Genetic determinants of diabetic nephropathy

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ABSTRACT

Diabetic nephropathy is the most serious complication of diabetes mellitus. Progression of the condition leads to end-stage renal failure, and other complications of diabetes are also common in this group of patients. The onset of overt albuminuria in a patient with diabetes heralds an increased risk of death, particularly from cardiovascular disease. There is considerable evidence to show that nephropathy is influenced by genetic factors. Epidemiological studies show that only a minority of patients with diabetes develop nephropathy irrespective of glycaemic control, suggesting that a subgroup of patients are at higher risk of nephropathy. Marked ethnic variation is observed, with nephropathy being more common in certain ethnic groups. Familial clustering of nephropathy is also observed. Parental history of hypertension, diabetes or cardiovascular disease appears to predispose to nephropathy in patients with diabetes. A number of methods are available to dissect polygenic disease: animal models, genetic association studies (case-control studies), affected sib-pair studies, discordant sib-pair studies and transmission distortion analysis. Most published work has been based on association studies. Association studies have shown conflicting results often due to small numbers of cases and controls, and poor phenotypic characterization. The angiotensin-converting enzyme gene insertion (I)/deletion (D) polymorphism has been studied in detail, but does not appear to be a strong risk marker for nephropathy. It does, however, appear to have a role in response to angiotensin-converting enzyme inhibition, with II homozygotes being the most responsive and DD homozygotes the least. A number of other genetic loci have also shown positive associations with nephropathy, including apolipoprotein E, heparan sulphate and aldose reductase. More recently, affected sib-pair analysis and discordant sib-pair analysis have suggested possible genetic loci on chromosomes 3, 7, 9, 12 and 20. These have yet to be reproduced in larger numbers of families, and the specific gene regions on these chromosomes remain elusive. The evidence presented in this review strongly supports the role of genetic factors in nephropathy. Detection of strong genetic risk markers for nephropathy will allow further insights into the pathogenesis of nephropathy, and possibly the development of novel therapeutic agents for its treatment. It will also allow preventive therapy to be directed at those patients with the greatest risk for development of diabetic nephropathy.

INTRODUCTION

In patients with diabetes mellitus, albuminuria is associated with significantly reduced life expectancy and quality of life, due to the dual complications of end-stage renal failure and atherogenic cardiovascular disease. The condition is the most serious complication of diabetes mellitus, accounting for a high proportion of new cases of end-stage renal failure, and a 40–100-fold excess mortality from cardiovascular disease in these patients [1]. Possibly

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Abbreviations: ACE, angiotensin-converting enzyme; HLA, human leucocyte antigen.
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as a result of improvements in glycaemic control and blood pressure therapy, the incidence of diabetic nephropathy appears to be declining, and overall prognosis is improving [2]. Although recent insights into the natural history of diabetic nephropathy have enabled treatment strategies to be considered earlier to modulate the progression of the condition [3], the pathogenic mechanisms involved in initiation are as yet unknown.

The pathogenesis of nephropathy has been widely investigated. Hyperglycaemia is necessary but not sufficient for the development of diabetic nephropathy. Haemodynamic and metabolic factors also appear to have a role, but there is also strong evidence that hereditary factors are pivotal in the development of nephropathy, particularly in Type I diabetes. Although a high proportion of patients with end-stage renal failure as a result of diabetic nephropathy have Type II diabetes, genetic research has concentrated on patients with Type I diabetes. This is probably because of the genetic heterogeneity of Type II diabetes, as illustrated by the variable phenotype of the condition (ranging from obese, insulin resistant to lean, insulin deficient), and also the fact that proteinuria in Type II diabetes may be due to non-diabetic factors such as hypertension or chronic infection [4].

This article considers the evidence for genetic factors in the pathogenesis of diabetic nephropathy and the methods by which these genetic determinants can be investigated, and reviews recent progress in the search for such factors. To date, most research has concentrated on Type I diabetes, but some work in Type II diabetes will be discussed.

