Molecular diagnosis of myotonic dystrophy

The data summarised in the editorial by Suthers et al on the molecular diagnosis of myotonic dystrophy are very useful, but I fear that the example of Bayesian calculation used to estimate the risk of congenital myotonic dystrophy (CMD) to a fetus may be incorrect. In table 2 the authors use the conditional probability of the size of fetal (CTG), amplification and the conditional probability of fetal (CTG), amplification being the same or greater than the mother's as independent probabilities (that is, they multiplied them). This is only valid if the two observations are independent in the statistical sense, in other words if information about the value of one of them gives you no information about the other. This is manifestly not the case, as to know that (to take an extreme example) the fetal (CTG), amplification is >4.5 kb also tells you that it is very likely to be greater than the mother's. In order to use both sets of data the authors would need to set up two joint conditional probability tables (one for children with CMD and one without). The data are not available in the editorial for this to be derived.

On a further point the authors use a probability of 0.01 as a conservative estimate of the true proportion of infants with CMD who have band sizes the same as their mother's. This was based on the observation that no instances were seen out of 22 cases. Assuming a binomial distribution the 95% confidence limits of the estimate of the true proportion of infants with CMD who have band sizes the same as their mother's from this sample size are 0.0 to 0.13, so that the estimate of 0.01 would not appear to be conservative enough.

Taking these two points together, readers should be cautioned against using the model calculations in table 2 to estimate risks in real clinical situations.

Graeme K Suthers

Kay E Davies

Molecular Genetics Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK

Susan M Hinson

Department of Medical Genetics, Churchill Hospital, Oxford OX3 7JQ, UK

This letter was shown to Dr Suthers et al who reply as follows.

Dr Winter's comments on our suggested approach to risk calculation in the differential prediction of congenital and non-congenital myotonic dystrophy (DM) are very reasonable. We are grateful for the opportunity to discuss the matter further.

It is not yet clear whether fetal (CTG), copy number and fetal (CTG), amplification being the same or greater than the mother's are truly independent events. Among congenitally affected children, copy number is independent of the probability of an increase in copy number as all congenitally affected children reported to date show an increase. It is not known whether these probabilities are independent among children who have the non-congenital form of DM. The experience with the (CGG)_n triple repeat at the fragile X locus would suggest that these probabilities are not independent.1 In the short term the concerns raised by Dr Winter can be addressed by careful calculation of the fetal (CTG), copy number as a conditional probability in a Bayesian risk calculation; the probability of the fetal copy number being larger than the maternal copy number contributes less to the posterior probability.

We acknowledge that our estimate (0.01) of the proportion of congenitally affected fetuses who do not show an increase in copy number may be too low. The number of congenitally affected children who have been examined is small, and we cautioned that the conditional probabilities were based on few observations. Using the data of Tshilidisi et al the best estimate of the proportion of congenitally affected children who fail to show an increase in (CTG), copy number is 0.0; however, as only 22 children were studied, the 95% confidence interval for this proportion is indeed 0.0-0.13. It could be argued that one should calculate a risk interval (rather than a point estimate) for the fetus being affected with congenital DM, thereby making allowance for the small number of congenitally affected children studied. On the other hand, in view of the limited data, it may be more appropriate to use the best estimate of this proportion.

This discussion touches on the general difficulty of taking raw biological data and applying it in a clinical setting. The dynamics of the DM mutation are complex and not well documented. Until more data are available to address the issues raised by Dr Winter, we would urge careful clinical consideration in the application of mutation analysis to the differential prediction of congenital and non-congenital DM.

