarded as entirely postcleavage, so we set b and d as, for example, 0.01 and 0.005. The precleavage factors are set $a = c = 1.0$ because the only necessary precleavage condition is survival. Inferences regarding the parameter values can be obtained once more by "reversing" the estimation processes.

It should be noted again that we are dealing in the frequencies of "necessary" causes. Since we are operating retrospectively, from the point of view that the disease has already occurred, the large parameter values—for example, 1.0—are relatively unimportant from the point of view of drawing inferences, and it is the smaller ones such as 0.001 or 0.25 that help us to locate and identify the important causes.

Results

Table 2 gives the observed distributions of the three data sets, together with the pooled leukaemia data—

that is, United States and United Kingdom. A distribution of Down's syndrome twin data taken from earlier publications^{5 6} is appended for purposes of comparison. Iterative fitting of parameter values for the leukaemia and cancer sets of twins was carried out both with p "floating"—that is, to be determined as one of the estimated values—and with p fixed at 0.38, the value for England and Wales. There were no differences of note in the two sets of results. The results for p -fixed are shown in table 2. For the Down's syndrome data, p was set at a lower value to allow for the greater age of mothers giving birth to infants with this disorder.

The relative frequency distribution of seven types of twins has six degrees of freedom; however, we reduce the degrees of freedom by 1 for every variable calculated from the data. Thus with four freely iterated variables, the χ^2 values shown in table 2 should be attached to two degrees of freedom. Values for $\chi^2_{(2)} < 5.99$ indicate an adequate fit ($p > 0.05$) between predictions and observations.

Despite smaller numbers, similar analyses were carried out for the United Kingdom with respect to individual tumours and several alternative groupings. They included (1) all central nervous system tumours (87 pairs), (2) medulloblastoma and astrocytoma (69 pairs), (3) leukaemia with lymphoma (148 pairs), (4) neuroblastoma with nephroblastoma (57 pairs), . . . and several others. The outcome was similar in every case. That is, the optimal values for the precleavage determinants (a, c) were always small, in the neighbourhood of 0.001, while those for the postcleavage determinants (b, d) were larger, in the ranges indicated in table 2.

Table 2 Twin-pair frequencies for leukaemia and cancer in childhood

		$\bar{M}\bar{M}$	$\bar{F}\bar{F}$	$\bar{M}\bar{M}$	$\bar{F}\bar{F}$	$\bar{M}\bar{F}$	$\bar{M}\bar{F}$	$\bar{M}\bar{F}$	Total	p	a	b	c	d	$\chi^2_{(2)}$
Leukaemias (US)	Obs	2	3	18	24	0	12	13	72	*0.38	0.001	0.308	0.001	0.384	0.38
	Exp	2.0	3.1	19.7	23.3	0.0	10.6	13.3	72.0						
Leukaemias (UK)	Obs	1	2	36	36	0	27	24	126	*0.38	0.001	0.180	0.001	0.173	4.66
	Exp	2.3	2.1	41.3	39.9	0.0	20.6	19.8	126.0						
Leukaemias (US + UK)	Obs	3	5	54	60	0	39	37	198	*0.38	0.001	0.242	0.001	0.256	3.79
	Exp	4.4	5.1	60.1	64.0	0.0	31.0	33.3	198.0						
Solid cancers (UK)	Obs	5	1	86	53	0	34	32	211	*0.38	0.001	0.169	0.001	0.114	2.94
	Exp	4.2	1.9	81.0	56.7	0.0	40.1	27.2	211.0						
Leukaemias** + solid cancers (UK)	Obs	6	3	122	90**	0	61	56	338	*0.38	0.001	0.162	0.001	0.128	2.37
	Exp	6.0	3.7	122.2	98.4	0.0	60.3	47.5	338.0						
Down's syndrome (UK)	Obs	10	13	31	31	0	25	19	129	*0.26	0.001	0.970	0.001	0.999	4.74
	Exp	9.4	9.6	27.7	27.8	0.0	27.1	27.5	129.1						

*Parameters marked thus are "preset." Other parameters are freely iterated within the arbitrary range 0.001 and 0.999.
 **Includes one "doubtful" FF pair, excluded from "Leukaemia UK." One twin with designated as leukaemia and the other as aplastic anaemia.
 The United Kingdom data also exclude (1) two "discordant" leukaemia pairs in which the sex of the stillborn opposite twin was not known: 1 $\bar{M} - \text{NK}$, 1 $\bar{F} - \text{NK}$; (2) nine "discordant" solid tumour pairs: 5 $\bar{M} \text{NK}$, 4 $\bar{F} \text{NK}$; (3) two triplet pairs, 1 $\bar{M} \text{MF}$ (glioma) and 1 $\bar{F} \text{FF}$ (hepatoblastoma).
 The six concordant pairs among the solid cancers were all concordant for type of cancer. They included medulloblastoma (two pairs), astrocytoma (two pairs), nephroblastoma (one pair), and teratoma (one $\bar{F}\bar{F}$ pair).

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The confidence limits of the precleavage and postcleavage parameter values were investigated through repeatedly "fixing" one of these sets at a time (such as the precleavage parameters), over a range of values, and allowing iterative estimation of the remaining parameters (such as the postcleavage) to take place. The minimum χ^2 value was used as the criterion of a good fit. A minimum value >5.99 was taken as an indication that a fixed value was not credible, on the grounds that a good fit could not be obtained from any adjusted value of the "floating" parameters.