EVIDENCE FOR GENETIC FACTORS

Epidemiological studies
It has been suggested that genetic factors are important in the pathogenesis of diabetic nephropathy for over two decades. This hypothesis was initially based on epidemiological observations, showing that diabetic nephropathy appears to affect only a minority of patients with Type I diabetes. The largest epidemiological study to date examined over 300 Scandinavian patients with Type I diabetes over a period of 40 years [5]. It was noted that the cumulative incidence of diabetic nephropathy rose to a peak at around 20 years from diagnosis of diabetes, to approximately 30%, and then plateaued, so that the initiation of diabetic nephropathy after 20 years of Type I diabetes was uncommon. Similar findings were also noted in a later study from Boston of 292 subjects with Type I diabetes over a period of 40 years [6]. These patterns of incidence were all the more remarkable because they contrasted markedly with those seen for diabetic retinopathy, where there is an increase in prevalence of retinopathy with longer duration of diabetes [7]. Some degree of retinopathy occurs almost universally in Type I diabetic subjects within 20 years of diagnosis, although around 75% progress to proliferative retinopathy after 40 years of diabetes. It is also of note that no major difference in glycaemic control was seen in the patients with nephropathy compared with those without, and a large number of patients remained free of nephropathy despite prolonged severe hyperglycaemia. It has thus been suggested that a subset of patients with Type I diabetes are at a particularly high risk of nephropathy due to non-metabolic (possibly hereditary) factors.

Ethnic variation
Prevalence rates of diabetic nephropathy in subjects with Type II diabetes show a marked ethnic variation. Higher rates of diabetic renal disease are seen in Indo–Asians in the U.K. [8], in African–Americans [9] and Mexican– Americans in the U.S.A. [10], and in Nauruans [11] and Pima Indians [12]. The reason for this inter-racial difference in incidence of diabetic nephropathy is unclear, but ethnic variation in genetic susceptibility to nephropathy is a possibility. It is of note that these ethnic groups not only have a very high incidence of Type II diabetes, but also a high incidence of hypertension. This may suggest that differences in genetic predisposition to hypertension may contribute to the higher prevalence of nephropathy in certain racial groups, although an alternative explanation is that the presence of hypertension may accelerate an already present renal disease and lead to the condition becoming clinically apparent more quickly.

Familial clustering
Strong evidence for genetic factors being important in nephropathy is provided by family studies. Seaquist et al. [13] examined probands of Type I diabetic patients with and without diabetic nephropathy, and found 83% of diabetic siblings of probands with nephropathy had evidence of nephropathy, whereas this figure was only 17% in diabetic siblings of probands without nephropathy. This finding was reproduced in a Danish cohort, although the figures were considerably lower at 33% and 10% respectively [14]. It was the magnitude of difference between these two studies that led Quinn et al. [15] to undertake a much larger study to clarify the issue. They examined 110 probands for nephropathy status and found that the cumulative risk of nephropathy was 71.5% if the proband had persistent proteinuria, but only 25.4% if the proband did not. The authors surmised that this difference was consistent epidemiologically with a major autosomal dominant gene effect. Further support for the genetic hypothesis was given by examination of the cohort investigated in the Diabetes Control and Complications Trial [16]. A total of 217 probands with Type I
Inherited predisposition to hypertension and cardiovascular disease

In 1987, Viberti et al. [18] examined 17 Type I diabetic patients with proteinuria and matched them with 17 normoalbuminuric Type I diabetic patients. Examination of surviving parents of these two cohorts found that the mean systolic blood pressure was 15 mmHg higher and the diastolic blood pressure 8 mmHg higher in the parents of the proteinuric subjects compared with the parents of the normoalbuminuric subjects. This finding was subsequently reproduced in larger cohorts by questionnaire [19,20]. Increased activity of the sodium/lithium countertransporter – a possible marker for hypertension [21] – has been noted in patients with diabetic nephropathy and their parents [22,23]. This possibly reflects overactivity of the sodium/hydrogen antiporter which has also been noted to be elevated in patients with diabetic nephropathy [24]. More recently, data from the EURODIAB Type I diabetes complications study have shown that mean age-adjusted blood pressure is significantly higher in those patients reporting parental hypertension (123/77 mmHg) than in those reporting no parental hypertension (120/75 mmHg), although the clinical significance of such a difference in blood pressure is unclear [25]. Thus, parental hypertension appears to increase the risk of hypertension in diabetic offspring, although this does not necessarily imply an increased risk of nephropathy.

