Letters to the Editor

Linkage analysis of the receptor gene and MODY

Dear Sir,

Dr. Elbein and colleagues have provided important data on the inheritance of insulin receptor haplotypes in two families with maturity onset diabetes of the young (MODY) [1]. The families (RW and J) have been reported previously and are well-characterised MODY pedigrees [3, 4] with mild diabetes occurring at a young age, transmitted in a strict autosomal dominant fashion. Spouses of diabetic probands had normal glucose tolerance. Recently, we have also excluded close linkage to the insulin receptor locus in a large MODY pedigree [2].

Elbein et al. also report a suggestive co-segregation with diabetes of an uncommon insertional SstI polymorphism in two further families, Va and Ho, but the findings in these families are complicated both by the structure of the pedigrees and the mode of analysis. Both families contain at least one pair of conjugal diabetic patients. This potentially confounding phenomenon is briefly referred to in the text but it greatly complicates analysis as either of the two parents could transmit diabetogenic genes to their children.

It is likely that pedigrees Va and Ho represent an entity separate from classical MODY as the mean age of onset of diabetes in the families is 57 and 41 years respectively. The early-onset diabetic members of these families have probably inherited diabetogenic genes from both parents and may be "homozygous" for a common Type 2 (non-insulin-dependent) diabetes genotype [5].

It is stated that in family Ho all the individuals in generation 3 share allele C with their affected mother, whereas they could equally have received quite separate diabetic determinants from their diabetic father, who himself had two diabetic parents. In family Va two of the members with the symbol I are considered normal, whereas Fajans et al. in earlier reports of this family, used this to represent impaired glucose tolerance as part of the spectrum of diabetes [3]. If the latter is true then this would imply the existence of a further conjugal diabetic pair. No information is given regarding the diagnostic tests used on individuals classified as normal. This pertains especially to pedigree V where all individuals in generation five are considered non-diabetic. These subjects cannot be more than 25 years of age, at which time diabetes may not have expressed itself.

The techniques of linkage analysis used in this study are not entirely appropriate to the family material. The assumption of complete penetrance for MODY assumes 100% confidence in a diagnosis of "non-diabetic", and implies that the subject, whatever his age, will never develop diabetes. In a condition like non-insulin-dependent diabetes, this assumption is not warranted. Some form of age-specific penetrance adjustment should be included in the linkage analysis to avoid obtaining spuriously elevated positive or negative log scores.

The authors also state that due to the rarity of the SstI "–" allele the probability that both pedigrees would demonstrate co-segregation by chance alone was $p < 0.01$; but as co-segregation was not proven the observation is limited to the simple occurrence of an uncommon allele in two separate families, which is not itself of note.

Linkage analysis in large pedigrees is an extremely powerful tool for the localisation of genetic markers for diabetes. However, families used in such a strategy must be rigorously tested for the presence or absence of diabetes, the criteria used for definition of affected and

non-affected must be clearly shown and conjugal diabetic parents avoided if possible. In addition, appropriate adjustments for age-specific prevalence must be included in the statistical analysis.

Yours sincerely,

S.O'Rahilly and R.C. Turner

References

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Response from the authors

Dear Sir,

Drs. O'Rahilly and Turner have raised some important issues with regard to our recent analysis of the potential role of the insulin receptor locus in the etiology of MODY. Firstly, it is interesting to note that these authors have a manuscript currently in press that replicates our major result: Linkage of the insulin receptor locus with MODY may be excluded, at least in the families studies thus far. To the extent that our major conclusion is concordant, there is no discrepancy in our thinking. However, these authors have raised questions regarding the structure of the two smaller pedigrees included in our study, and the issue of age-specific penetrances in modelling the genetic transmission of MODY, and we should like to respond to these comments here.

Our conclusion was based on a linkage analysis using the well-established lod-score method. Calculation of the likelihood is based on a probable expression that encompasses the genotypes at the

marker loci in conjunction with the MODY-affected status of each individual, and their cosegregation pattern under a hypothesized recombination fraction. The presence of conjugal diabetic patients does not complicate the analysis per se, although it is not the optimal mating type for detecting linkage. The information from an affected by affected mating will depend on several factors including the marker genotypes themselves, and phase information. However, this is no a priori reason to exclude such families from analysis. The data contained in the two smaller pedigrees (Ho and Va) will not bias the results; in this case, they simply were less informative than the two larger pedigrees (i.e. the lod score for these families $\rightarrow 0$). The evidence that allows us to reject close linkage ($0 < 0.15$) primarily comes from the two large pedigrees, which Drs. O'Rahilly and Turner accept as well-characterised MODY pedigrees. We agree that families with two diabetic parents are not the *optimal* mating type for linkage analysis, but the results are not biased.

The reduced information in the Ho and Va pedigrees, owing to the conjugal diabetic parents, in conjunction with their small size (recognising that marker data were obtained on a subset of individuals of each pedigree), leads us to the expectation that results of individual analyses of these pedigrees will not reach statistical significance, as stated in our paper. However, an unproved cosegregation between and uncommon insertional SStI polymorphism and MODY was observed. This was noted because it is of interest that a test of the hypothesis that this marker and MODY occurred together as a result of chance alone *could* be rejected ($p \leq 0.01$).