On this basis, the upper (95%) limit for the precleavage factors relating to the pooled leukaemia data was 0.17. The lower value could not be defined on the basis of the twin data alone and most of the iterative exercises returned the lower arbitrary limit of 0.001. Estimates of the cumulative prevalence of leukaemia in childhood in the United Kingdom suggest that it could not be much less than this. Indeed, given the "penetrance" estimates—that is, the values for b and d —a value of 0.002 might be regarded as a more plausible lower limit. The 95% lower and upper confidence limits of the postcleavage values in the pooled leukaemia data, supposing $b = d$, were approximately 0.16 and 0.39.

For the 211 solid cancers in the OSCC series, the confidence limits were rather wider. For the precleavage values no firm limits could be established at all with $\chi^2_{(2)} = 2.94$ for $a = c = 0.001$; and $\chi^2_{(2)} = 5.77$ for $a = c = 0.99$. The 95% confidence limits for the postcleavage factors (supposing $b = d$) were approximately 0.08 and 0.26. The obvious heterogeneity of the solid cancers adds further to the uncertainties of interpreting these data. The concordance patterns here (see footnote to table 2) suggests that we are dealing with multiple diseases with different detailed aetiologies.

Interpretation and discussion

The results of these analyses were similar both for the leukaemias and for the solid tumours taken as a whole and, so far as could be assessed from the limited numbers, for the detailed subdivisions of both groups. In each case they suggest that the greater part of the differentiation of diseased from normal has already occurred by the time of cleavage. This would suggest that within a few days of conception about two in every thousand embryos have already suffered a determining event, while the remainder have escaped and are immune from subsequent risk. The event may have occurred to the embryo itself, or in the germ cells giving rise to it. The determining event, however, is not in itself a "sufficient" cause, and it has a less-than-complete penetrance. Only one quarter of

the preselected embryos will develop leukaemia or cancer by the age of 15 years. Except for this last feature, the parameter evaluations suggest analogies with Down's syndrome, as illustrated in table 2. The fact that leukaemia also has a high incidence in children with Down's syndrome adds to the force of the analogy.

The individual parameter estimates on which these inferences are based have rather wide confidence limits, but indirect arguments can be used to exclude several aetiological possibilities. Firstly, the pooled leukaemia observations are not compatible with setting $a, c = 1.0$, the values which would imply the existence of *sufficient* postcleavage determinants. If the postcleavage determinants are not sufficient then it follows that precleavage determinants must exist. The minimum $\chi^2_{(2)}$ for postcleavage sufficiency—that is, for $a, c = 1.0$ —was 11.24, a highly significant non-fit; so this statement can be made with some confidence.

Conversely, it is not possible to set the postcleavage determinants to values for which the precleavage causes could be regarded as sufficient—that is, $b = d = 1.0$; $\chi^2_{(2)}$ was 50.0. Postcleavage causes must also therefore exist. We are thus led to the conclusion that neither the precleavage nor the postcleavage causes of leukaemia are sufficient causes, and therefore that a *combination* of non-sufficient precleavage and non-sufficient postcleavage causes must be inferred. Strictly, we are referring to "causes of occurrence before age 15"; if some of the non-affected twins developed leukaemia later in life we might have to modify our conclusions. We must also note that although the parameter estimates are similar for the solid cancers, they have wider confidence limits. This, together with the possibility of gross aetiological heterogeneity between the different solid cancers (which would disturb the conditions of the model) invites corresponding caution there.

Another firm inference can be drawn regarding leukaemia. The products ab and cd , which indicate the incidence of the disease in boys and girls within the substratum of the population from which the affected twins were drawn, are close to the known cumulative prevalences, by age 15, in the total population. We conclude from this that the whole population of matings is at risk, and that there is no substantial prior selection of a high risk subgroup of matings, definable in terms of parental—for example, genetic—characteristics. If the determining events were in the germ cells, preceding conception, then they must be seen as occurring in a minority of germ cells within all parental pairs, rather than within a minority of parents. In addition we note that the estimated parameters a and c are not reconcilable

with any simple—for example, single-locus—mechanism of genetic segregation within affected sibships. In these respects the results again resemble those derived from the Down's syndrome data.

There is, of course, a *general* caveat concerning the validity of the model. Any mechanism that went outside this rather simple and closely defined frame of reference could invalidate or modify all the inferences which have been drawn. Examples would include interactions between twin fetuses, selective abortion or resorption of concordant pairs, or specific associations between twinning and the occurrence of cancer or leukaemia. This wider problem of the validity of the frame of reference of an argument is a general one in scientific inference, and there is no way of guarding against it in any instance. Our conclusions, as in any such situation, must be regarded as interim. They are, nevertheless, interesting and serve to focus our attention on the possibility of physical, chemical, or infective injuries to very early zygotes or to germ cells in a manner analogous with our present day understanding of the aetiology of karyotype abnormalities. In contrast with Down's syndrome, however, the inferred existence of postcleavage determining factors offers potential scope for devising preventive procedures during late fetal or postnatal life.

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