Genetics of B27-associated diseases—1

J. C. WOODROW

From the Department of Medicine, University of Liverpool, Liverpool

The genetic analysis of those conditions that show familial aggregation, in the absence of recognisable patterns of Mendelian segregation, has proved to be very difficult. A generalised model would allow for the possibility of more than one disease susceptibility gene and for the influence of environmental factors. The pedigree illustrated in Fig. 1 includes patients with Reiter's syndrome (RS), ankylosing spondylitis (AS) and uveitis, ulcerative colitis, and psoriasis. It can be seen that the associations with the HLA-B locus genes enable us to achieve an understanding of some genetic structure in pedigrees of this kind. With regard to the arthropathies being considered at this meeting, clearly the HLA-linked 'ankylosing spondylitis gene' (which may or may not be B27 itself) and genes that play a role in susceptibility to psoriasis and inflammatory bowel disease have to be considered.

If we look first at AS itself I think we must look at two phenomena. Firstly, there are the genetic and environmental factors that determine whether an individual gets or does not get the disease. Secondly, there is the question of the severity. Experience in recent years increasingly suggests that we are dealing here with a very graded phenotype from mild asymptomatic radiologically detectable sacroiliitis to the full picture of severe spinal disease. This evidence comes from the study of members of the families of patients with spondylitis and from studies of populations, particularly those made up of B27-positive individuals.

It seems to me that clinically there is a much greater difference between a B27-positive individual with asymptomatic radiological sacroiliitis and one with very severe sacroiliitis than between the former and a normal B27-negative individual. I think it is going to be just as important to ask why some B27-positive individuals get very severe disease while in others the only evidence of disease is a slight radiological change in the sacroiliac joints. The source of variation in relation to both these questions must be either genetic or environmental or both.

If we accept for the moment that the 'spondylitis gene' is B27 itself then clearly B27 has an essential role in determining whether any degree of sacroiliitis or spondylitis occurs. We can produce a general model (Fig. 2) in which the much increased relative risk associated with B27—something of the order of 100 to 150—is evident but which allows for further variation in liability within B27-negative and B27-positive individuals. This underlying variability could be environmental or genetic. Evidence of important environmental factors comes from a study of identical twins in which it can be
shown that a B27-positive identical twin of a severe spondylitic may be, as far as we can tell, completely free of disease. Fig. 3a, b shows the sacroiliac joints in a pair of identical B27-positive twins, one with grade 4 sacroiliitis and the other clinically and radiologically normal.

There are several ways of looking at the genetics of AS in relation to B27. We can look to see if the risk for homozygous B27-positive individuals is the same as that for heterozygotes, and I think it probably is. We can look at the association of B27 and spondylitis in different populations. Previously I have suggested that the relative risk in B27-positive individuals is apparently constant in different populations* in spite of great variation in the incidence of B27. Other contributors have data at variance with this statement—Editor.

Table 1 presents data from four studies of populations of apparently healthy B27-positive blood donors. Although there is some overall agreement about the incidence of sacroiliitis or spondylitis—that is, of the order of 20%—there are important differences. No doubt there is appreciable selection bias here, but among our own subjects we found only one male who had suggestive symptoms and definite radiological changes. The remaining five males had very mild radiological changes and no relevant history. We found nothing in the females. This contrasts with the study from California.*

Table 1 Incidence of sacroiliitis or spondylitis in four studies of B27-positive individuals

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<th>Authors</th>
<th>B27-positive subjects</th>
<th>Spondylitis</th>
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<td>No.</td>
<td>M. F.</td>
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<tr>
<td>Calin and Fries</td>
<td>78</td>
<td>30 48</td>
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<td>Cohen et al.</td>
<td>24</td>
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<td>Truog et al.</td>
<td>44</td>
<td>8</td>
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<td>Nichol and Woodrow</td>
<td>48</td>
<td>33 15</td>
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spondylitis, firstly, in B27-positive individuals in the general population and, secondly, in the families of affected probands. By relating these we can attempt to dissect out the genetics of the disease a little further.
IV. Aetio-pathogenetic features

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Fig. 3 Appearance of sacroiliac joints in a pair of identical twins aged 52 years, one showing grade 4 sacroiliitis (a) and the other normal appearance (b).

which puzzles me a little because of (a) the high incidence of what seems to have been severe disease, and (b) the relatively high incidence in women. There are obvious problems here because it is extremely difficult to get a completely random sample of B27-positive individuals. I believe we are all selecting a possibly biased population when we do these studies, and perhaps we have been selecting in different ways.

