Original Papers

Carrier detection for X-linked agammaglobulinaemia (Bruton Type) in an Irish family using linked DNA probes

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Abstract

Being an X-linked condition, the sisters of men with X-linked agammaglobulinaemia have a 50% risk of being carriers of the disease gene (provided the disease has not developed as a result of a new mutation). We demonstrate how this risk can be modified very significantly by DNA analysis using linked DNA probes. The value of such tests for genetic purposes is discussed.

Introduction

Among the eight immunodeficiency conditions which are inherited in an X-linked manner, X-linked agammaglobulinaemia (McKusick No 30030)[1] is the second most common, with an incidence of one per 100,000 births.[2] The primary defect has been shown to be in the maturation of the B lymphocyte series.[3] Without intervention the natural course of the disease is of recurrent bacterial infections with death in early childhood. Present day treatment with regular immunoglobulin infusions has altered the traditional prognosis and survival into the fourth decade has been achieved but life long supportive care is required.[4]

Being inherited as an X-linked recessive trait, it is a disease of males although girls may inherit the carrier status from their mothers and so be placed at 50% risk of having a son with the disease. There is no immunological test which can predict whether or not a girl in this situation has inherited the gene for Bruton's agammaglobulinaemia. However, the localisation of the gene for Bruton's agammaglobulinaemia to the q21.3-q22 region of the X chromosome[5,6] has provided the means by which carrier status may be evaluated in the sisters of affected boys.

The pedigree documented in Fig l(a) is that of an Irish family with a confirmed diagnosis of Bruton's agammaglobulinaemia. Patients III and 112 have both succumbed to the condition but their affected brother 113 has survived with treatment. Their sisters 115 and 116 were very concerned as to their own risks of being carriers and of transmitting the disease. Patient 115 was 34 years old and had been married eight years. She and her husband urgently wished to start a family but she felt unable to take the chance of having an affected son and sought carrier detection, as did her younger sister 116.

Materials and Methods

Blood was collected for all surviving family members and high molecular weight DNA was extracted from lymphocytes according to the protocol of Jeanpierre.[7] DNA was cleaved using the commercially available restriction endonuclease TaqI, separated by electrophoresis and immobilised on nylon filters using the technique of Southern.[8] The radioactively labelled linked DNA probes S21 and 212-9 were hybridised to these filters which were then exposed to Kodak x-ray film for 48 hours before being developed.

Results

As demonstrated in Fig l(b) the results allow the mother's (12) X chromosomes to be distinguished and the inheritance of these chromosomes by her children to be determined. This enables predictions to be made as to which of her X chromosomes carries the gene for X-linked agammaglobinulaemia and thus to predict carrier status in her daughters. The results shown are those obtained with probe 212-9. These results were confirmed by a second probe, S21, which is also linked to XLA.

Discussion

The advances in molecular biology of the last decade have heralded a period of extraordinary progress for clinical genetics and its practitioners. For the medical profession this progress has led to a better understanding of the defects in some inherited conditions[9,10] and a corollary of this knowledge is the ability to detect carrier status in some diseases. Particularly among families where an inherited disease is fatal there exists a demand for genetic counselling and accurate detection of carrier status. Broadly speaking, this can be accomplished by one of two ways.[11]

Firstly, mutation detection methods may be used. A prerequisite of this method is that the precise location of the gene is known and a specific probe available which will selectively bind to that part of the gene in which the mutation is known to occur. Variations of this technique involving the polymerase chain reaction have recently been employed to determine the frequency of the deletion most commonly observed in cystic fibrosis. For most diseases however this approach is impossible as the nature of the mutation may vary from one family to the next and it is impracticable to search a whole gene, which may be several kilobases long, for a mutation. This is particularly true for X chromosomal diseases.

The second method is the so called "gene tracking" method. This applies to the situation where the gene is unknown but its location within a region of a designated chromosome is known. DNA markers are used from that same chromosomal region to determine whether or not a person has inherited the same region of the chromosome as an affected family member. In this way carrier status can be determined. Implicit to the technique of gene tracking is a risk of wrong prediction due to a crossover occurring between the flanking marker used for predictive purposes and the gene itself. Were this to occur, then the gene would be on the opposite chromosome to the expected one and, in consequences of the crossover, the risks given to the patient with regard to offspring would be wrong. To cover this possibility geneticists, in calculating risk which

includes information derived by gene tracking, allow a “recombination fraction” (-0) which takes account of the genetic distance between the DNA probe used and the gene and therefore of the propensity for a crossover to occur in this region of the chromosome and lead to incorrect risk evaluation for the patient. The recombination fraction between the probe 212-9 and Bruton’s agammaglobulinaemia is 0.05 (ie 1 in 20).

