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Use of Bayes' Formula to Make a Genetic Risk Estimate

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Izabella and her partner sought pre-implantation genetic diagnosis (PGD) because Izabella had retinoblastoma due to a deletion in chromosome 13 and they want to have children not at genetic risk of retinoblastoma. Fortunately, Izabella's tumor was unilateral and was treated successfully and she is well. Izabella's chromosome abnormality is mosaic with 70% of lymphocytes having the deletion. This mosaicism may not be present in Izabella's ovaries. The couple went through PGD on two occasions and 13 embryos were tested. None had the deleted chromosome 13. IVF and PGD failed to produce a pregnancy. The couple wished to know what the experience provides as to the risk to their offspring: in particular, does it indicate a risk low enough to be acceptable if they go ahead with a natural pregnancy instead of another resort to PGD? Also, the couple did not want prenatal diagnosis. The situation therefore requires an estimate of the probability that an embryo will have the deletion. Counseling is problematic because there is no obvious way of selecting a prior probability from which to compute a Bayesian estimate of risk. Two solutions are offered, depending on the amount of information available about genes transmitted from the maternal grandparents.

■ **Keywords:** Bayes' formula, Bayes' theorem, genetic risk

Winston (2007, p. 131) writes: 'It has been suggested that, around the globe, some one million babies have been born from IVF'. He notes that there are now IVF practitioners in many countries throughout the world. Thus the birth of babies by this technology is of some importance. The case described here is not of a child born from IVF but the couple in question originally came to Sydney for IVF because they feared that a child born naturally might have a high risk of retinoblastoma. The prospective mother Izabella had already been treated for retinoblastoma. Izabella and her partner were referred to Dr. G. Morgan for advice.

The next section introduces the case and the following one gives an outline of the development of eggs carried by each woman. This follows various sections in the monograph of Robert Winston (2007). Additional material on vertebrate reproductive cycles is given by Bullough (1961). Then follows a description of the logic used to calculate the probability of risk. The details of two methods of calculation are given in Appendix A.

The Case

Chromosomal analysis established that Izabella's retinoblastoma was associated with a deletion in chromosome 13. The abnormality is mosaic with 70% of lymphocytes having the deletion. This mosaicism may not be present in Izabella's ovaries. The couple went through pre-implantation genetic diagnosis (PGD) on two occasions and 13 embryos were tested. None had the deleted chromosome13. IVF and PGD failed to produce a pregnancy.

Winston (p. 142) comments briefly on genetic or chromosomal disorders:

> Some families with a damaged gene may be at high risk of having an abnormal baby that may die of the disease to which the family is prone. ... A treatment that may be considered in such cases is IVF associated with screening of the embryos. Only embryos free of the specific defect causing the problem are replaced in the uterus. This procedure is called 'pre-implantation diagnosis' (or sometimes, in the case of chromosome problems, 'aneu-

ploidy screening'). This is a complex area of medicine, and the scientific and ethical problems associated with these techniques are discussed separately in chapter 7.

The Source of a Woman's Eggs

In order to give advice to the couple it is necessary to consider the process by which eggs are produced. Winston (p. 23) writes:

> A woman's ability to achieve a pregnancy is decided when she herself is in her mother's uterus — when she is a tiny embryo less than 2 millimetres long. This is when her own eggs start to be created. She will carry these throughout her adult reproductive life, even though at this stage of development she has an unformed heart and no other recognizable organs. Astonishingly, her ability to create further life is in place well before her own ability to survive outside the womb has been secured.

Where do these eggs come from? They are created from the germ cells, a collection of tiny, primitive cells that grow outside the embryo, in the yolk sac. Around 100 of these progress along the primitive highway that in later pregnancy becomes the umbilical cord and stream into the embryo itself, where they divide and multiply astonishingly. Nobody knows precisely how many eggs are made, but the best estimate is that by mid-pregnancy a female fetus may well have around 7 million eggs in her ovaries.

Gloomy poets and novelists have pointed out that we begin to die almost from the moment we are born. The truth is, if anything, even darker. By the time she is born, the female will have lost most of these eggs — and, as far as we know, no more eggs are formed after birth. My colleagues at Hammersmith, Kate Hardy and Stephen Franks, estimate that probably only 600,000 are left when a girl is born. By the time she reaches puberty — the point at which her body is potentially capable of using these eggs to create and sustain life — she will have perhaps 100,000 or fewer in each ovary. In the western world, around two or three of these eggs will probably go on to become children.'

In the section entitled 'Examining eggs and refining PCR', Winston (p. 337) writes:

> Around the time we were starting to think about transferring biopsied embryos, Yuri Verlinsky and his colleagues in Chicago were advocating screening eggs. The procedure they favored was to analyse the polar body — the part of the egg that is discarded when one-half of the paired set of chromosomes is extruded. Like all cells, the egg starts with paired chromosomes, but in order for fertilization to take place without increasing the number of chromosomes, the egg has to get rid of one half of the pair. Sperm do this as well, but at an earlier stage of development.

On the last point relating to eggs, Bullough (1961, p. 58) writes: 'Apparently in all vertebrates it is common for the final divisions of the primary and secondary oocytes (with the shedding of polar bodies) to take place in the oviduct after ovulation.'