If we accept, for the sake of further argument, that 20% of B27-positive males get some degree of disease there are three simple independent possibilities that can be put forward. (1) That B27 is itself the 'spondylitis gene' and that 20% of individuals are exposed to the appropriate environmental stimulus. (2) That B27 is the 'spondylitis gene' but that 20% of individuals inherit the additional gene or genes necessary for the full genotype. (3) That the 'spondylitis gene' is not B27 but is in linkage disequilibrium with B27 to the extent that it occurs on 20% of chromosomes having B27.

Evidence for the first possibility comes from the study of identical twins previously referred to. Some suggestive evidence for the second possibility—that is, that there are other interacting genes—comes from families of the type shown in Fig. 4. The father, who has a brother with severe spondylitis, is not B27-positive but may have supplied a gene or genes which interacted with B27 from the mother to produce the full genotype in the affected son. I have seen other families like this, and in theory an extensive family study could be designed to prove whether this hypothesis is true.

Evidence for the third possibility has been put forward by those who describe families in which there is disassociation of spondylitis from B27. It would, 

Fig. 4 Pedigree of family of B27-positive proband with ankylosing spondylitis. The B27-negative father has a brother with severe spondylitis.
However, be premature to talk in terms of recombination here without further evidence. In our own family studies, with one interesting exception, over 30 affected relatives of B27-positive (affected) probands were themselves B27-positive. If the hypothesis were true we should now have entirely B27-negative families in which there are two or more spondylitics. Some suggestive evidence for the association between B27 and the Bf polymorphism has been briefly reported from Iceland, but this needs confirmation.

If we now turn to family studies we can ask what are the implications of these simple hypotheses for the expected incidence of disease in B27-positive relatives of probands (Fig. 5). We will look at the parents of spondylitic patients and presume a B27-positive father, because this largely avoids the problem of the variable age of onset and disease expression according to sex. For each of the following models we can suppose an expected frequency of disease.

Assuming, on the first model, that there is no particular environment relevant to AS shared by the members of the family over a considerable period of time, we would expect something like 20% of fathers to have some evidence of disease—that is, no different from the risk for B27-positive individuals in the general population.

On the second model the incidence in fathers would depend on the genetic behaviour of the additional genes. These are unlikely to be recessive. Therefore on a simple basis one might suppose an independently segregating gene of dominant effect which would be present in the B27-positive father in 50% of cases, and therefore we would expect that 50% or thereabouts of the B27-positive fathers to have the disease.

On the third model we would expect all the fathers, if positive for B27, to have the disease, assuming a low rate of recombination.

We have examined 21 B27-positive fathers. Five have definite ankylosing spondylitis and five have only radiological sacroilitis (Fig. 5). Thus 50% of B27-positive fathers had evidence of sacroilitis or spondylitis. This is higher than expected on the first model, and suggests that being B27-positive and exposed to an environmental factor is not the sole basis for development of disease.

The fact that the observed incidence fits the second model raises two difficulties. Firstly, that there is no room for an environmental factor, and, secondly, that identical twins (with the full presumed two gene-genotype) do not always get detectable disease. Out of 15 reported pairs nine have been concordant for AS and six discordant. (However, the problems associated with the ascertainment and selective reporting of disease in twins must lead to considerable caution in drawing general conclusions from the concordance rate.)

If, nevertheless, we accept these data as they stand—that is, that only 60% of those with the right genotype get disease—then on the second model we would expect 30% of B27-positive fathers to show disease in some degree. If we turn to the findings in random B27-positive individuals the implication would be that an independently segregating gene of dominant effect would have to have a gene frequency of 0.2 to give a 20% incidence of disease in this group.

The expected incidence on the third model—that is, a ‘spondylitis gene’ in linkage disequilibrium with B27—is clearly too high to fit in with the observed incidence.

Combining the first and third models, and again assuming that an environmental factor occurs in 60% of individuals, the data do not fit too badly. The implication when we come to the incidence of disease in B27-positive individuals in the population is that the second ‘spondylitis gene’ would have to be present on 35%-40% of chromosomes that have the B27 gene.

In summary, none of these simple models will by themselves explain all the available evidence. The data in regard to concordance in identical twins and to the incidence of disease in B27-positive relatives of probands and in B27 random individuals are all subject to the dangers of biased ascertainment. I hope that with the collection of further data in these fields a more definitive genetic analysis may be possible.
You will notice that in the mothers in the Liverpool study (Fig. 5) there was a much lower incidence. I think this supports the general experience that the disease is much less common in women and does not support the thesis of Calin and Fries in this respect. The pedigree shown in Fig. 6 gives food for thought. One of two B27-positive identical twins has severe spondylitis and the other has asymptomatic radiological sacroiliitis. A further brother, also B27-positive, is clinically and radiologically normal. Yet all three have produced severely affected children.

