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MESTRADO E DOUTORADO

Valor da Excreção Urinária de Albumina como Marcador de Risco para o Desenvolvimento de Nefropatia Diabética e Relação entre a Estrutura Glomerular e a Função Renal em Pacientes com Diabete Melito

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LISTA DE ABREVIATURAS

DM: diabete melito

DCCT: *Diabetes Control and Complications Trial*

ECA: enzima conversora da angiotensina

EUA: excreção urinária de albumina

IGF: *insulin like growth factor*

NF-κB: fator de transcrição nuclear kappa B

PAS: ácido periódico de Schiff

PKC: proteína quinase C

TGF-β: *transforming growth factor* β

UKPDS: *United Kingdom Prospective Diabetes Study*

Vv(Int/córtex): volume fracional de interstício por córtex

Vv(Mes/glom): volume fracional de mesângio por glomérulo

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INTRODUÇÃO

Importância e Epidemiologia

A nefropatia diabética é a principal causa de morte em pacientes com diabete melito (DM) tipo 1 (1) e está associada com um aumento da mortalidade de aproximadamente 100 vezes (2). Nos Estados Unidos da América, cerca de 45% dos pacientes que ingressam em programas de substituição renal são portadores de DM (3). Também na Europa, o DM é a doença renal primária mais freqüente em pacientes com doença renal terminal (4). Pode-se concluir que a nefropatia diabética representa um importante problema de saúde pública.

Tradicionalmente, o diagnóstico de nefropatia clínica é estabelecido quando a excreção urinária de proteínas é superior a 0,5 g em 24 horas. Nesta fase, a perda de função renal é progressiva, sendo as medidas de intervenção terapêutica capazes de postergar, mas não de impedir, a progressão para insuficiência renal terminal.

A prevalência de nefropatia diabética clínica varia de 22-40% nos pacientes com DM tipo 1 (2, 5-9). Os estudos da história natural demonstram que a incidência anual de nefropatia clínica aumenta rapidamente a partir de 5 anos de duração de DM, atingindo um pico de cerca de 3% ao ano entre 15-17 anos de duração e, após, declina até atingir

uma incidência anual de 1% nos pacientes com 40 anos de duração da doença (10). Este padrão sugere que somente um certo número de pacientes é susceptível às complicações renais, já que apenas a exposição cumulativa ao DM não é suficiente para explicar o desenvolvimento da nefropatia clínica.

Alguns autores relatam que a proporção de pacientes com DM tipo 1 que desenvolve nefropatia diabética vem declinando ao longo dos anos (11-13). Entretanto, isto não foi confirmado em estudos mais recentes (14) e, na última década, um rápido e contínuo aumento no número de pacientes com DM ingressando em programas de substituição renal vem sendo observado (3). É interessante notar que este aumento na incidência de doença renal terminal causada pelo DM tem sido observado apesar das recomendações, por parte de associações como a Associação Americana de Diabetes (15), de avaliações periódicas da excreção urinária de albumina (EUA), na tentativa de identificar pacientes com risco aumentado de nefropatia diabética e de intensificar o controle glicêmico e da pressão arterial nestes pacientes. Mudanças nos critérios de indicação para o início do tratamento da doença renal terminal em pacientes com DM e aumento na sobrevida destes pacientes podem ter contribuído para o aumento no número de pacientes com DM ingressando em programas de substituição renal.

Curso Clínico

A nefropatia diabética tem sido classificada, para fins didáticos, em três etapas principais: a fase inicial, a fase de microalbuminúria e a fase de nefropatia clínica (macroalbuminúria ou proteinúria). As informações sobre o caráter evolutivo e involutivo destas etapas ainda não são completas, devendo-se ter em mente que o uso rígido desta classificação pode incorrer em erros.

Fase inicial

Na fase inicial da nefropatia diabética, os pacientes predispostos seriam caracterizados pela presença de indicadores de risco para nefropatia. Estes indicadores estariam relacionados aos fatores ligados à patogênese da nefropatia diabética. Neste sentido, o grau de controle metabólico, as alterações hemodinâmicas, os fatores genéticos e outros fatores associados ao acúmulo de matriz extracelular estariam entre os possíveis candidatos.

Tem sido geralmente aceito que pacientes com DM de longa duração e EUA normal apresentam baixo risco de progressão para nefropatia diabética. Entretanto, como será discutido em detalhe no Capítulo I, um número significante de pacientes normoalbuminúricos com DM tipo 1 de longa duração evolui para nefropatia clínica. Apesar de certa controvérsia (16, 17), é importante notar que pacientes normoalbuminúricos com DM tipo 1 podem apresentar alterações histopatológicas renais (18). Entretanto, a gravidade das lesões renais nestes pacientes é variável, e pacientes com lesões mais avançadas podem apresentar risco mais elevado de

progressão para nefropatia diabética (19). Estes achados sugerem que o estado diabético *per se* é estímulo suficiente para que alterações estruturais renais iniciem, mas que outros fatores governam a progressão destas lesões, uma vez que apenas uma fração dos pacientes com DM tipo 1 desenvolverá nefropatia clínica.

Fase de microalbuminúria

Estudos realizados no início da década de 80 (20-22) estabeleceram que níveis de EUA acima de determinados valores (15, 30 ou 70 μg/min, dependendo do estudo) eram preditivos para o desenvolvimento de nefropatia clínica. Nestes estudos, cerca de 80% dos pacientes que apresentavam níveis de EUA acima dos valores considerados críticos desenvolveu proteinúria. Entretanto, estudos mais recentes (23, 24) sugerem que o risco de progressão para proteinúria, apesar de aumentado em pacientes com microalbuminúria, não é tão elevado como sugerido inicialmente. Com base nos estudos iniciais acima descritos (20-22), foi publicado um consenso que estabeleceu que valores entre 20 e 200 μg/min caracterizavam a etapa de microalbuminúria (25). A Associação Americana de Diabetes, além de referendar estes valores, sugere também, como valores diagnósticos de microalbuminúria, níveis de albumina de 30 a 300 mg em urina de 24 horas, ou 30 a 300 mg/g de creatinina em amostra urinária (15). A concentração de albumina em amostra casual de urina também tem sido utilizada para diagnóstico e rastreamento de microalbuminúria (26, 27). Usando estes critérios, é estimado que a microalbuminúria esteja presente em 30 a 45% dos pacientes com mais do que 10 anos de DM tipo 1.

Os pacientes microalbuminúricos apresentam, em média, alterações histopatológicas renais mais graves do que os pacientes normoalbuminúricos (18). Entretanto, parece existir uma sobreposição importante entre a gravidade das lesões observadas nestes pacientes e a de pacientes com normoalbuminúria ou com proteinúria.