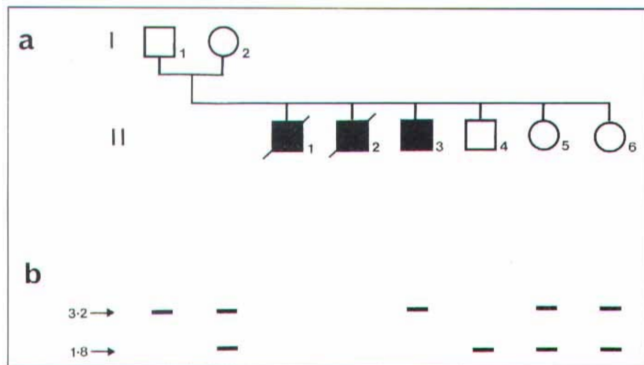

Figure l(a) Pedigree data. I and II refer to the different generations in the pedigree. 1,2,3 etc refer to the individuals within a particular generation.
▯ = affected male
❑ = unaffected male
❍ = female
Figure l(b) beneath each individual indicated in figure l(a) is represented the hybridisation pattern obtained for that probe 212.9.

The key manoeuvre in assessment of carrier status for any x-linked condition is to find a linked probe for which the mother is heterozygous. This enables the X chromosomes to be separated into carrier and non carrier chromosomes depending on which chromosome the affected son inherited. In Fig l(b) the affected son (113) has inherited the 3.2 kilobase long X chromosome segment from his mother which suggests that the disease segregates with the X chromosomes allele represented by the 3.2kb polymorphism. This is supported by the fact that his healthy brother (114) inherited the x chromosome allele 1.8 kb from the mother. The sisters 115 and 116 both have x chromosome alleles 3.2 and 1.8kb but in their cases, the 3.2 allele must have come from the father and so they have inherited the 1.8 kb allele from the mother and are therefore unlikely to be carriers. There are two ways in which this reasoning could break down. The first is if a crossover took place in the mother’s ovary at oogenesis with the result that the mutant gene in one or both sisters is on the opposite X chromosome to that in their brothers 113 and 114 from whom we derived which chromosome the disease gene is on.

Figure 2

Consider the mother (I_2)		Disease on 3.2Kb allele		1.8kb allele
Prior probability		.5		.5
Conditional probability	(II_3)	.95		.05
	(II_4)	.95		.05
		= .45125		.00125
Daughet (II_5)	Carrier	Non-carrier	Carrier	Non-carrier
	.05	.95	.95	.05
	= .0225625	.4286875	.0011875	.0000625
Total	= .45125		.00125	

II_5 Risk of being a carrier = $\frac{.0225625 + .0011875}{.45125 + .00125}$

= 5.2%

This possibility is covered by incorporating a recombination value (0) of 0.05 in the calculation of risk. The second way in which an incorrect carrier risk might be counselled is if the girls 115 and 116 got their 3.2 kb allele from their mother and the 1.8kb allele from a man other than II. Hence to outrule this possibility paternity must be confirmed, as it was in the example shown.

Calculation of risk[12] (See figure 2) - The empirical risk to 115 or 116 of being carried is 50%. They have a 50% chance of passing the disease bearing X chromosome on to their children. Hence the chance of having an affected boy for both 115 and 116 is 1/2 x 1/2=1/4. If 115 and 116 can be shown to be carriers, their risk of a boy being affected rises to 50%, where as if they can be shown to be at a low risk of being carriers the risk of having an affected boy is 1/2 of their own carrier risk.[12]

I 2 is a carrier, with 2 X chromosome alleles designated 3.2 kb and 1.8kb. Theoretically she is equally likely to carry the disease on either chromosome. But we know from 113 that she transmitted the disease with allele 3.2 kb and hence we are 95% confident that the disease is on this allele (we are not 100% sure because of crossover risk Θ = 0.05). However, there is a 5% chance that the disease is on allele 1.8kb. Looking at her well son, 114 we see that he inherited allele 1.8 kb. Therefore we believe that I 2 carriers the gene for X-linked agammaglobulinaemia on the X chromosome represented by the 3.2 kb allele. Consider now patient 115. As she has inherited the 1,8kb band from her mother, the risk that she is a carrier is approximately 5% - the risk of a crossover taking place between the mutant gene and the linked DNA marker employed. Five per cent represents the risk of 115 or 116 being a carrier. Their risk of having an affected boy is 2.6% (1/2 x carrier risk). Thus the incorporation of information available on DNA analysis allows carrier risk to be refined from an empirical risk of 50% to a new risk of 5%. The anxiety relieving aspects of such tests are self evident. Obviously not all cases will have the same happy outcome as the situation outlined here. However patients seeking this information should have genetic counselling before testing and be aware of the equal likelihood of being confirmed as definite carriers. In general, patients who are shown to be carriers are relieved that the uncertainty has been overcome, although disappointed to be confirmed as carriers. It should be emphasised that carrier detection can still be offered if all affected family members have died, provided that healthy males are available so that the disease bearing allele in the mother can be inferred.

We submit this report to bring the subject of carrier detection to the notice of clinicians, particularly those involved with the management of inherited disease. We suggest that optimal management of X-linked agammaglobulinaemia involves not only treating the patient but also offering genetic counselling and its attendant services to the families involved.

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