While we stated (and agree) that this does not achieve proof of the rigorous standards of 1000:1 odds for linkage in a given pedigree, and we agree that these pedigrees are not perfect in having clear autosomal dominant inheritance, the reality of frequent and complex disease is that (a) pedigrees may not be large enough to be individually informative; (b) inheritance may not be simple and clear cut autosomal; (c) genes may enter from both sides of the pedigree. We would strongly disagree with the argument that a potentially significant finding (a statistically impressive segregation of an unusual but not rare RFLP known to represent an insertion in the tyrosine kinase coding portion of the gene) should not be reported because it fails to meet strict linkage criteria which can only be met in very large pedigrees with simple patterns of inheritance. Such arguments suggest that we ignore potentially important clues to the aetiology of Type 2 (non-insulin-dependent) diabetes mellitus (MODY or otherwise). Proof of linkage (cosegregation) is of necessity more difficult than proof of non-linkage, and consequently suggestive results, (especially in a disease which may be heterogeneous and may preclude pooled data) must be considered worthy of further evaluation, which is all we suggested.

With regard to age of onset, this is really age of diagnosis and as such may vary according to type of testing and age at which testing was done. We have commented on this problem in the Methods. In the Va pedigree, the individuals in generation 5 were used only to establish phase, and not for linkage analysis. The analysis as performed included only diabetic individuals, not unaffected individuals; and the comments regarding potential diabetes in these individuals are not relevant.

Drs. O'Rahilly and Turner raise a substantive issue regarding the use of age-specific penetrances in modelling the transmission of the MODY phenotype. We actually analysed the data with several different combinations of age-specific penetrances, estimated gene frequencies and mutation rates, and no substantive differences were observed. This stems from several reasons. First, most of the information comes from the single large pedigree RW, in which there are few young individuals still potentially at risk. For this pedigree, the assumption of complete penetrance serves well enough. When you go from a situation of complete confidence in the diagnosis (i.e. complete penetrance), to a situation in which some degree of "error" in the diagnosis is permitted (incomplete penetrance), the analysis becomes less informative, and you would expect the lod scores to be nearer zero. This is what we observed; however, the general shape of the lod surface was unchanged, and still permitted rejection of linkage for $0 < 0.15$, with no evidence for linkage at any recombination value.

Furthermore, Drs. O'Rahilly and Turner do not indicate how such a penetrance function would be derived. If a penetrance function based on typical Type 2 diabetes age of onset curve is included, then the diabetes corrected for is likely not to be MODY in any sense of the word - in other words, MODY implies an early age of onset, and if one is performing linkage with MODY in a pedigree which *has been screened*, development of diabetes after age 40 or 50 must be suspect for linkage. Use of a penetrance function which cannot be justified is certainly worse than assuming complete penetrance (which would increase the significance of a positive finding, not lower it as these investigators suggest). Many geneticists do not include penetrance functions in the analysis and prefer instead to use sporadic frequency as the only correction factor, since good penetrance functions are difficult to derive. We have in fact included a penetrance function in our analysis of Type 2 pedigrees for the insulin gene (in press data) where reasonable data was available to derive such a function.

Yours sincerely,
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Treatment failures in patients with Type 2 (non-insulin-dependent) diabetes

Dear Sir,
We have with interest read the comments by Pontiroli et al. [1] to our paper "Relationship between B-cell function and HLA antigens in patients with Type 2 (non-insulin-dependent) diabetes" [2]. Although we are pleased by the attention our submission has received, we disagree with several of their conclusions. Therefore, we think a reply is warranted.

In contrast to what is claimed in the letter by Pontiroli et al. [1], we did not conclude that patients requiring insulin treatment because of secondary failure to oral hypoglycaemic agents showed reduced insulin release and a HLA phenotype characteristic of Type 1 (insulin-dependent) diabetes. In fact, the term secondary failure was never mentioned in the paper. However, we have dealt with this issue in another report [3].

It is correct that a definition of secondary failure is still lacking. In our mind, patients who cannot be controlled with oral hypoglycaemic agents are failures on these agents, if reasons such as diet failure and intercurrent illness have been excluded. If the goal of treatment is to (nearly) normalise blood glucose concentrations (i.e. to attain a fasting blood glucose concentration < 7.0 mmol/l and postprandial blood glucose < 10.0 mmol/l), failure to achieve this goal should be considered as treatment failure. A subdivision into primary and secondary failures is unnecessary, as the former represents the use of oral hypoglycaemic agents in the wrong place. The statement that treatment failures primarily affects lean patients with a reduction in insulin release seems an oversimplification of the problem. Treatment failures are frequent in obese patients even after controlling for diet and compliance [3, 4]. Insulin resistance, therefore, seems to have an important role in the pathogenesis of treatment failure in obese subjects. To include body weight in the definition of treatment failure would exclude a major part of the patients. Importantly, Pontiroli et al. [1] confirmed our observation of an increased frequency of HLA-Cw4 in patients with Type 2 diabetes. The fact that they could not observe a significant increase in DR5