Fase de nefropatia clínica

No estágio da nefropatia clínica a concentração de proteínas totais em urina de 24 h é superior a 500 mg. Os valores de albuminúria correspondentes a este valor de proteinúria são: 200 μg/min, 300 mg/24 h, 300 mg/g creatinina (15). Nos pacientes com DM tipo 1, a partir do diagnóstico de nefropatia clínica, ocorre uma redução média nos valores de filtração glomerular da ordem de 1 ml/min/mês (28-30). Aproximadamente 50% dos pacientes com DM tipo 1 desenvolvem insuficiência renal terminal após 10 anos do início da proteinúria e cerca de 75% após 15 anos. Sem intervenção terapêutica específica, a sobrevida mediana após o surgimento de proteinúria persistente se situa entre 7 e 10 anos (2, 12). Nestes pacientes, as lesões renais são ainda mais avançadas e há um aumento na proporção de glomérulos esclerosados.

A evolução desfavorável observada neste estágio reforça a necessidade de se indentificar pacientes com risco aumentado de nefropatia diabética cedo no curso da doença, num período em que intervenções terapêuticas específicas possam ser mais efetivas.

Patologia

As alterações na estrutura renal causadas pelo DM são específicas, criando um padrão que não é visto em outras doenças renais. A gravidade das lesões parece estar relacionada à duração do DM, ao grau de controle metabólico e a fatores genéticos. Finalmente, estas alterações estruturais progressivas levam aos distúrbios funcionais da nefropatia diabética. Entretanto, a relação entre a duração do DM e a extensão das lesões glomerulares não é precisa. Isto está de acordo com a marcada variabilidade na susceptibilidade à nefropatia diabética, onde alguns pacientes podem apresentar insuficiência renal após terem DM tipo 1 por 15 anos ou menos, enquanto outros não apresentam complicações apesar de apresentarem DM por várias décadas.

Microscopia Óptica

A alteração estrutural mais precoce do DM tipo 1, a hipertrofia renal, não se reflete em alterações específicas na microscopia óptica. Em muitos pacientes, a estrutura glomerular permanece normal, ou quase normal, apesar de várias décadas de DM (17, 18, 31). Outros, desenvolvem expansão mesangial difusa progressiva, que se apresenta como um aumento na quantidade de material na matriz mesangial que se cora pela técnica do ácido periódico de Schiff (PAS). Áreas de expansão mesangial extrema, chamadas de nódulos de Kimmelstiel-Wilson, ou expansão mesangial nodular, ocorrem em cerca de 40-50% dos pacientes que desenvolvem proteinúria. Nestes nódulos, os núcleos das células mesangiais estão situados em paliçadas na periferia da lesão, circundando massas de matriz mesangial. É possível que estes nódulos resultem de microaneurismas que se formam nos capilares glomerulares (32).

Apesar de serem diagnósticos de DM, os nódulos de Kimmelstiel-Wilson não são necessários para que disfunção renal se desenvolva. Alterações renais mais precoces incluem a presença de material hialino nas arteríolas aferentes e eferentes. A gravidade destas lesões é diretamente relacionada à freqüência de glomeruloesclerose global que, por sua vez, parece resultar da isquemia glomerular. É também possível detectar, por microscopia óptica, espessamento das membranas basais glomerular e tubular, entretanto estas alterações são mais facilmente detectadas por microscopia eletrônica. Finalmente, em fases tardias da doença, atrofia tubular avançada e fibrose intersticial, alterações comuns à maioria das doenças renais, são geralmente observadas.

Imunofluorescência

Estudos imuno-histoquímicos indicam que as alterações da matriz extracelular no mesângio e nas membranas basais glomerular e tubular representam, pelo menos em parte, expansão de componentes intrínsecos da matriz, incluindo colágenos tipo IV e tipo VI, laminina e fibronectina (33). Entretanto, a natureza exata do material que se acumula não é completamente conhecida (34, 35).

Microscopia Eletrônica

A estrutura glomerular no início do DM é normal e alterações morfométricas podem ser detectadas somente após 1,5 a 2,5 anos de doença (36). Entretanto, enquanto alguns indivíduos apresentam estrutura glomerular normal por vários anos, outros desenvolvem lesões graves rapidamente. Usando técnicas morfométricas, a

primeira alteração renal detectável no DM é o espessamento da membrana basal glomerular (37), sendo que o espessamento da membrana basal tubular ocorre quase em paralelo (38, 39). O aumento na área relativa do mesângio pode ser observado, de maneira objetiva, entre 4 e 5 anos de DM. Estudos de morfometria renal em pacientes com DM tipo 1 sugerem que o volume fracional do mesângio por glomérulo, [Vv(Mes/glom)], aumenta de 0,2, no estado normal, para cerca de 0,4 quando a proteinúria inicia , atingindo 0,6 a 0,8 em pacientes cuja filtração glomerular está reduzida em 50-60% (17, 18, 40-42). Entretanto, como será discutido posteriormente, existe uma importante variação individual na gravidade das lesões associadas a aumentos na EUA.

Em pacientes com DM também são observadas alterações qualitativas e quantitativas no interstício renal. Entretanto, diferente do que é observado no glomérulo (43), nossos estudos iniciais sugerem que o acúmulo de matriz extracelular no interstício é um evento tardio, ocorrendo somente quando a filtração glomerular já se encontra reduzida (44). Nas fases iniciais de expansão intersticial as alterações celulares predominam e não se observa aumento quantitativo no colágeno (44).

Relações Estruturais e Funcionais na Nefropatia Diabética

Se acredita que a expansão mesangial, causada principalmente pelo acúmulo de matriz extracelular, leve à redução do lúmen capilar, reduzindo a superfície de filtração glomerular e, conseqüentemente, a taxa de filtração glomerular. As alterações intersticiais renais também parecem se correlacionar às alterações

funcionais em pacientes com DM tipo 1 (45), contudo esta relação parece ser complexa e mudar com a progressão da doença. De fato, em pacientes normoalbuminúricos e microalbuminúricos com DM tipo 1 (46), o aumento na EUA está associado ao aumento no Vv(Mes/glom), mas não ao aumento no volume fracional de interstício por córtex [Vv(Int/córtex)], enquanto que em pacientes proteinúricos (45, 47) ambos, Vv(Mes/glom) e Vv(Int/córtex), estão relacionados à EUA e à taxa de filtração glomerular. A atrofia tubular progressiva, a hialinose arteriolar, a arterioesclerose e a glomeruloesclerose também são componentes importantes da nefropatia diabética que provavelmente contribuem para uma redução adicional na filtração glomerular. Por último, a aterosclerose de vasos de maior calibre, especialmente em pacientes com DM tipo 2, pode levar a dano renal isquêmico. Em pacientes com DM tipo 1 as lesões renais tendem a progredir em paralelo, enquanto que em pacientes com DM tipo 2 isto freqüentemente não é o caso.