Calculating the Risk

In this case the focus of interest is the copy of chromosome 13 present in the embryo. This comes either from the father of Izabella or the mother. Denote the chromosome from the father as A and that from the mother as B. Assume that A is the chromosome which may have some kind of copying error (leading to a deletion). Suppose that such a copying error occurs which leads to two lines of A, say A_d and A_n. Suppose that a proportion x are of type A_d and the others are of type A_n.

The problem is how to estimate x by looking at embryos. Assume that embryos are formed as either AF or BF, the F coming from the father, with probability 1/2 for each of AF and BF. However, when one looks at a BF it is 'uninformative' because of the assumption that the copying error occurred in only one parental line. Of course one does not know whether one is looking at an AF or a BF. When one looks at an AF it provides information about x — it is 'informative'. Thus, with probability 1/2, each embryo is informative.

The first of the two methods of estimating x uses the binomial probability distribution with probability of 'success' 1/2 to give weight to the information coming from the sample of tested embryos. The second method uses knowledge of the identity of the chromosome being tested, that is whether it comes from Izabella's father or from her mother. It turned out that both methods of estimating the risk gave the same result — 5%.

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Appendix A
Method 1

The object of this section is to present a method of estimating the probability that, if Izabella supplies a further ovum, it will be heterozygous for del(13) and from that the probability of any embryo of having the del(13).

Suppose that n embryos have been tested and of these y have been found to have del(13). The number of informative embryos, denoted i, follows the binomial distribution with parameter $p = \frac{1}{2}$, that is, the probability of obtaining i informative embryos is given by

$$^nC_i p^i(1-p)^{n-i} = {}^nC_i(\tfrac{1}{2})^n, \ i = 0,1,2,\ldots,n. \quad (1)$$

The term $^nC_i = n!/(i!(n-i)!)$.

Suppose that the unfertilized ovum comes from a source whose prior probability of heterozygosity x follows the beta distribution with parameters α and β. That is the probability of having a value of x in a small interval of width dx is

$$(x^{\alpha-1}(1-x)^{\beta-1}/B(\alpha, \beta))dx, \ 0 \le x \le 1 \ ; \alpha, \beta > 0,$$

where $B(\alpha, \beta)$ is the beta function with parameters, α, β given by

$$B(\alpha, \beta) = \int u^{\alpha-1}(1-u)^{\beta-1}du \ ,$$

the integral being taken over the interval $(0, 1)$.

The properties of the beta distribution are given in Kendall and Stuart (1977, p. 35). In particular the first two moments about the origin are:

$$\mu_{1'} = \alpha/(\alpha + \beta)$$

$$\mu_{2'} = \alpha(\alpha + 1)/((\alpha + \beta)(\alpha + \beta + 1)).$$

From these the mean of x is

$$\mu_{1'} = \alpha/(\alpha + \beta) \quad (2)$$

and the standard deviation of the distribution of x is

$$\sigma_x = \sqrt{(\alpha,\beta/((\alpha + \beta)^2(\alpha + \beta + 1)))}. \quad (3)$$

Given a number of informative ova i, the Bayesian estimate of x given by Good (1968, p. 17) and Leonard and Hsu (1999, p. 108) is

$$\dot{x}_i = (\alpha + y)/(\alpha + \beta + i). \quad (4)$$

The weighted estimate of x obtained by combining (1) and (4) is

$$\ddot{x} = \Sigma^nC_i(\tfrac{1}{2})^n.(\alpha + y)/(\alpha + \beta + i), \quad (5)$$

the sum being taken over $i = 0$ to n.

In the actual case under discussion, y is zero so formula (5) reduces to

$$\Sigma^nC_i(\tfrac{1}{2})^n.\alpha/(\alpha + \beta + i). \quad (6)$$

A further simplification can be achieved if α and β are chosen so that $\alpha + \beta = 1$. Then formula (6) reduces to

$$\alpha(2 - 2^{-n})/(n + 1). \quad (7)$$

Substituting $\alpha = 0.7$ and $\beta = 0.3$ into formula (7) yields $\ddot{x} = 0.1$ and the risk for the embryo 0.05.

Method 2

The logic is simpler than for Method 1. Assume that if deletion occurs it does so in only one grand-parental line, with probability $\frac{1}{2}$ in A and in B. In the case in question there were 6 embryos from each of A and B so there was no need to obtain more data from the grandparents. Taking each line in turn and assuming as before $\alpha = 0.7$ and $\beta = 0.3$ gives posterior probability for the line 0.1. A deletion is transmitted with probability $\frac{1}{2}$ so the probability for carriage to the embryo from each line is 0.025. The combined probability is therefore 0.05.

All of this depends on the values of α and β that have been used in the calculations. It was noted above that Izabella's abnormality is mosaic with 70% of lymphocytes having the deletion. It is an open question as to whether and to what degree mosaicism is present in Izabella's ovaries. Use of the beta distribution allows flexibility in the choice of the prior probability. The values of α and β used above are rather 'conservative' in giving a fairly high prior probability that one set of Izabella's chromosomes is carrying the deletion.