SUMMARY - The role of genetics in diabetic renal disease has been suspected on the basis of follow-up and familial studies. Barely half of Type 1 patients who develop a diabetic retinopathy also develop nephropathy, and the relative risk of nephropathy for a diabetic proband is around 3 if a sib is affected. Candidate genes for diabetic nephropathy can be divided into two categories: those affecting glucose metabolism in target organs of diabetic microangiopathy, and those affecting renal changes in response to hyperglycaemia. The role of angiotensin-I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has been suspected for several years. Evidence of its possible role in the development and progression of diabetic renal disease is presented here.

Key-words: genetics, follow-up studies, family studies, candidate genes, diabetic nephropathy, angiotensin-I-converting enzyme (ACE), polymorphism.

RÉSUMÉ - Facteurs héréditaires dans le développement de la néphropathie diabétique.
Le rôle de la génétique dans le développement de la néphropathie diabétique a été suspecté à partir d’études de suivi de cohortes et d’études familiales. A peine la moitié des diabétiques de type 1 développent une néphropathie diabétique lorsqu’ils ont développé une rétinopathie diabétique, et le risque relatif de néphropathie pour un germain de sujet affecté est de trois environ. Les gènes candidats pour la néphropathie diabétique peuvent être classés en deux catégories : ceux qui peuvent affecter le métabolisme du glucose dans les organes cibles de la microangiopathie diabétique, et ceux qui peuvent modifier la réponse rénale hyperglycémique. Le rôle du polymorphisme d’insertion/délétion de l’enzyme de conversion a été suspecté depuis plusieurs années. Les arguments en faveur de ce rôle dans le développement et la progression de la néphropathie diabétique sont présentés ici.

Mots-clés : génétique, néphropathie diabétique, suivi de cohortes, études familiales, gènes candidats, polymorphisme, enzyme de conversion.
A study published by Siperstein et al. in 1968 suggested that capillary basement membrane enlargement (a typical sign of diabetic microangiopathy) precedes diabetes and that Type 1 diabetic complications could thus be genetically determined independently of glycaemic level [1]. However, considerable experimental and clinical data have accumulated that contradict this possibility and indicate that anatomical and functional signs of diabetic nephropathy are acquired and secondary to hyperglycaemia and its consequent disorders [2, 3]. Pipart [4] produced a large 25-year prospective follow-up study in diabetic patients, establishing that the development of diabetic complications (including diabetic nephropathy) is proportional to the duration of diabetes and to diabetes control (as assessed by the amount of glycosuria and by random blood glucose measurements). Last but not least, intervention studies in Type 1 diabetes (the DCCT trial [5] being the most important quantitatively), or in Type 2 diabetes [6], have indicated that a reduction of hyperglycaemia decreases the risk of diabetic nephropathy, as assessed by the incidence of microalbuminuria and proteinuria. However, prospective, follow-up studies have suggested that long-term, uncontrolled Type 1 diabetes is a necessary, but not sufficient, condition for the development of diabetic nephropathy. For instance, Pipart [4] noted that concordance was not perfect between the onset of each of the three specific complications (retinopathy, neuropathy, and nephropathy). Barely one-fourth of patients developing retinopathy or neuropathy display nephropathy. Diabetic nephropathy occurs in just about half of Type 1 diabetic patients, presenting with a peak of onset between the 10th and 25th year of Type 1 diabetes duration, as documented by follow-up studies in the Joslin Clinic in Boston and in the Steno Memorial Hospital in Copenhagen [7-9].

The concept of a genetic basis for diabetic nephropathy is also supported by familial aggregation of this complication within families in which several members are affected by Type 1 diabetes [10-12]. Moreover, phenotypes attached to diabetic nephropathy, such as high blood pressure, seem to segregate with diabetic nephropathy in families of Type 1 diabetic patients [13]. The major drawback of familial studies is that members can share the same environmental conditions, in addition to the same genes, as indicated by a study of families with several Type 1 diabetic patients [14]. However, studies in Pima Indians are of importance in this respect because these families share similar environmental conditions. The fact that familial aggregation for proteinuria was found among diabetic siblings of this ethnic group is important evidence for a genetic basis of diabetic nephropathy, even though these patients mostly have Type 2 diabetes [15]. This result was also confirmed in Caucasian Type 2 diabetic patients. As in Type 1 diabetic patients [12], the risk for diabetic nephropathy is three- to four-fold higher for Type 2 diabetic siblings when the diabetic probands display proteinuria [16]. The heritability of urinary albumin excretion and of a risk for diabetic nephropathy has been established very recently [17-20].