Patogênese da Nefropatia Diabética

As lesões renais da nefropatia diabética são causadas pelo acúmulo de componentes da matriz extracelular, tais como colágeno e fibronectina (33), assim como de moléculas ainda não bem definidas. Este acúmulo de matriz extracelular ocorre primeiro nas membranas basais glomerular (37) e tubular (39), é a principal causa da expansão mesangial e também contribui para a expansão intersticial (44). O acúmulo da matriz extracelular é secundário ao desequilíbrio entre a síntese e a degradação de seus componentes. Os componentes que se acumulam em pacientes

com rápido *versus* lento desenvolvimento de nefropatia diabética não são os mesmos (34, 35, 48), sugerindo que a resposta celular levando ao acúmulo de matriz extracelular é regulada geneticamente.

A concentração de vários dos componentes da matriz extracelular em células mesangiais aumenta quando estas células são cultivadas em meio rico em glicose (49-51). Estudos *in vitro* sugerem que a atividade das metaloproteinases (enzimas responsáveis pela degradação da matriz mesangial) (52) e de seus inibidores parece ser regulada pela proteína quinase C (PKC) e pelo *transforming growth factor beta* (TGF-β) (53-57).

Entre os fatores relacionados e os mecanismos envolvidos na patogênese da nefropatia diabética podemos citar, entre outros, a hiperglicemia, as alterações hemodinâmicas e os fatores genéticos.

Hiperglicemia

Apesar da existência de fatores moduladores, a nefropatia diabética é secundária às alterações metabólicas encontradas no DM. Estudos em pacientes com DM tipo 1 e tipo 2 demonstraram que um melhor controle glicêmico pode levar à redução no desenvolvimento de nefropatia diabética. O *Diabetes Control and Complications Trial* (DCCT) demonstrou, em pacientes com DM tipo 1 acompanhados por 6 anos, que o controle intensivo do DM reduz o risco de progressão para microalbuminúria e proteinúria (58), efeito este ainda evidente 4-6 anos após o término do estudo (59, 60). Após 10 anos de controle glicêmico intensivo, foi

demonstrada redução na incidência de nefropatia diabética nos pacientes com DM tipo 2 do *United Kingdom Prospective Diabetes Study* (UKPDS) (61). Resultados semelhantes foram descritos em pacientes Japoneses com DM tipo 2 submetidos a controle intensivo da glicemia por 8 anos (62). Também foi demonstrado que o controle glicêmico intensivo pode ter efeito no desenvolvimento e na progressão das lesões glomerulares da nefropatia diabética. O controle glicêmico intensivo em pacientes com DM tipo 1 que receberam transplante renal como tratamento para doença renal terminal causada pela nefropatia diabética impediu o desenvolvimento de lesões glomerulares diabéticas no rim transplantado (63). Em pacientes microalbuminúricos com DM tipo 1 o tratamento intensivo do DM reduziu a taxa de espessamento da membrana basal glomerular e a expansão da matriz mesangial (64). Finalmente, foi observada regressão de lesões glomerulares estabelecidas após 10 anos de normoglicemia induzida por transplante de pâncreas isolado em pacientes com DM tipo 1 (65). Estes estudos sugerem que a hiperglicemia é necessária tanto para o desenvolvimento quanto para a manutenção das lesões renais, e que a remoção da mesma permite que mecanismos de reparo sejam expressos, levando ao desaparecimento das lesões glomerulares da nefropatia diabética.

Mecanismos de ação da hiperglicemia

É possível que a hiperglicemia leve à nefropatia diabética através de vários mecanismos, tais como (a) aumento na atividade de fatores do crescimento (66), incluindo TGF-β, hormônio do crescimento, *insulin-like growth factor* (IGF), fator endotelial de crescimento vascular e fator epidérmico do crescimento; (b) ativação da

PKC (67-71); (c) ativação de citocinas, como renina, pró-renina (72-76), angiotensina (77-80), endotelina (81-84) e bradicinina (85, 86); (d) formação de radicais livres de oxigênio (87); (e) aumento na expressão do fator de transcrição nuclear kappa B (NF-κB) (88); (f) aumento na formação de produtos da glicação não-enzimática (89-95); (g) aumento na atividade da aldose-redutase (96-98); e (h) diminuição no conteúdo de sulfato de heparano na membrana basal glomerular (99, 100).

Estas hipóteses se sobrepõem e parecem interagir uma com a outra. O aumento na formação de radicais livres de oxigênio secundário ao excesso de glicose pode ser, por exemplo, responsável pela maioria das anormalidades bioquímicas acima descritas (101-103). Como discutido anteriormente, estes mecanismos podem ser influenciados por determinantes genéticos da susceptibilidade ou da resistência ao dano renal causado pela hiperglicemia. Estudos *in vitro,* avaliando o comportamento de enzimas antioxidantes em fibroblastos cultivados em concentrações de glicose normais ou elevadas, reforçam esta hipótese (104). Em concentrações normais de glicose, não houve diferença na atividade da catalase e da glutationa oxidase entre pacientes com DM tipo 1 com nefropatia, pacientes com DM tipo 1 sem nefropatia e controles não diabéticos. Por outro lado, em altas concentrações de glicose, pacientes com nefropatia diabética não apresentaram o aumento esperado na atividade e no RNA mensageiro para estas enzimas (104).

Entretanto, para que fosse possível concluir que as rotas metabólicas citadas acima estão definitivamente envolvidas na gênese do dano renal causado pelo DM, seria necessário demonstrar que a ativação destes sistemas pode levar ao

desenvolvimento de lesões típicas da nefropatia diabética (expansão mesangial e espessamento da membrana basal glomerular) ou que o bloqueio farmacológico destas rotas pode prevenir o desenvolvimento destas lesões.

Alterações Hemodinâmicas

O DM é associado a aumento na filtração glomerular e alguns estudos sugerem que a presença de hiperfiltração glomerular é um fator de risco para o desenvolvimento de microalbuminúria (20, 105, 106), entretanto ainda há controvérsias (107, 108).

Diversos mecanismos têm sido propostos como mediadores da hiperfiltração induzida pelo DM, entre eles o aumento dos níveis renais de bradicininas (109) e de óxido nítrico (110-113).