Weyers' unlar ray/oligodactyly syndrome

We read with interest the paper by Turn- penny et al on 'Weyers' unlar ray/oligodac- tyly syndrome and the association of mid- dle malformations with unlar ray defects'. In 1985 we published a case of craniosynostosis and unilateral unlar apsia associated with pulmonary stenosis. The child was a 5 month old male who showed synostosis of the coronal and metopic sutures, supraorbital flattening, downward slanting palpebral fissures, low set and poste- riorly rotated ears with poorly developed helices, severe microretrognathia, a high arched palate, and a shortened, wide, and redun- tant retromandibular skin. He also had a heart murmur, markedly hypoplastic right forearm with radial deviation, and oligodactaly (only the thumb and the second finger were pre- sent). On x ray examination, beside aplasia of the ulna there was aplasia of the third, fourth, and fifth metacarpals and corre- sponding fingers and a short, proximally dislocated radius. He was thought to have a maldevelopment of the left arm and the lower extremities. At the time of publication the following syndromes were considered for the differential dia- gnosis: Baier-Gerold syndrome which in- cludes craniosynostosis but in which only the radius is involved; Lowry syndrome which shows craniosynostosis but involvement of the fibula only; Herrmann-Pallister-Ot- zinc syndrome which shows craniosynostosis and severe, symmetrically malformed limbs; and Sakat syndrome which shows craniosynos- tosis and polydactyly with shortening of the limbs. The case was considered to be sporadic, since the parents were normal and non- consanguineous and the family history was negative for craniofacial anomalies or limb defects. We think that our patient, who had craniosynostosis, isolated ulnar aplasia, microretrognathia, and un radial defect could add further evidence of a possible defect of the same developmental field involving the limb buds and midline.

Nevertheless, the hypothesis of the pres- ence of a single gene which could cause dysmorphogenesis of midline body struc- tures and loss of specificity in radioulnar development is limited and seems not to fit with the unilaterality of the limb anomaly present in our patient. This observation suggests that the develop- mental timing of these two areas of the body, even though correlated in some way, are also influenced by more complex and independent mechanisms.

The contribution of genetic factors to the pathogenesis of type I (insulin dependent) diabetes mellitus

In a recent review article on the genetics of type I (insulin dependent) diabetes mellitus, Cavan et al correctly state that diabetes is probably "influenced by several genes as well as by environmental factors". To support this view they mention the low concordance rate in identical twins and the average disease risk of about 6% in sibs of a diabetic pro- band. The discussion emphasizes the importance of the HLA antigens, particu- larly DR and DQ, by applying such terms as
'susceptibility determinants', 'protective genes', and 'susceptibility alleles'. The authors conclude that type 1 diabetes is not a single genetic entity but that "to date its genetic basis is unclear, although the HLA-DQ loci are strongly implicated."1

Why is the genetic basis still unclear despite the enormous effort that has been devoted to this subject? For the past 15 years the Medline alone lists over 1700 entries on the genetics of type I diabetes. Many of these reports used susceptibility genes inherited in a simple ( mendelian) mode to explain the genetic basis, some postulating these genes to be linked to the HLA locus.

General agreement seems to exist concerning the importance of the HLA locus. However, understanding is lacking on how the HLA locus and the incidence of diabetes are interrelated. It is the aim of this letter to present a unifying concept for the genetics of type 1 diabetes.

The British twin studies

Identical twins showed a concordance rate of non more than 36%, implying that the true figure is even less owing to inevitable ascertainment bias.2 Because identical twins are genetically identical, such a low concordance rate indicates that non-genetic (environmental) factors contribute importantly to the pathogenesis of type I diabetes. The concordance rate of much less than 100%, provides strong evidence against type I diabetes being conditioned by any simple ( mendelian) mode of inheritance. However, the concordance rate of not more than 36% is of a similar magnitude to the 37.7% concordance rate found in identical twins for cleft lip, a condition which has long been accepted as a multifactorial trait.3

Testing of the immune changes in non-diabetic co-twins of diabetics showed that, once initiated, the autoimmune reaction does not necessarily progress to the total destruction of the β cells, but rather it may remit before insulin production is markedly impaired.4 Thus, the immune changes and the presence of 100% concordance in identical twins both point to a complex rather than a simple aetiology. The DR antigens in identical twins displayed a high prevalence of DR3 and DR4, and low prevalence of DR2.5 However, the most relevant information disclosed by the twin studies is that the genetic contribution to type I diabetes is complex, and that the concordance rate in identical twins falls into the range of a multifactorial trait.