CANDIDATE GENE VERSUS WHOLE GENOME SCREENING APPROACH

The whole genome screening approach is a promising strategy currently applied to monogenic diseases. In multifactorial diseases, this strategy may be less effective, but it has already been used to study the determinants of blood pressure, a variable very closely linked to glomerular disease. For instance, an original methodological approach recently indicated that a genetic region at or near the lipoprotein lipase gene locus was related to blood pressure in humans [21]. Moreover, the techniques of reverse genetics have led to the discovery of a link between severe familial hypertension and angiotensinogen gene polymorphisms (and subsequently angiotensinogen plasma levels) [22].

The strategy of whole genome scanning was applied to screening for determinants of microvascular disease related to diabetes. In Type 2 diabetes, linkage analysis detected two regions in chromosomes 7 and 20 that could determine susceptibility to nephropathy in Pima Indians [23]. These regions contain possible candidate genes such as NO synthase or aldose reductase. In Type 1 diabetic patients, when discordant sibling pairs for diabetic nephropathy were used, a susceptibility locus was found on chromosome 3q – Candidate genes in this region involved angiotensin II sub-type 1 receptor (AT1R), but the role of the AT1R gene itself was not evidenced when known polymorphisms were used in this gene [24]. However, the candidate-gene approach has been the most fruitful one to date in the domain of cardiovascular risk [25-27], and this strategy has been widely applied to diabetic nephropathy.

WHICH CANDIDATE GENES ARE TO BE TESTED FOR DIABETIC NEPHROPATHY?

The target tissues/organs susceptible to diabetic complications are those for which insulin is not required for trapping and/or metabolising glucose, i.e. nerves, lens, kidneys, blood cells, and epithelial and endothelial cells. Glucose metabolism is altered in these tissues/organs through a few biochemical pathways, e.g. the polyol pathway or non-enzymatic glycation of proteins. On the other hand, the vascular (and especially the renal) complications encountered in Type 1 diabetes can be explained by haemodynamic...
factors [28]. Increased arteriolar vasodilatation due to high glucose [3] creates high hydrostatic capillary pressure [29], which results in arteriosclerosis and glomerulosclerosis [30, 31]. In this context, screening for candidate genes capable of modulating the risk of renal complications due to long-term hyperglycaemia can use two approaches: first, by searching for the gene polymorphism of enzymes driving glucose metabolism in tissue/target organs; and secondly, by testing gene polymorphism affecting background vascular risk in the general population. When the first strategy was used, dinucleotide repeat polymorphism was found at the 5' end of the aldose reductase gene associated with early onset of retinopathy in Chinese Type 2 diabetes patients [32]. Later on, another group found this polymorphism in association with diabetic nephropathy in Caucasian Type 1 diabetic patients, although the interaction with the presence or absence of retinopathy was not clearly delineated [33]. This positive result was challenged by some [34] but not all authors [35]. Thus, it is possible that aldose reductase, an enzyme able to affect glucose metabolism within target tissues/organisms of diabetic microangiopathy, may affect the vascular prognosis for Type 1 diabetic patients, including the condition of nephropathy, as a result of effects on variable, genetically determined levels of its activity.

A second strategy consists in applying candidate genes indicative of cardiovascular risk (especially those for alterations in microcirculation) to prediction of the risk for diabetic nephropathy. The working hypothesis relies on the assumption that global capillary vasodilatation induced by hyperglycaemia and/or insulinopenia in Type 1 diabetes [3, 29] also affects renal circulation, and that glomerular capillary hypertension (a universal cause for progression towards glomerulosclerosis and renal failure [36]) is due to an imbalance between hyperglycaemia-induced afferent glomerular vasodilatation and constitutive, efferent, glomerular relative vasoconstriction (Fig. 1). There is a series of regulatory systems that can affect glomerular haemodynamics. Within each of these systems, enhanced or reduced activity of one component can lead to high/low glomerular hydraulic pressure. In fact, pharmacological alterations in these systems can affect glomerular haemodynamics, as indicated by changes in albuminuria. For instance, angiotensin-I-converting enzyme inhibitors can block the renin-angiotensin and kallikrein-kinin systems and reduce micro- or macroalbuminuria [37, 38]. However, a reduction in urinary albumin can also be obtained through the blockade of prostanooids with indomethacin [39], or alterations of haemostasis and proteoglycans with heparin [40]. Thus, it is worth testing the new polymorphisms within each component of these various regulatory systems to determine their possible roles in the development of Type 1 diabetic complications, especially diabetic nephropathy [41, 42]. This is especially true in the event that gene polymorphisms are associated with variable levels of expression of the concerned protein.