A further finding of interest is the observation of clustering of cardiovascular disease in parents of patients with nephropathy. This was first reported by Earle et al. [26] in 1992, who examined 61 Type I diabetic patients with proteinuria matched with 61 Type I diabetic patients with normoalbuminuria. Cardiovascular disease was a more frequent cause of death in the parents of the proteinuric patients than in the parents of the normoalbuminuric patients (40% compared with 22%). A more rigorous examination was carried out recently by De Cosmo et al. [27], who studied the parents of 31 Type I diabetic patients with an albumin excretion rate > 45 mg/min matched with 31 Type I diabetic patients with normoalbuminuria. The parents of the proteinuric group died at an earlier age, and had a significantly higher prevalence of arterial hypertension, hyperlipidaemia, lipoprotein(a) and insulin resistance than the parents of the normoalbuminuric patients.

The link between albuminuria, cardiovascular disease and hypertension may be modulated by an inherited predisposition to insulin resistance. It has been noted that Type I and Type II diabetic patients with nephropathy have increased insulin resistance compared with normoalbuminuric diabetic patients [28,29]. Increased insulin resistance has also been noted in the parents of Type I diabetic patients with proteinuria [30]. In Type II diabetes, first degree relatives of patients with nephropathy have been noted to have an increased albumin excretion rate and increased insulin resistance [31].

METHODS FOR INVESTIGATING GENETIC DETERMINANTS OF NEPHROPATHY

A number of methods are available for examination of genetic determinants of diabetic nephropathy (Figure 1).

Animal models

Animal studies can provide powerful tools to localize disease genes. Selection of a particular disease, and then sibling mating over multiple generations, will give rise to progeny enriched with disease-causing genes. Thus, such animal models for renal disease may provide a clue to the genetic aetiology of human renal disease. Recently, two distinct gene regions – Rf-1 and Rf-2 – have been found to be linked to renal disease independent of hypertension in the fawn-hooded rat [32]. This is a rat strain that is genetically hypertensive, and develops early onset chronic renal failure. Using synteny maps (maps of conserved gene regions among mammalian species), it may be possible to test for regions of the human genome homologous to those containing Rf-1 and Rf-2. This
Figure 1 Methods for examining genetic determinants of diabetic nephropathy

**Association studies**

**Case-control analysis**

A frequently used approach to examine genetic determinants in diseases with complex inheritance is to undertake genetic association studies. This approach has been used with some success to investigate the genetics of diabetes mellitus, multiple sclerosis, hypertension, coronary artery disease and a number of other polygenic conditions. Essentially this type of study requires large numbers of patients with a condition of interest, and a control group of patients with no evidence of the condition, and who are unlikely to develop the condition. In Type I diabetes, diabetic nephropathy can be adequately phenotyped using simple clinical criteria such as the presence of persistent proteinuria in the absence of other causes, hypertension and retinopathy. Absence of nephropathy can be reasonably surmised if the patient has normoalbuminuria after duration of diabetes of more than 20 years. The numbers of cases and controls required in this type of study are empirical as the number of genes involved is unknown, but cohorts in excess of 200 patients in each group are probably required for adequate power.

The candidate genes examined in these types of studies are selected by virtue of their possible role in the pathogenesis of the condition (Table 1). For example, as hypertension and cardiovascular disease are common in diabetic nephropathy, genes that modulate these two conditions may also be involved in the pathogenesis of nephropathy. Although this method may be useful for screening a number of genes for involvement in nephropathy, there are many putative candidate genes, and examination of them all could be time consuming and inefficient. Nevertheless, the majority of studies in this field are genetic association studies.