É provável que as alterações na hemodinâmica intraglomerular possam influenciar a velocidade com que as lesões diabéticas se desenvolvem, mas não é possível explicar a gênese da nefropatia diabética pela presença isolada de hiperfiltração. A redução na massa renal através de uninefrectomia (114) ou de nefrectomia bilateral quase-total (115), por exemplo, produz alterações hemodinâmicas semelhantes às vistas no DM (116), mas não produz as lesões diabéticas clássicas em animais (117-119). Em pacientes com DM tipo 2 e rim único foi observada maior prevalência de microalbuminúria do que em pacientes com DM tipo 2 com ambos rins ou em controles não-diabéticos com rim único (120). Entretanto, apesar da proporção de pacientes proteinúricos ter sido maior no grupo

com DM tipo 2 e rim único do que no grupo controle não-diabético com rim único, a prevalência de proteinúria nos grupos com DM foi semelhante (120). Em ratos, a redução da pressão capilar glomerular com inibidores da enzima conversora da angiotensina (ECA) previne o desenvolvimento de glomeruloesclerose segmental e focal (121), mas não o espessamento da membrana basal glomerular e o aumento do Vv(Mes/glom) (122). Entretanto, apesar do bloqueio do sistema renina-angiotensina não prevenir o desenvolvimento das lesões precoces do DM (122), é provável que o bloqueio deste sistema possa reduzir a progressão de lesões estabelecidas e de nefropatia clínica (123). Recentemente foi demonstrado que inibidores da ECA podem prevenir o aumento progressivo na espessura da membrana basal glomerular em pacientes microalbuminúricos com DM tipo 1 (124). Estas drogas também parecem reduzir a progressão da expansão intersticial em pacientes com DM tipo 1 (125) e tipo 2 (126). Entretanto, esses efeitos talvez não sejam específicos desta classe de medicamentos, uma vez que, nestes estudos, efeitos similares foram observados com o uso de beta-bloqueadores (124, 125). Estes achados estão de acordo com os dados do UKPDS, que não observou diferenças na taxa de progressão para nefropatia diabética entre pacientes tratados com inibidores da ECA ou com beta-bloqueadores (123, 127). Considerados em conjunto, estes dados suportam a hipótese de que as alterações hemodinâmicas possam ser mais importantes na progressão do que na gênese das lesões estruturais da nefropatia diabética.

Contudo, deve-se também considerar a possibilidade de que alterações na hemodinâmica renal possam afetar o desenvolvimento de lesões da nefropatia

diabética através de outros mecanismos. O sistema renina-angiotensina poderia, por exemplo, agir como mediador da hipertrofia renal e da produção e degradação da matriz extracelular renal. Estudos da proliferação celular e da produção de matriz extracelular em células mesangiais (128), assim como respostas destas células (129, 130) e de animais diabéticos (131) ao tratamento com inibidores da ECA, suportam esta hipótese. Além disto, a expressão celular do TGF-β parece ser regulada em paralelo com a da renina (132, 133) e poderia ser, portanto, modulada por inibidores da ECA (134). A hiperfiltração glomerular poderia, então, levar a aumento na expressão local de TGF-β e, conseqüentemente, a acúmulo renal da matriz extracelular.

Fatores Genéticos

A predisposição genética parece ser o determinante de risco mais importante para o desenvolvimento da nefropatia diabética (135). Diferenças na prevalência de nefropatia diabética em diferente populações suportam este ponto de vista (3, 136-138). Além disto, apenas cerca de metade dos pacientes com mau controle glicêmico desenvolve nefropatia diabética (139), enquanto alguns pacientes desenvolvem nefropatia apesar de razoável controle do DM. Estes achados são consistentes com a presença de fatores genéticos modulando o risco para nefropatia diabética.

Estudos de Agregação Familiar

Desde o primeiro relato, cerca de 10 anos atrás (140), a predisposição genética para nefropatia diabética tem sido sugerida por múltiplos estudos transversais

avaliando irmãos com DM tipo 1 (141, 142) e tipo 2 (143-146). Além disto, associação entre nefropatia diabética e predisposição à hipertensão e à doença cardiovascular também tem sido demonstrada (147-152), ligando a patogênese da nefropatia diabética a fatores que favoreçam o desenvolvimento de arterioesclerose (147, 148, 153).

Contratransporte de Sódio-Lítio

O contratransporte de sódio-lítio é geneticamente determinado e está associado à hipertensão e à doença cardiovascular em indivíduos não-diabéticos (154, 155). Em pacientes com DM tipo 1 a atividade deste sistema parece estar aumentada tanto em pacientes proteinúricos e microalbuminúricos quanto em normoalbuminúricos (148, 156-158). A atividade aumentada deste sistema em pacientes com DM tipo 1 inicialmente normoalbuminúricos foi associada a um aumento de 4 vezes na incidência de microalbuminúria (159). O contratransporte de sódio-lítio representa o contratransporte de sódio-hidrogênio, que está envolvida na regulação do pH intracelular e na reabsorção tubular de sódio (160). A atividade deste sistema está aumentada nos leucócitos (161) e nos fibroblastos (162, 163) de pacientes microalbuminúricos e proteinúricos com DM tipo 1 e tipo 2. Além disto, existe uma forte concordância na atividade da bomba de sódio-hidrogênio nos fibroblastos de irmãos com DM tipo 1 que apresentam lesões glomerulares similares em gravidade (164), e esta concordância parece ser independente de fatores ambientais. Estes achados sugerem que o comportamento dos fibroblastos é regulado geneticamente e pode refletir o risco para nefropatia diabética.

Genes Associados à Nefropatia Diabética

Diversos estudos tentaram identificar os genes associados à nefropatia diabética através da avaliação do genoma e de genes específicos. Nenhuma destas abordagens gerou, até o presente, resultados definitivos, mas há indicações de que mais do que um gene possa estar envolvido (135, 165). Vários genes, geralmente selecionados de acordo com o conhecimento vigente da patogênese da nefropatia diabética, têm sido propostos como candidatos plausíveis.

Polimorfismos genéticos associados a risco cardiovascular aumentado na população em geral, tais como o dos genes relacionados ao sistema renina-angiotensina, têm sido avaliados. Ainda não está claro se o polimorfismo do gene da ECA está associado (166-173), ou não (165, 174-181), à gênese da nefropatia diabética. Entretanto, este polimorfismo parece estar associado à taxa de progressão da doença renal estabelecida (182), à resposta ao tratamento com inibidores da ECA (183, 184) e à gravidade das lesões glomerulares da nefropatia diabética (185). Associações entre nefropatia diabética e polimorfismos do gene do angiotensinogênio (166, 186-188) e do receptor tipo 1 da angiotensina (189) foram observadas em alguns mas não em todos os estudos (171, 179, 180, 190-194). É de interesse notar que interações entre genes (166, 171) e entre genes e controle glicêmico (189) têm sido relatadas.