**THE RENIN-ANGIOTENSIN SYSTEM AND RISK FOR DIABETIC NEPHROPATHY**

**Background hypothesis**

Angiotensin II (AII) generation results from a series of enzymatic reactions. All interacts with a well-defined membrane receptor whose sub-type 1 AII receptor (AT1R) is of particular interest. Several of these components have displayed genetic polymorphisms, but in only two cases were the polymorphisms related to variable expressions of the protein, *i.e.* the
The effect of diabetes on glomerular circulation resembles that of AI [44, 45]. Furthermore, ACE inhibition prevents diabetic nephropathy [46] or halts its progression [47]. If the ancient hypothesis proposed by Vane [48] is accepted, i.e. that ACE availability can limit the transformation of angiotensin I into II within the glomerular circulation [48], then it is tempting to test the hypothesis of a role for ACE I/D polymorphism in the risk of diabetic nephropathy for Type 1 diabetic patients. Moreover, genetically determined AGT levels could affect AI since the amount of substrate for renin (an enzyme without relevant genetic polymorphism) may limit angiotensin I production [22]. However, the proportion of inter-subject variance of AGT due to genetic factors is relatively low (10-15%), compared to that of ACE, which is accounted for by genetic factors (75%), including a minimum of 50% due to I/D polymorphism [27]. Finally, A1166C AT1R polymorphism has been reported in relation to essential hypertension [49], although no study to date has demonstrated this.

Case-control studies on diabetic nephropathy and ACE I/D polymorphism in Type 1 diabetic patients

A case-control study conducted by our group indicated that the homozygotes for the ACE I allele in Type 1 diabetic subjects seem to be protected against diabetic nephropathy by low circulating ACE levels [50]. In this work, nephropathy control subjects were carefully matched with cases for short- and long-term glycaemic control (as assessed by HbA1c and the severity of retinopathy) and not just for age, sex, and Type 1 diabetes duration. Our results were challenged by some other, apparently negative, case-control studies, although bias resulting from better glycaemic control of control subjects than of cases was observable in these studies [51-54].

In an attempt to reduce the uncertainty due to variable Type 1 diabetes control and duration on the role of a given form of protein polymorphism in kidney prognosis for Type 1 diabetic patients, our group organised a multicentre cross-sectional study of Type 1 patients who had an expressed risk of kidney disease due to diabetes, i.e. those who developed proliferative retinopathy, a clear hallmark for uncontrolled Type 1 diabetes [55]. It was subsequently determined that the severity of renal involvement is dependent on ACE I/D polymorphism, with a dominant effect of the ACE D allele (adjusted odds ratio for renal involvement attributable to the D allele = 1.889; 95% confidence interval: 1.209-2.952). There was no independent effect of AGT, or AT1R polymorphism on the risk for diabetic nephropathy, but a significant interaction between ACE I/D and AGT M235T polymorphisms, which suggests that genetically determined AGT levels can affect the risk for diabetic nephropathy through angiotensin I generation (if angiotensin I transformation into AI is not restrained by ACE availability) (i.e. in patients with the ACE II genotype) [55]. A family-based study showed that the T allele of the M235 T polymorphism of AGT was preferentially transmitted to male Type 1 diabetic patients with nephropathy [56]. Other cross-sectional studies have produced discordant results concerning the role of AT1R on diabetic nephropathy. Some authors have shown an interaction between the A1166C polymorphism of the AT1R gene and poor glycaemic control [57], whereas others failed to reproduce this result [58] or did not find any effect of this genotype on diabetic nephropathy [59, 60].

Finally, some meta-analyses of all currently available studies on ACE I/D polymorphism and diabetic nephropathy have indicated that the II genotype may confer relative protection against diabetic nephropathy [61-63].