Association studies can give misleading results as a consequence of a number of confounding factors [33]. Population admixture (mixture of various races analysed together) can cause artificial association if a study includes genetically distinct populations. Furthermore, population stratification (founder effect) can also give rise to false positive results. Multiple hypothesis testing and subgroup analysis, compounded by publication bias (publication of only positive results), will also give an overestimate of the significance of positive associations. Also, inadequate numbers of cases and controls, or inadequate phenotyping (i.e. ensuring presence of the
Table 1  Putative candidate genes for diabetic nephropathy

<table>
<thead>
<tr>
<th>Hypertension/Cardiovascular disease</th>
<th>Angiotensin-I converting enzyme</th>
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<td></td>
<td>Angiotensinogen</td>
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<td>Angiotensin II type 1 receptor</td>
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<td>α-Adducin</td>
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<td>Atrial natriuretic peptide</td>
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<td>Platelet activator inhibitor-I</td>
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<td>Platelet glycoprotein IIIa</td>
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<td>Paraoxonase</td>
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<td>Extracellular matrix proteins</td>
<td>Heparan sulphate core protein</td>
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<td>Type IV collagen</td>
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<td>Lipids</td>
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<td>Apolipoprotein E (ApoE)</td>
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<td>Cholesterol ester transfer protein</td>
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<td>Others</td>
<td>Apolipoprotein A1</td>
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<td>Human leucocyte antigen</td>
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<td>β-Adrenergic receptor</td>
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<td>Transforming growth factor-β</td>
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reference condition of interest), can give rise to false positive or false negative results (type 2 error).

Transmission disequilibrium analysis
A second method of association study is to use a family-based analysis of disease susceptibility genes using the transmission disequilibrium (or distortion) test [34]. This method requires DNA from an affected patient, and their parents, and examines the transmission of alleles of the gene in question from a heterozygous parent to the affected offspring. Significant variance from the expected Mendelian ratio of 50:50 would suggest that the allele has a role in susceptibility to the disease in question, and association is demonstrated. Although this method removes the risk of population stratification giving rise to false positive results, it can give rise to false positive or false negative results if the numbers used are inadequate.

Linkage studies
As a result of the above concerns regarding association studies, it has been propounded that any positive association needs to be replicated in a separate population, and also in family studies. These have been used to great effect in the dissection of the genetics of Type I diabetes itself [35], and can potentially also be of use in diabetic nephropathy. One method of using families to examine genetic determinants of disease is the affected sib-pair approach. This requires a large number of families with two or more siblings with Type I diabetes who also both have diabetic nephropathy, and their parents (generally unaffected), and areas of interest are screened for linkage to disease. Using sib-pair families, the entire human genome can be screened using highly polymorphic markers throughout the human genome to test for linkage of chromosomal regions to disease (genome-wide screening). This has been facilitated using semi-automated fluorescent-based technology allowing large-scale genotyping of large numbers of samples. Recently, discordant sib-pair analysis has been used, as it requires fewer families to achieve the same power as the affected sib-pair approach [36].

GENETIC ASSOCIATION STUDIES IN DIABETIC NEPHROPATHY

Human leucocyte antigen and insulin genes
A large number of studies have been undertaken to determine which genetic factors are of importance in diabetic nephropathy. Most of these studies have taken the form of case-control or association studies using a candidate gene approach. The earliest studies examined the human leucocyte antigen (HLA) loci, because twins with Type I diabetes showed marked concordance for retinopathy status, especially those with the DR3/4 genotype [37]. Examination of HLA identical non-diabetic siblings of Type I diabetic probands showed basement membrane expansion, which is not observed in HLA non-identical siblings, suggesting that even in the absence of diabetes, microangiopathy may occur due to HLA-related genetic factors [38]. Barbosa and Saner [39] have reviewed the early studies of HLA associations with nephropathy, and positive and negative associations with A2, B8, DR4 and DR3/4 have been reported. In our association studies of HLA loci in large cohorts of Type I diabetic patients with and without nephropathy (n = 675), we found no positive or negative associations with diabetic nephropathy [40].