Também foram estudadas, entre outras, associações entre a nefropatia diabética e os genes associados ao metabolismo da matriz extracelular (177, 195-199) e aos mecanismos fisiopatológicos da nefropatia diabética (200-213).

Pode-se concluir que os determinantes genéticos da nefropatia diabética ainda não são completamente conhecidos e que pesquisas nesta área podem levar a descobertas importantes relacionadas aos fatores de risco, à patogênese e ao tratamento da nefropatia diabética.

Outros Fatores

Além dos fatores acima descritos, o conteúdo protéico da dieta, os níveis de lipídios e o hábito de fumar, entre outros, são fatores possivelmete associados ao desenvolvimento de nefropatia diabética. O conteúdo protéico da dieta pode ser relacionado à filtração glomerular, sendo que a ingestão de dietas hiperprotéicas promove hiperfiltração glomerular (214-216) e a diminuição da quantidade de proteínas ingeridas é capaz de reduzir a FG em pacientes com DM tipo 1 (217, 218). Além disto, em estudos transversais, o conteúdo aumentado de proteínas da dieta, assim como o conteúdo de proteínas de origem animal, tem sido associado a níveis mais altos de EUA (219, 220). Alguns estudos sugerem que a origem da proteína também possa ser importante, já que as dietas isoprotéicas à base de galinha e de peixe são tão eficazes quanto as dietas hipoprotéicas em promover redução da hiperfiltração glomerular (221). Além disto, o consumo aumentado de proteínas derivadas de carne de peixe parece estar associado a uma menor prevalência de microalbuminúria (222). Níveis mais elevados de lipídios (223) também têm sido implicados no desenvolvimento da nefropatia diabética. O aumento dos lipídios poderia levar à glomeruloesclerose e a presença de monócitos repletos de lipídios foi demonstrada no mesângio de pacientes com níveis elevados de colesterol (224). O hábito de fumar

também foi relacionado à nefropatia diabética (225-228). O tabagismo poderia levar à hipóxia tecidual e a dano vascular (229) e, ainda, a aumento dos níveis pressóricos, afetando a hemodinâmica renal (230).

Comentários Gerais

A nefropatia diabética, por sua prevalência, morbidade e mortalidade associadas, é uma importante complicação do DM. Até o presente momento, não existem marcadores que permitam identificar de forma precisa os pacientes predispostos ao desenvolvimento de nefropatia. O papel da EUA como marcador de risco para a nefropatia diabética e a sua relação com a gravidade das lesões glomerulares não estão bem definidos. A avaliação mais precisa destes aspectos fornecerá subsídios para o melhor entendimento da associação entre estes fatores e o desenvolvimento de nefropatia clínica. A identificação precoce de pacientes susceptíveis permitiria que medidas terapêuticas, como o controle intensivo da glicemia e da pressão arterial e a manipulação protéica da dieta, fossem direcionadas a estes indivíduos. Além disto, o conhecimento dos fatores e mecanismos associados à patogênese da nefropatia diabética poderia levar ao desenvolvimento de estratégias preventivas e terapêuticas mais específicas e eficazes a serem oferecidas a pacientes com risco aumentado.

BIBLIOGRAFIA

1. Deckert T, Andersen AR, Christiansen JS, Andersen JK: Course of diabetic nephropathy. Factors related to development. *Acta Endocrinol Suppl* 242:14-15, 1981.

2. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in Type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496-501, 1983.

3. *USRDS 1999 Annual Data Report.* 1999. Bethesda, MD: The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

4. Valderrabano F, Jones EH, Mallick NP: Report on management of renal failure in Europe, XXIV, 1993. *Nephrol Dial Transplant* 10:1-25, 1995.

5. Mangili R, Deferrari G, Di Mario U, Giampietro O, Navalesi R, Nosadini R, Rigamonti G, Spezia R, Crepaldi G: Arterial hypertension and microalbuminuria in IDDM: the Italian Microalbuminuria Study. *Diabetologia* 37:1015-1024, 1994.

6. Parving HH, Hommel E, Mathiesen E, Skott P, Edsberg B, Bahnsen M, Lauritzen M, Hougaard P, Lauritzen E: Prevalence of microalbuminuria, arterial hypertension, retinopathy and neuropathy in patients with insulin dependent diabetes. *Br Med J (Clin Res Ed)* 296:156-160, 1988.

7. Warram JH, Gearin G, Laffel L, Krolewski AS: Effect of duration of type I diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol* 7:930-937, 1996.

8. Ebeling P, Koivisto VA: Occurrence and interrelationships of complications in insulin-dependent diabetes in Finland. *Acta Diabetol* 34:33-38, 1997.

9. Microalbuminuria in type I diabetic patients. Prevalence and clinical characteristics. Microalbuminuria Collaborative Study Group. *Diabetes Care* 15:495-501, 1992.

10. Borch-Johnsen K, Andersen PK, Deckert T: The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 28:590-596, 1985.

11. Kofoed-Enevoldsen A, Borch-Johnsen K, Kreiner S, Nerup J, Deckert T: Declining incidence of persistent proteinuria in type I (insulin-dependent) diabetic patients in Denmark. *Diabetes* 36:205-209, 1987.

12. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR: The changing natural history of nephropathy in type I diabetes. *Am J Med* 78:785-794, 1985.

13. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J: Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* 330:15-18, 1994.

14. Rossing P, Rossing K, Jacobsen P, Parving HH: Unchanged incidence of diabetic nephropathy in IDDM patients. *Diabetes* 44:739-743, 1995.

15. American Diabetes Association: Diabetic nephropathy. *Diabetes Care* 24:S69-S72, 2001.

16. Berg UB, Torbjornsdotter TB, Jaremko G, Thalme B: Kidney morphological changes in relation to long-term renal function and metabolic control in adolescents with IDDM. *Diabetologia* 41:1047-1056, 1998.

17. Østerby R: Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia* 35:803-812, 1992.

18. Fioretto P, Steffes MW, Mauer M: Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. *Diabetes* 43:1358-1364, 1994.

19. Caramori ML, Fioretto P, Mauer M: Long-term follow-up of normoalbuminuric longstanding type 1 diabetic patients: Progression is associated with worse baseline glomerular lesions and lower glomerular filtration rate (Abstract). *J Am Soc Nephrol* 10:126A, 1999.

20. Mogensen CE, Christensen CK: Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89-93, 1984.

21. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1:1430-1432, 1982.