None of us can be certain at present of the genetic structure of AS, but the evidence I have put forward and the fact of the enormous variability in expression of the disease itself should make us think of other possible genetic factors. Of course, variation in exposure to the relevant environmental agents could also account for variability of expression.

It is interesting to consider the relationship between sacroiliitis or spondylitis and inflammatory bowel disease (IBD) and psoriasis, each of which has its own genetic predisposition. The facts that 40% of patients with spondylitis and IBD are B27-negative, that two groups of workers have shown that there is a considerable incidence of asymptomatic sacroiliitis in B27-negative individuals with IBD, and that it can be estimated that about 50% of B27-positive patients with IBD get spondylitis (which is more than expected from controls) point to genes underlying IBD giving rise to susceptibility to sacroiliitis and spondylitis. Two pedigrees are exceptional in our studies. The first (Fig. 7) shows our only case of disassociation between B27 and spondylitis within a family but ulcerative colitis is present in the B27-negative spondylitic. The second (Fig. 8) shows two brothers with spondylitis, both B27-negative but one with ulcerative colitis.

There is little doubt that genes underlying susceptibility to psoriasis play a role in the genetics of some cases of sacroiliitis and spondylitis. About 30% of patients with spondylitis and psoriasis are B27-negative. Our analysis suggests (a) that genes for psoriasis provide susceptibility to peripheral psoriatic arthritis and to sacroiliitis or spondylitis, and (b) that B27 also provides susceptibility to these two patterns of joint involvement. Some of the cases of spondylitis with psoriasis are, I think, the purely coincidental occurrence of the two diseases in the same patient. The pedigree shown in Fig. 9 illustrates the probable interaction of B27 and genes for psoriasis in predisposing to peripheral psoriatic arthritis.
RS has important genetic relationships with both spondylitis and psoriasis. We would expect RS and spondylitis to occur in the same individuals and in related individuals to a degree greater than chance expectation. This is in fact the case. The pedigree (Fig. 10) shows an example in a woman who developed chlamydia-positive RS on her honeymoon and who shares the same haplotype, including B27, with an uncle and cousin who have spondylitis. Lawrence’s studies confirm the supposition.199

This must lead to a modification of the way we look at sacroiliitis or spondylitis and RS. Finding the former does not mean that it must be a complication of the latter. Six of my patients already had evidence of established spondylitis when they had their first attack of RS. Indeed, we would expect at least 15% of patients with RS to develop sacroiliitis or spondylitis at some stage independently of the RS.

In addition to spondylitis, B27-positive individuals may get any one or more of the following more episodic diseases: lower limb arthropathy of a characteristic type; RS; arthritis reactive to salmonella, shigella, yersinia, and possibly brucellosis184; and anterior uveitis. These may occur at any time before or after the onset of the spondylitis and in varying combinations in the same patient. I have seen one patient who had both shigella arthritis and sexually acquired RS, the genetic basis being the same with the environmental agent being different.

The incidence of spondarthropathies in B27-positive individuals exposed to various environmental stimuli is interesting (Table 2). In those conditions that appear to follow a clear-cut infective stimulus a near 20% incidence of arthritis is rather striking and inevitably suggests a common basis.

Table 2  Incidence of various arthropathies in B27-positive individuals calculated from published data

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<tr>
<th>Clinical syndrome</th>
<th>Authors</th>
<th>% B27-positive individuals affected</th>
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<tr>
<td>Ankylosing spondylitis</td>
<td>Calin and Fries54</td>
<td>20–25</td>
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<td></td>
<td>Cohen et al.64</td>
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<td></td>
<td>Truong et al.315</td>
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<td></td>
<td>Nichol and Woodrow236</td>
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<td>Salmonella arthritis</td>
<td>Friis and Svejgaard31</td>
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<td>Hakansson et al.144</td>
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<td>Aho et al.3</td>
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<tr>
<td>Shigella arthritis</td>
<td>Calin and Fries54</td>
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<td>Keat et al.173</td>
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Within each group all individuals have had the same environmental stimulus. The occurrence of disease in only 20% of B27-positive individuals must be attributed to other genes being necessary for susceptibility or, as mentioned before, to the 'susceptibility gene' being not B27 but close to this B locus and present on 20% of chromosomes having the B27 allele.