A further region examined in diabetic nephropathy is the insulin gene region, which has been implicated in premature atherosclerosis, and hence is a suitable candidate gene for nephropathy [41]. An excess of class I allele homozygotes in patients with nephropathy has been described [42], but again this finding has not been confirmed in our own larger cohorts [40].

Genes of the renin–angiotensin system
Among the candidate genes suggested for nephropathy, genes of the renin–angiotensin system have attracted the most interest. There is considerable evidence to suggest...
disturbance of the renin-angiotensin system in diabetic nephropathy. Prorenin, renin, angiotensin-converting enzyme (ACE) and angiotensin II levels are all noted to be elevated in diabetic nephropathy [43]. Furthermore, genes of the renin–angiotensin system have been suggested as being genetic determinants for both hypertension and cardiovascular disease, both of which are common in patients with diabetic nephropathy. Thus, genes of the renin–angiotensin system are suitable candidate genes for diabetic nephropathy.

**Angiotensin I-converting enzyme gene**

The Insertion (I)/Deletion (D) polymorphism of the ACE gene is responsible for a large proportion of the genetic variation in serum ACE levels [44]. Thus the II genotype is associated with low levels of serum ACE, whereas the DD genotype is associated with high levels, with ID genotypes having intermediate levels of ACE. This gene polymorphism has been investigated for a role in the susceptibility to coronary artery disease, and DD genotype carriers have been noted to be at higher risk of coronary artery disease compared with other genotypes. In diabetic nephropathy two small studies have suggested an association between the D allele of the ACE gene and nephropathy [45,46]. However, our own and other subsequent large studies examining over 1000 patients with and without nephropathy have shown no association between nephropathy and the D allele [47–49]. A recent meta-analysis of published data has suggested a weak association of the D allele with nephropathy in Type I diabetes, although the major proviso of publication bias (lack of publication of negative results) has not been addressed in the study [50]. Overall, the cumulative results from a large number of studies suggest that if the ACE gene has any effect, it is likely to be small, and it is not useful as a screening marker for nephropathy. The ACE D allele has been suggested as a risk factor for coronary artery disease in patients with nephropathy [51]. This subgroup analysis had no *a priori* hypothesis, and involved very small numbers of patients; hence the results are likely to be due to a type 2 error. The ACE D allele may have a role in the progression of, rather than susceptibility to, diabetic nephropathy. This is supported by the observation that the presence of the DD genotype confers a reduced responsiveness to ACE inhibition and an increased rate of decline of glomerular filtration rate [52]. More recently, examination of 530 patients with Type I diabetes as part of the EURODIAB study has shown that the ACE gene polymorphism predicts the beneficial effect of ACE inhibition [53]. Thus, patients with the II genotype are expected to have a 51.3 % lower albumin excretion rate after 2 years of lisinopril, compared with a 14.8 % reduction in the ID group and a 7.7 % reduction in the DD group. This failure of renoprotective therapy according to ACE genotype has also been described in non-diabetic renal failure [54].

However, only long-term prospective studies will truly ascertain whether the ACE gene modulates disease progression.

**Angiotensinogen gene**

Linkage of the M235T polymorphism of the angiotensinogen gene has been demonstrated in essential hypertension [55]. This polymorphism has also been examined in diabetic nephropathy, again with conflicting results. In a small study of 95 patients with nephropathy, the presence of the TT genotype was associated with nephropathy [56]. In our own much larger study [49], and others [57], no such association was found, although one study has suggested an association of the TT genotype with elevated blood pressure in patients with nephropathy [58]. Again, this subgroup analysis is based on small numbers of patients.

**Angiotensin II type 1 receptor gene**

The A1166C polymorphism of the angiotensin II type 1 receptor gene has also been linked to essential hypertension [59], and thus has been examined in diabetic nephropathy. Two large studies suggested no role for this polymorphism in diabetic nephropathy [60,61], although one smaller study suggests that the presence of the C allele in combination with poor glycaemic control is associated with nephropathy [62].