Environmental factors

The seasonal variation in onset of disease which has been recorded in various population studies has to be ascribed to environmental rather than genetic causes.6 An association between type I diabetes and antecedent viral infections has long been postulated. Mumps, rubella, and coxsackie B viruses have frequently been implicated, but a number of different viruses are probably involved if viruses constitute an important factor in the aetiology of the disease.7 Of 173 patients with childhood diabetes treated in the City of New York, 16 (9%) were insulin dependent.8 This is 18 times higher than the estimated 0.5% lifetime average for type I diabetes in a human population.9 The DR3 antigen was significantly more and the DR2 significantly less frequent in diabetic patients than in controls. The DR4 antigen was slightly but not significantly more frequent in diabetic patients than in controls. Congenital rubella patients also displayed increased autoimmune abnormalities, including pancreatic islet cell surface antibodies.10 Thus, type I diabetes in congenital rubella patients displays a similar DR antigen association and similar immune abnormalities as those observed in classical type I diabetes.

Familial accumulation of disease

An accumulation of type I diabetes in families has been well documented. The average risk was estimated to be 4.9% for an offspring of a diabetic parent and 6.6% for the sib of a proband.11 These risks are much higher than the average population incidence of 0.5% reported for the Danish population,12 but they are lower than the 25% and 50% recurrence risks expected for autosomal recessive and dominant modes of inheritance, respectively. However, the estimated risks are of similar magnitude, possibly slightly higher, to those obtained for other multifactorial traits such as anencephaly, spina bifida, congenital dislocation of the hip, and cleft lip.13

The association between type I diabetes and the HLA antigens (HLA-B, DR, and DQ) has been well established. Based upon samples from the Danish population, the absolute risk was estimated to be 75 times higher for the DR3/DR4 than for the DR2 positive phenotype and it was 37.5 times higher for the DR3/DR4 than for the non-DR3/non-DR4 phenotype.4 For the DR3/ DR4 phenotype, 3%, or in 33 subjects, are expected to develop the disease. This contrasts with 0.08% or 1 in 1250 for the non-DR3/non-DR4 phenotype and 0.04%, or 1 in 2500, for the DR2 positive phenotype. These data disclose very wide variation in disease risk from person to person depending on the DR antigens present. Interrelations between HLA-B8 and DR as well as between DR and DQ have also been recorded.11-13 The HLA antigens are inherited. The following example illustrates how the members of a certain family can inherit relatively high risks. A DR3/DR4 offspring inherits the DR3 antigen from one parent and the DR4 antigen from the other parent. A sib has at least a 1/4 probability of also inheriting DR3/ DR4 and at least a 1/4 probability of inheriting either the DR3 or DR4 antigen. In contrast, families lacking the DR3 and the DR4 antigens on average are bestowed with a relatively lower risk of developing disease under similar environmental conditions. Because a high risk is associated (and inherited) with certain HLA antigens, any meaningful disease model should incorporate the contribution of the HLA antigens in addition to allowing for the contribution of non-genetic (environmental) factors.

Synthesis

In accordance with the foregoing observations it is proposed that the HLA antigens represent the principal inherited risk factors, and that environmental factors, particularly certain viral infections, also contribute importantly to the aetiology of type I diabetes. Such wide variation in disease risk is displayed by the HLA antigens, particularly DR and DQ, that they can readily account as the principal liability factors. No susceptibility genes are postulated. Both inherited risk and the severity of detrimental environmental effects are crucial as to whether or not disease will develop. For a subject who has inherited a relatively high risk, relatively less detrimental environmental conditions are needed for the development of disease and vice versa. Consequently, no simple relationship exists between the genetic information and the development of disease. The model is illustrated in the figure.

This is a quantitative multifactorial system because certain risks are associated with the various HLA antigens. In this respect it differs from the general multifactorial model in which multiple genes interact with the environmental factors but the individual genes cannot usually be recognised. The proposed model can incorporate all HLA antigens and antigen combinations (DR, DQ, DP, B, and others, if relevant) as well as the possible contribution of the hypervariable segment adjacent to the insulin locus on chromosome 11.14-15

In absolute terms, the HLA antigens can be arranged according to their risk, even though this may be small for some and as high as 1 in 33 for DR3/DR4 subjects.16 On both chromosomes 6 each normal subject carries a series of HLA antigens. Interactions between antigens have also been recognised.17-19 The higher liability displayed by heterozygous DR3/DR4 than by homozygous DR3/DR3 and DR4/DR4 subjects is explained in quantitative genetics on the basis of heterosis. The possibility that a molecular configuration may furnish a reason...
for the increased risk associated with DR3/DR4 makes the case even more interesting.