We now can relate this to the incidence of spondylitis in B27-positive individuals. I think we must be careful about concluding that there is a common mechanisms here, because this would immediately imply that all B27-positive individuals are exposed to the relevant agent. The evidence from identical twins suggests the contrary. Moreover, the implication would be that all who develop these postinfective arthropathies must get sacroiliitis or spondylitis in some degree. This seems unlikely on the evidence. In other words, the genetic mechanism for these postinfective arthropathies may differ from that for spondylitis.

We have, I think, made progress in understanding the genetic structure underlying these disorders. Much more needs to be done. In particular, further definitive family studies should help to clarify some of the present uncertainties.

General discussion

DR. E. ALBERT: It is difficult not to be impressed by the many families that Dr. Woodrow has shown. Indeed, one of the best ways of identifying previously recognised or new clinical entities is to study families. We have seen beautiful data from Professor Bitter’s computer analysis rounding-up a neat clinical picture. I would like to see such a disease segregate in one or two families with that particular expression. Only then would I say that we have a new disease entity.

Another issue, the genetic background of B27-associated diseases, raises the major question whether B27-associated gene(s) are different when coding for such different diseases as AS, or RS, or reactive arthritis, and so on. Let us assume that they
are different (for this hypothesis is easier to test). A different gene for RS, for AS, and for reactive arthritis would call for allelic forms of a disease susceptibility gene which is associated with B27. This seems unlikely to me. Indeed, if we had different genes, different allelic forms, different susceptibility coding associated with B27, each causing a different form of rheumatic disease, then within a given family the disease expression and set of rheumatological symptoms should be constant.

Alternatively, if we have one gene that broadly determines reactivity to all sorts of environmental risk factors that cause the disease these will determine, to a certain extent, how much variability we are going to see expressed within a population and within a family. But there again the degree of variability of disease expression is going to be best illustrated in monozygotic twins. If environmental influences play a major role we expect discordance. However, we would expect a high degree of concordance if the environmental impact was rather negligible and the gene played the major role. Dizygotic twins should be very different if both environmental conditions and genetic coding are operative.

Dr. Woodrow has shown us discordant pairs of monozygotic twins. That at once indicates that we have a strong environmental influence in the expression of the disease. That in a way—I am sorry, Dr. Woodrow—invalidates right away some of the models that you have produced. You don’t actually have to assume the action of a second gene if a father of a B27-positive ankylosing spondylitic child either lacks the B27 or does not have AS. You don’t have to assume that there is a different gene inherited by the child, you don’t have to assume that there is a second locus involved. To me, the picture is somewhat more simple. I think that we are dealing with one gene that determines the broad susceptibility and that we are dealing with a great variety of environmental influences (chlamydia, mycoplasma, etc.) that may or may not influence more or less strongly the expression of a disease.

For that reason I presume RS and AS are based on the same genetic mechanism. Indeed, we have explored the various possibilities of another gene action (Fig. 11) but, by and large, we do not have any evidence for such a hypothesis—either for an allelic form of B27-associated gene or even for cross-reacting antigens such as B7, B22, B13, and B40. On the other hand, we have looked at the distribution of B27-positive haplotypes associated with ankylosing spondylitis—that is, a certain degree of linkage disequilibrium with an antigen of the A locus (as had been reported by Terasaki)—but we did not find any evidence. We have not ourselves searched for a gene or an allele in the D locus, but the UCLA group (with Dr. Bluestone) has not found any such association.

Finally, Dr. Woodrow has mentioned the study of the Bf locus in AS. This is not all that puzzling to me. Why should there not be a linkage disequilibrium between the AS-associated B27 gene and a Bf type found in that Icelandic population? However, we have no direct information for a second gene.

One other definitely genetic factor I haven’t mentioned is the sex, which directly or indirectly influences the expression of AS and perhaps RS. I do not believe that—as Cepellini used to say—women complain less about their back and therefore we see fewer clinical cases of AS in women. I would rather think that the sex has a major influence on the expression of the disease on the one hand, and, on the other, despite dominant inheritance, we might actually be dealing with a very low degree of penetrance and hence a major environmental impact. Thus the B27-positive son of a B27-positive diseased father does not have to have the disease. From the dominant mode of inheritance of the B27-associated susceptibility genes we conclude that the pathogenesis of, for example, AS is based on a ‘too much’ rather than a ‘too little’, while in recessive diseases we usually have a lacking gene product and one of the two genes required for the product are missing. A dominant disease, on the other hand, is usually caused by a gene that codes for a pathological development such as we see, for example, in ankylosing spondylitis, but we could also assume that the dominant gene codes for too little co-ordination, for example, or defective immune reactivity to bacteria.