**Aldose reductase**

A number of other candidate genes have been examined in diabetic nephropathy. The enzyme aldose reductase has been implicated in a number of microangiopathic complications of diabetes. A dinucleotide repeat polymorphism of the aldose reductase gene locus has been suggested to be strongly associated with nephropathy in Type I diabetes [63]. This finding, however, is based on small numbers and has not been replicated in our own larger cohorts [63a] and other groups [64].

**Extracellular matrix proteins**

Loss of the proteoglycan, heparan sulphate, from the glomerular basement membrane may be of major importance in the pathogenesis of diabetic nephropathy, and forms the basis of the ‘Steno Hypothesis’ [65]. One study has shown a significant association with nephropathy of a *BamH1* polymorphism of the gene encoding heparan sulphate core protein (HSPG2) [66]. This has been replicated in two separate cohorts of diabetic subjects. The polymorphism is located within a domain of the core protein responsible for binding heparan sulphate chains, and the high risk 250 bp allele may attenuate binding of the heparan sulphate chains, and hence lead to loss of heparan sulphate from the basement membrane. Examination of the polymorphism within the collagen IV α1 chain gene has shown no association with nephropathy [67].
Genes affecting lipid metabolism
We have recently reported a positive association of the $\varepsilon2$ allele of the apolipoprotein E gene polymorphism, which has been reproduced in a second separate cohort, and is also confirmed by stratification of patients with recent onset diabetes by albumin excretion rate [68]. This finding is of particular interest as physiological studies suggest lipid abnormalities may play a role in the pathogenesis of diabetic nephropathy, and lipid-lowering therapy may attenuate the progression of nephropathy [69]. Similar findings have been published recently by a group examining 162 German subjects with Type I diabetes – patients with the $\varepsilon2$ allele were noted to have reduced creatinine clearance and elevated albumin excretion rate compared with those without the $\varepsilon2$ allele [70]. Furthermore, examination of this locus using the transmission disequilibrium test has confirmed this positive association [71].

Other genes
Polymorphisms of the interleukin-1 gene cluster have also been examined, as this locus appears to have a role in mediation of inflammation. One study has suggested an association with the interleukin-1 gene RN$^2$ allele and diabetic nephropathy [72], although this finding has been refuted in a much larger study [73]. Other polymorphisms examined in Type I and Type II diabetes where no associations have been found include transforming growth factor-$\beta$ [74], $\beta$_2-adrenergic receptor [75], platelet glycoprotein IIIa [76], nitric oxide synthase [77], atrial natriuretic peptide [77], bradykinin receptor and paraoxonase [77], although a weakly positive association with the endothelial nitric oxide synthase gene has recently been reported [78]. A polymorphism of plasminogen activator inhibitor-1 has also been reported as showing a weak association with nephropathy [79]. The methylenetetrahydrofolate reductase gene polymorphism has also been examined in patients with Type II diabetes and nephropathy [80]. This is an enzyme involved in the transmethylation pathway by which homocysteine is converted into methionine, and polymorphism of the gene encoding methylenetetrahydrofolate reductase has been implicated in coronary artery disease [81]. Sixty-four percent of patients with nephropathy had a mutated allele compared with 36% of patients without nephropathy, but again the number of cases and controls in this study was small.

FAMILY STUDIES IN DIABETIC NEPHROPATHY
Genetic association studies are subject to the biases that affect all case-control studies; namely selection bias, publication bias and population stratification (founder effect), all of which can give rise to false negative and false positive results [82]. The chances of this type of bias influencing results can be reduced in association analyses by using large numbers of cases and controls, adequately phenotyping cases and controls, and ensuring ethnic homogeneity. As illustrated above, highly conflicting results can be obtained as a result of small sample sizes and poor phenotyping of cases. It is thus imperative that any positive association analyses should be followed up by family analyses. Linkage analysis using concordant or discordant sib-pairs is very difficult in diabetic nephropathy, as adequate numbers of sib pairs may not be found due to the high premature mortality rate of patients with nephropathy.