Clinical investigations on renal haemodynamics and ACE I/D polymorphism

Miller et al. [64] recently reported that Type 1 diabetic patients with the II genotype displayed a higher glomerular filtration rate and effective renal plasma flow during normoglycaemia than did Type 1 diabetic patients within the ID or DD genotypes. These results are consistent with the effect of ACE inhibition on renal haemodynamics reported in normoalbuminuric [65] and microalbuminuric [38] subjects, and consistent with a global, systemic vasodilation due to low circulating AI levels. These alterations give protection against diabetic nephropathy [46]. Conversely, our group recently studied the effect of acute hyperglycaemia in normotensive, normoalbuminuric Type 1 diabetes patients, and found that those with the ID or DD genotypes displayed alterations in glomerular haemodynamics consistent with a rise in glomerular capillary hydraulic pressure, whereas this condition was not observed in patients with the II genotype [66]. Thus, these investigations provide further evidence that ACE I/D polymorphism and related ACE levels can affect the constitution and progression of diabetic nephropathy.

Follow-up studies and intervention studies according to ACE I/D genotypes

A follow-up study in Austria indicated that ACE I/D polymorphism can affect the course of kidney disease in Type 1 diabetic patients [67]. This suggests that prospective follow-up of kidney function must be organised according to ACE genotypes and other possibly important polymorphisms. In a prospective follow-up study of a cohort of 310 Type 1 diabetic
patients, our group confirmed that the D allele is significantly and independently associated with a risk of development and progression of diabetic nephropathy [68]. Moreover, Parving et al. [69] have reported that ACE I/D polymorphism can affect the course of the glomerular filtration rate once diabetic nephropathy is established. These authors also suggested that ACE inhibition was less effective in preventing progression towards renal failure when Type 1 diabetic patients displayed the DD genotype than when they did not [69]. Conversely, Penno et al. [70], in a study of normo- or microalbuminuric Type 1 diabetes patients, failed to find a substantial interaction between ACE inhibition and ACE I/D polymorphism. However, these studies must be considered with due caution because of possible survival bias and other unidentified hidden biases. Intervention studies (especially with ACE inhibitors) must be designed according to ACE I/D polymorphism, using an appropriate method, probably involving a surrogate endpoint such as urinary albumin excretion as the main outcome in a first step.

ACE I/D polymorphism and diabetic nephropathy in Type 2 diabetic patients

ACE I/D polymorphism has been reported to be associated with risk of coronary heart disease in Type 2 diabetes [71]. As microalbuminuria or proteinuria predicts, or is associated with, coronary heart disease in Type 2 diabetes [72-74], it is worth looking for an association between urinary albumin and ACE I/D polymorphism in Type 2 diabetes. This association was found to be positive in the UKPDS study [75]. In addition, positive studies on the association between ACE I/D polymorphism and diabetic nephropathy have been reported in Japanese Type 2 diabetic patients by most [76-78] if not all studies [79]. Some cross-sectional studies in Caucasian subjects have found discordant results. When pooled in a meta-analysis, the effect of ACE I/D polymorphism was statistically significant in Japanese but not Caucasian Type 2 diabetes patients [63]. However, in a longitudinal study of Caucasian Type 2 diabetic patients, the decline of the glomerular filtration rate during the follow-up period was lowest in those patients with the II genotype [80]. Thus, the issue of an association between ACE I/D polymorphism remains debatable, because microalbuminuria or proteinuria is attributable to diabetic nephropathy in only a portion of cases with Type 2 diabetes [81], and because the interaction between elevated urinary albumin, coronary heart disease and ACE I/D polymorphism must be clarified. Finally, the frequency of the ACE II genotype is higher among Asian (40-45%) than Caucasian (20-25%) subjects, and the clinical characteristics and age at onset of Type 2 diabetes are not similar in Japanese and Caucasian patients. Thus, the portion of risk for (or protection against) diabetic nephropathy/coronary heart disease may differ among Asian and Caucasian Type 2 diabetic patients.

Acknowledgements – The authors are grateful to Mrs Line Godiveau, Isabelle Gouleau, and Françoise Rieuse for their excellent secretarial assistance.

REFERENCES


27 Cambien F, Costerusse O, Tiret L, et al. Plasma level and gene polymorphism of angiotensin converting enzyme in relation to myo-


30 Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Glomerular hemodynamics in diabetic nephropathy and diabetic nephropathy and proliferative retinopathy in IDDM pa-


34 Ko BCB, Lam KSL, Wat NMS, Chung SSM. An (A-C)n dinucleotide repeat polymorphic marker at the 5’ end of the aldose reductase gene is associated with early-onset diabetic retinopathy in NIDDM pa-


38 1620.


41 Marre M, Bernadet P, Gallois Y, et al. Relationships between angio-


45 Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Glomerular hemodynamics in diabetic nephropathy and diabetic nephropathy and proliferative retinopathy in IDDM pa-