22. Parving HH, Oxenboll B, Svendsen PA, Christiansen JS, Andersen AR: Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *Acta Endocrinol (Copenh)* 100:550-555, 1982.

23. Forsblom CM, Groop PH, Ekstrand A, Groop LC: Predictive value of microalbuminuria in patients with insulin-dependent diabetes of long duration. *Br Med J* 305:1051-1053, 1992.

24. Rossing P, Hougaard P, Borch-Johnsen K, Parving H-H: Progression of microalbuminuria in type 1 diabetes: 10 years observation and follow-up (Abstract). *Diabetologia* 43 (Suppl 1):A254, 2000.

25. Mogensen CE, Chachati A, Christensen CK, Close CF, Deckert T, Hommel E, Kastrup J, Lefebvre P, Mathiesen ER, Feldt-Rasmussen B, et al.: Microalbuminuria: an early marker of renal involvement in diabetes. *Uremia Invest* 9:85-95, 1985.

26. European Diabetes Policy Group 1998: Guidelines for diabetes care. A desktop guide to type 1 (insulin-dependent) diabetes mellitus. *Diabetes Nutr Metab* 12:345-378, 1999.

27. Zelmanovitz T, Gross JL, Oliveira JR, Paggi A, Tatsch M, Azevedo MJ: The receiver operating characteristics curve in the evaluation of a random urine specimen as a screening test for diabetic nephropathy. *Diabetes Care* 20:516-519, 1997.

28. Mogensen CE: Progression of nephropathy in long-term diabetics with proteinuria and effect of initial anti-hypertensive treatment. *Scand J Clin Lab Invest* 36:383-388, 1976.

29. Viberti GC, Bilous RW, Mackintosh D, Keen H: Monitoring glomerular function in diabetic nephropathy. A prospective study. *Am J Med* 74:256-264, 1983.

30. Parving HH, Andersen AR, Smidt UM, Svendsen PA: Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* 1:1175-1179, 1983.

31. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143-1155, 1984.

32. Saito Y, Kida H, Takeda S, Yoshimura M, Yokoyama H, Koshino Y, Hattori N: Mesangiolysis in diabetic glomeruli: its role in the formation of nodular lesions. *Kidney Int* 34:389-396, 1988.

33. Falk RJ, Scheinman JI, Mauer SM, Michael AF: Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. *Diabetes* 32 Suppl 2:34-39, 1983.

34. Zhu D, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM: Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. *Kidney Int* 45:425-433, 1994.

35. Moriya T, Groppoli TJ, Kim Y, Mauer M: Quantitative immunoelectron microscopy of type VI collagen in glomeruli in type I diabetic patients. *Kidney Int* 59:317-323, 2001.

36. Østerby R: Early phases in the development of diabetic glomerulopathy. *Acta Med Scand Suppl* 574:3-82, 1974.

37. Østerby R: Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes. I. Development of initial basement membrane thickening. *Diabetologia* 8:84-92, 1972.

38. Steffes MW, Sutherland DE, Goetz FC, Rich SS, Mauer SM: Studies of kidney and muscle biopsy specimens from identical twins discordant for type I diabetes mellitus. *N Engl J Med* 312:1282-1287, 1985.

39. Brito PL, Fioretto P, Drummond K, Kim Y, Steffes MW, Basgen JM, Sisson-Ross S, Mauer M: Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. *Kidney Int* 53:754-761, 1998.

40. Walker JD, Close CF, Jones SL, Rafftery M, Keen H, Viberti G, Østerby R: Glomerular structure in type-1 (insulin-dependent) diabetic patients with normo- and microalbuminuria. *Kidney Int* 41:741-748, 1992.

41. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM: Glomerular lesions and urinary albumin excretion in type I diabetes without overt proteinuria. *N Engl J Med* 320:966-970, 1989.

42. Bangstad HJ, Østerby R, Dahl-Jorgensen K, Berg KJ, Hartmann A, Nyberg G, Frahm Bjorn S, Hanssen KF: Early glomerulopathy is present in young, type 1 (insulin-dependent) diabetic patients with microalbuminuria. *Diabetologia* 36:523-529, 1993.

43. Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Cell and matrix components of the glomerular mesangium in type I diabetes. *Diabetes* 41:679-684, 1992.

44. Katz A, Caramori MLA, Sisson-Ross S, Groppoli T, Basgen JM, Mauer M: An increase in the fraction of the cortical interstitium occupied by cells antedates interstitial fibrosis in type 1 diabetic patients. *Kidney Int,* in press.

45. Lane PH, Steffes MW, Fioretto P, Mauer SM: Renal interstitial expansion in insulin-dependent diabetes mellitus. *Kidney Int* 43:661-667, 1993.

46. Fioretto P, Steffes MW, Sutherland DE, Mauer M: Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. *Kidney Int* 48:1929-1935, 1995.

47. Taft JL, Nolan CJ, Yeung SP, Hewitson TD, Martin FI: Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. *Diabetes* 43:1046-1051, 1994.

48. Kim Y, Kleppel MM, Butkowski R, Mauer SM, Wieslander J, Michael AF: Differential expression of basement membrane collagen chains in diabetic nephropathy. *Am J Pathol* 138:413-420, 1991.

49. Ayo SH, Radnik RA, Glass WFd, Garoni JA, Rampt ER, Appling DR, Kreisberg JI: Increased extracellular matrix synthesis and mRNA in mesangial cells grown in high-glucose medium. *Am J Physiol* 260:F185-191, 1991.

50. Pugliese G, Pricci F, Pugliese F, Mene P, Lenti L, Andreani D, Galli G, Casini A, Bianchi S, Rotella CM, et al.: Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. *Diabetes* 43:478-490, 1994.

51. Sharma K, Ziyadeh FN: Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. *Diabetes* 44:1139-1146, 1995.

52. Davies M, Coles GA, Thomas GJ, Martin J, Lovett DH: Proteinases and the glomerulus: their role in glomerular diseases. *Klin Wochenschr* 68:1145-1149, 1990.

53. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA: Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 4:197-250, 1993.

54. Kahari VM, Saarialho-Kere U: Matrix metalloproteinases and their inhibitors in tumour growth and invasion. *Ann Med* 31:34-45, 1999.

55. Murphy G: Matrix metalloproteinases and their inhibitors. *Acta Orthop Scand Suppl* 266:55-60, 1995.

56. Matrisian LM: Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 6:121-125, 1990.

57. Bruijn JA, Roos A, de Geus B, de Heer E: Transforming growth factor-beta and the glomerular extracellular matrix in renal pathology. *J Lab Clin Med* 123:34-47, 1994.

58. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. The Diabetes Control and Complications (DCCT) Research Group. *Kidney Int* 47:1703-1720, 1995.

59. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med* 342:381-389, 2000.

60. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Sustained reduction in albuminuria six years after the diabetes control and complications trial (DCCT) (Abstract). *Diabetes* 50:A63, 2001.

61. United Kingdom Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-853, 1998.

62. Shichiri M, Kishikawa H, Ohkubo Y, Wake N: Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care* 23 Suppl 2:B21-29, 2000.

63. Barbosa J, Steffes MW, Sutherland DE, Connett JE, Rao KV, Mauer SM: Effect of glycemic control on early diabetic renal lesions. A 5-year randomized controlled clinical trial of insulin-dependent diabetic kidney transplant recipients. *Jama* 272:600-606, 1994.

64. Bangstad HJ, Østerby R, Dahl-Jorgensen K, Berg KJ, Hartmann A, Hanssen KF: Improvement of blood glucose control in IDDM patients retards the progression of morphological changes in early diabetic nephropathy. *Diabetologia* 37:483-490, 1994.

65. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M: Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 339:69-75, 1998.

66. Flyvbjerg A: Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease. *Diabetologia* 43:1205-1223, 2000.

67. Ziyadeh FN, Fumo P, Rodenberger CH, Kuncio GS, Neilson EG: Role of protein kinase C and cyclic AMP/protein kinase A in high glucose-stimulated transcriptional activation of collagen alpha 1 (IV) in glomerular mesangial cells. *J Diabetes Complications* 9:255-261, 1995.

68. Fumo P, Kuncio GS, Ziyadeh FN: PKC and high glucose stimulate collagen alpha 1 (IV) transcriptional activity in a reporter mesangial cell line. *Am J Physiol* 267:F632-638, 1994.

69. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL: Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science* 272:728-731, 1996.

70. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL: Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100:115-126, 1997.

71. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Ways DK, King GL, Kikkawa R: Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *Faseb J* 14:439-447, 2000.

72. Bryer-Ash M, Fraze EB, Luetscher JA: Plasma renin and prorenin (inactive renin) in diabetes mellitus: effects of intravenous furosemide. *J Clin Endocrinol Metab* 66:454-458, 1988.

73. Wilson DM ,Luetscher JA: Plasma prorenin activity and complications in children with insulin-dependent diabetes mellitus. *N Engl J Med* 323:1101-1106, 1990.

74. Daneman D, Crompton CH, Balfe JW, Sochett EB, Chatzilias A, Cotter BR, Osmond DH: Plasma prorenin as an early marker of nephropathy in diabetic (IDDM) adolescents. *Kidney Int* 46:1154-1159, 1994.

75. Allen TJ, Cooper ME, Gilbert RE, Winikoff J, Skinni SL, Jerums G: Serum total renin is increased before microalbuminuria in diabetes. *Kidney Int* 50:902-907, 1996.

76. Deinum J, Ronn B, Mathiesen E, Derkx FH, Hop WC, Schalekamp MA: Increase in serum prorenin precedes onset of microalbuminuria in patients with insulin-dependent diabetes mellitus. *Diabetologia* 42:1006-1010, 1999.

77. Zhang SL, Filep JG, Hohman TC, Tang SS, Ingelfinger JR, Chan JS: Molecular mechanisms of glucose action on angiotensinogen gene expression in rat proximal tubular cells. *Kidney Int* 55:454-464, 1999.

78. Tang SS, Diamant D, Rhoads DB, Ingelfinger JR: Angiotensin II regulates glucose uptake in immortalized rat proximal tubular cells (Abstract). *J Am Soc Nephrol* 6:748, 1995.

79. Wolf G, Mueller E, Stahl RA, Ziyadeh FN: Angiotensin II-induced hypertrophy of cultured murine proximal tubular cells is mediated by endogenous transforming growth factor-beta. *J Clin Invest* 92:1366-1372, 1993.

80. Kagami S, Border WA, Miller DE, Noble NA: Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. *J Clin Invest* 93:2431-2437, 1994.

81. Hargrove GM, Dufresne J, Whiteside C, Muruve DA, Wong NC: Diabetes mellitus increases endothelin-1 gene transcription in rat kidney. *Kidney Int* 58:1534-1545, 2000.

82. Simonson MS, Dunn MJ: Renal actions of endothelin peptides. *Curr Opin Nephrol Hypertens* 2:51-60, 1993.

83. Peppa-Patrikiou M, Dracopoulou M, Dacou-Voutetakis C: Urinary endothelin in adolescents and young adults with insulin-dependent diabetes mellitus: relation to urinary albumin, blood pressure, and other factors. *Metabolism* 47:1408-1412, 1998.

84. Shin SJ, Lee YJ, Tsai JH: The correlation of plasma and urine endothelin-1 with the severity of nephropathy in Chinese patients with type 2 diabetes. *Scand J Clin Lab Invest* 56:571-576, 1996.

85. Campbell DJ, Kelly DJ, Wilkinson-Berka JL, Cooper ME, Skinner SL: Increased bradykinin and "normal" angiotensin peptide levels in diabetic Sprague-Dawley and transgenic (mRen-2)27 rats. *Kidney Int* 56:211-221, 1999.

86. Tschope C, Reinecke A, Seidl U, Yu M, Gavriluk V, Riester U, Gohlke P, Graf K, Bader M, Hilgenfeldt U, Pesquero JB, Ritz E, Unger T: Functional, biochemical, and molecular investigations of renal kallikrein-kinin system in diabetic rats. *Am J Physiol* 277:H2333-2340, 1999.

87. Dunlop M: Aldose reductase and the role of the polyol pathway in diabetic nephropathy. *Kidney Int* 58 Suppl 77:S3-12, 2000.

88. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D: Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269:9889-9897, 1994.

89. Chen S, Cohen MP, Ziyadeh FN: Amadori-glycated albumin in diabetic nephropathy: pathophysiologic connections. *Kidney Int* 58 Suppl 77:S40-44, 2000.

90. Anderson SS, Kim Y, Tsilibary EC: Effects of matrix glycation on mesangial cell adhesion, spreading and proliferation. *Kidney Int* 46:1359-1367, 1994.

91. McLennan SV, Fisher EJ, Yue DK, Turtle JR: High glucose concentration causes a decrease in mesangium degradation. A factor in the pathogenesis of diabetic nephropathy. *Diabetes* 43:1041-1045, 1994.

92. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR: Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *N Engl J Med* 314:403-408, 1986.

93. Singh AK, Mo W, Dunea G, Arruda JA: Effect of glycated proteins on the matrix of glomerular epithelial cells. *J Am Soc Nephrol* 9:802-810, 1998.