The proposed quantitative multifactorial model differs from numerous earlier models which attempted to explain the genetics of type I diabetes on the basis of one or more susceptibility genes. Most earlier models equated phenotypic variation with genetic variation, thereby neglecting the importance of environmental factors that are evident from the British twin studies and described effects of viruses. Also, many earlier studies seem to have been conducted with the foregone conclusion that type I diabetes is caused by one or more mendelian genes and multiplex families were often selected for study. However, for traits with a complex aetiology the selection of certain families will lead to distorted conclusions. The following data may illustrate this point. In a series of 331 newly diagnosed cases of type I diabetes among Caucasians in Pittsburgh, USA, only 26 (8%) had first degree relatives (parent and/or sib), while the others were sporadic cases. Familial and sporadic patients displayed similar autoimmune characteristics, but heterozygous DR3:DR4 patients occurred slightly but not significantly more frequently among the familial cases. No compelling evidence seems to exist for any marked differences in the pathogenesis between familial and sporadic cases. However, for a genetic study the outcome will be different depending on whether (1) only multiplex families, (2) only sporadic cases, or (3) all cases of type I diabetes are included. To limit a genetic study to multiplex families alone or to simplex cases alone (an unlikely approach) is bound to furnish erroneous results. It should therefore become obvious that for a disease with a complex aetiology, such as type I diabetes, all cases need to be considered in order to obtain a meaningful conclusion concerning the genetic contribution to the disease development.

Information pertinent to the multifactorial model, such as risk figures for various HLA phenotypes and recurrence risks for sibs, has already been published. When estimating the lifetime risk for 11 different DR phenotypes, Sveigaard et al. concluded that "quite a number of different alleles may be involved" in the pathogenesis of type I diabetes. This is in full agreement with the multifactorial model. However, the authors encountered problems in resolving the higher risk for sibs of probands than for individual subjects in the population. With the proposed model this is explained by an increased similarity of environmental factors and possibly also by a higher similarity of other HLA antigens, such as DQ, among sibs than among unrelated subjects.

The risk to sibs according to DR antigens shared, as presented by Thomson et al., agrees nicely with the multifactorial model. The overall average risk was 6% for sibs of a proband. The lowest average risk (1.8%) was estimated for a sib sharing no haplotype with the proband, and the highest average risk (19%) for a DR3:DR4 sib sharing two haplotypes with the proband.

A higher ratio of type I diabetes was detected among the children of fathers with the disease than among the children of affected mothers. This difference could not be explained by a possible sex difference in the threshold of the disease nor by a difference in perinatal mortality. The transmission of protective antibodies from the mother to her offspring could be one cause for the distortion, since only the mother and not the father, can transmit protective antibodies to the offspring. Viral infections which contributed to the development of type I diabetes in the mother may have induced the production of protective antibodies which she could transmit to her offspring and furnish protection against certain potentially damaging infections early in life.

The nature of the pathogenesis of a defect may provide an indication of the involvement of genetic factors. Biochemical defects are usually conditioned by single gene differences, while developmental defects frequently result from an interaction of genetic and environmental factors. In type I diabetes, the loss of the $\beta$ cells constitutes the principal lesion of the defect, which may result from viral invasion, or presumably more often, from an autoimmune reaction against the $\beta$ cells. Thus, the main disease process takes place at the cellular and not at the molecular level. Also, the autoimmune reaction, once initiated, may progress to the total destruction of the $\beta$ cells, or it may remit before insulin production is markedly impaired.

The disease process in type I diabetes clearly points to a complex rather than a simple course, thus favouring the proposed multifactorial model over any single gene model.

By incorporating the known effects of the HLA antigens and allowing for contribution of environmental factors, the proposed quantitative multifactorial model can integrate the inherited and environmental effects contributing to the pathogenesis of type I diabetes. Only a certain disease liability and not the disease itself is inherited, that is, the inherited disease liability is a quantitative rather than a qualitative entity.

FRED J GRUNDBACHER
Department of Basic Sciences,
University of Illinois College of Medicine at Peoria,
Box 1649,
Peoria, IL 61614, USA.


The contribution of genetic factors to the pathogenesis of type I (insulin dependent) diabetes mellitus.

F J Grundbacher

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