Sib-pair analyses
Despite the difficulties of sib-pair analysis, a number of groups are now reporting findings from such studies, using family-based cohorts. In one early study examining 38 African–American affected sib-pairs, no evidence of linkage was demonstrated, although the numbers may have been too small to detect linkage [83]. In Caucasian Type II diabetic sib-pairs with diabetic nephropathy, linkage has been demonstrated to markers on chromosome 12 and 20 although once again the number of sib pairs in this study was small [84]. In a total genome screen of 98 Pima Indian sib-pairs with nephropathy, linkage with four regions has been demonstrated, the strongest being on chromosome 7 with other regions on chromosomes 3, 9 and 20 [85]. On chromosome 7, there are three known genes which may affect the development of diabetic nephropathy: aldose reductase, T-cell receptor $\beta$-chain and nitric oxide synthetase, all of which need to be examined further in large association and family studies. Most recently, results of a discordant sib-pair analysis have been reported [36]. A total of 66 discordant sib-pairs were examined, and linkage was demonstrated to a region within a 20 cM area on the long arm of chromosome 3. This study demonstrated no evidence of linkage to the angiotensinogen or ACE gene loci, but the region on chromosome 3 contains the angiotensin II type 1 receptor gene, which has previously been suggested as associated with nephropathy in the presence of poor glycaemic control. Other known genes in this region include the sodium/potassium ATPase $\beta$ subunit, glyco- genin and glucose transporter type 2 genes, although their relevance to nephropathy is not clear.

Transmission disequilibrium analyses
Family-based association analysis using the transmission disequilibrium test can be used instead of linkage analysis. This involves analysis of the frequency of transmission of designated alleles from heterozygous parents to affected offspring, and has been used to confirm linkage of Type I diabetes to the insulin gene region [34]. This method has
been used for the first time in the genetics of nephropathy to examine the angiotensinogen gene [86]. Data from the parents of 148 Type I diabetic subjects with nephropathy and 62 without nephropathy were collected. Overall, there was no evidence of preferential transmission of the T allele from parents to patients with nephropathy. However, on subgroup analysis, there was evidence of preferential transmission of the T allele to males with nephropathy, and to those patients with end-stage renal failure. The validity of this subgroup analysis is uncertain, particularly as the numbers with end-stage renal failure were small, and gender subdivision significantly reduces the power of the study. This method has, however, shown a positive association for the apolipoprotein E ε2 allele [71] and the endothelial nitric oxide synthase gene [78].

CONCLUSIONS

Diabetic nephropathy remains an important long-term complication of diabetes, responsible for greatly increased morbidity and premature mortality. Epidemiological data and family studies show that inherited factors are likely to be important in the pathogenesis of the condition. Numerous association analyses have now been carried out in large numbers of small patient cohorts, with predictably conflicting results, and it remains unclear from these studies which genetic determinants are important in the condition. Large collaborative and prospective studies are required to determine which associations may be of greatest relevance.

It is also extremely important that family-based studies are undertaken. This will almost certainly involve a multi-centre collection of patients, parents and siblings in order to conduct family-based association analyses. The British Diabetic Association is currently sponsoring a multi-centre collection of trio pedigrees (patients with nephropathy and their parents) in the United Kingdom. The use of affected sib-pairs is complementary to this, as loci found on linkage analysis can be further investigated using the transmission disequilibrium test. A number of groups are undertaking this approach in large numbers of small patient cohorts, with predictably conflicting results, and it remains unclear from these studies which genetic determinants are important in the condition. Large collaborative and prospective studies are required to determine which associations may be of greatest relevance.

The search for genetic determinants of diabetic nephropathy is an important area of research. Finding genes that contribute to nephropathy may enable targeting of therapy to patients at risk, the development of novel therapeutic agents and insights into the pathogenesis of the condition. In this way, we may be able to reduce the burden of end-stage renal failure and cardiovascular morbidity seen in these patients.

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