94. Soulis-Liparota T, Cooper M, Papazoglou D, Clarke B, Jerums G: Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* 40:1328-1334, 1991.

95. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315-1321, 1988.

96. Nadkarni V, Gabbay KH, Bohren KM, Sheikh-Hamad D: Osmotic response element enhancer activity. Regulation through p38 kinase and mitogen-activated extracellular signal-regulated kinase kinase. *J Biol Chem* 274:20185-20190, 1999.

97. Fazzio A, Spycher SE, Azzi A: Signal transduction in rat vascular smooth muscle cells: control of osmotically induced aldose reductase expression by cell kinases and phosphatases. *Biochem Biophys Res Commun* 255:12-16, 1999.

98. Forster HG, ter Wee PM, Hohman TC, Epstein M: Impairment of afferent arteriolar myogenic responsiveness in the galactose-fed rat is prevented by tolrestat. *Diabetologia* 39:907-914, 1996.

99. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 32:219-226, 1989.

100. Raats CJ, Van Den Born J, Berden JH: Glomerular heparan sulfate alterations: mechanisms and relevance for proteinuria. *Kidney Int* 57:385-400, 2000.

101. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404:787-790, 2000.

102. Nishikawa T, Edelstein D, Brownlee M: The missing link: a single unifying mechanism for diabetic complications. *Kidney Int* 58 Suppl 77:S26-30, 2000.

103. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M: Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A* 97:12222-12226, 2000.

104. Ceriello A, Morocutti A, Mercuri F, Quagliaro L, Moro M, Damante G, Viberti GC: Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. *Diabetes* 49:2170-2177, 2000.

105. Chiarelli F, Verrotti A, Morgese G: Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. *Pediatr Nephrol* 9:154-158, 1995.

106. Dahlquist G, Stattin EL, Rudberg S: Urinary albumin excretion rate and glomerular filtration rate in the prediction of diabetic nephropathy; a long-term follow-up study of childhood onset type-1 diabetic patients. *Nephrol Dial Transplant* 16:1382-1386, 2001.

107. Caramori ML, Gross JL, Pecis M, de Azevedo MJ: Glomerular filtration rate, urinary albumin excretion rate, and blood pressure changes in normoalbuminuric normotensive type 1 diabetic patients: an 8-year follow-up study. *Diabetes Care* 22:1512-1516, 1999.

108. Yip JW, Jones SL, Wiseman MJ, Hill C, Viberti G: Glomerular hyperfiltration in the prediction of nephropathy in IDDM: a 10-year follow-up study. *Diabetes* 45:1729-1733, 1996.

109. Harvey JN, Edmundson AW, Jaffa AA, Martin LL, Mayfield RK: Renal excretion of kallikrein and eicosanoids in patients with type 1 (insulin-dependent) diabetes mellitus. Relationship to glomerular and tubular function. *Diabetologia* 35:857-862, 1992.

110. Corbett JA, Tilton RG, Chang K, Hasan KS, Ido Y, Wang JL, Sweetland MA, Lancaster JR Jr., Williamson JR, McDaniel ML: Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. *Diabetes* 41:552-556, 1992.

111. Graier WF, Wascher TC, Lackner L, Toplak H, Krejs GJ, Kukovetz WR: Exposure to elevated D-glucose concentrations modulates vascular endothelial cell vasodilatory response. *Diabetes* 42:1497-1505, 1993.

112. Houben AJ, Schaper NC, Slaaf DW, Tangelder GJ, Nieuwenhuijzen Kruseman AC: Skin blood cell flux in insulin-dependent diabetic subjects in relation to retinopathy or incipient nephropathy. *Eur J Clin Invest* 22:67-72, 1992.

113. Chiarelli F, Cipollone F, Romano F, Tumini S, Costantini F, di Ricco L, Pomilio M, Pierdomenico SD, Marini M, Cuccurullo F, Mezzetti A: Increased circulating nitric oxide in young patients with type 1 diabetes and persistent microalbuminuria: relation to glomerular hyperfiltration. *Diabetes* 49:1258-1263, 2000.

114. Azar S, Johnson MA, Hertel B, Tobian L: Single-nephron pressures, flows, and resistances in hypertensive kidneys with nephrosclerosis. *Kidney Int* 12:28-40, 1977.

115. Bregman R, Boim MA, Santos OF, Ramos OL, Schor N: Effects of systemic hypertension, antidiuretic hormone, and prostaglandins on remnant nephrons. *Hypertension* 15:I72-75, 1990.

116. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410-415, 1981.

117. Steffes MW, Brown DM, Mauer SM: Diabetic glomerulopathy following unilateral nephrectomy in the rat. *Diabetes* 27:35-41, 1978.

118. Steffes MW, Vernier RL, Brown DM, Basgen JM, Mauer SM: Diabetic glomerulopathy in the uninephrectomized rat resists amelioration following islet transplantation. *Diabetologia* 23:347-353, 1982.

119. Rasch R: Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. The mesangial regions. *Diabetologia* 17:243-248, 1979.

120. Silveiro SP, da Costa LA, Beck MO, Gross JL: Urinary albumin excretion rate and glomerular filtration rate in single-kidney type 2 diabetic patients. *Diabetes Care* 21:1521-1524, 1998.

121. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925-1930, 1986.

122. O'Brien RC, Cooper ME, Jerums G, Doyle AE: The effects of perindopril and triple therapy in a normotensive model of diabetic nephropathy. *Diabetes* 42:604-609, 1993.

123. United Kingdom Prospective Diabetes Study Group: Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. *Br Med J* 317:713-720, 1998.

124. Rudberg S, Østerby R, Bangstad HJ, Dahlquist G, Persson B: Effect of angiotensin converting enzyme inhibitor or beta blocker on glomerular structural changes in young microalbuminuric patients with Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 42:589-595, 1999.

125. Østerby R, Bangstad HJ, Rudberg S: Follow-up study of glomerular dimensions and cortical interstitium in microalbuminuric type 1 diabetic patients with or without antihypertensive treatment. *Nephrol Dial Transplant* 15:1609-1616, 2000.

126. Cordonnier DJ, Pinel N, Barro C, Maynard M, Zaoui P, Halimi S, de Ligny BH, Reznic Y, Simon D, Bilous RW: Expansion of cortical interstitium is limited by converting enzyme inhibition in type 2 diabetic patients with glomerulosclerosis. The Diabiopsies Group. *J Am Soc Nephrol* 10:1253-1263, 1999.

127. United Kingdom Prospective Diabetes Study Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *Br Med J* 317:703-713, 1998.

128. Ray PE, Bruggeman LA, Horikoshi S, Aguilera G, Klotman PE: Angiotensin II stimulates human fetal mesangial cell proliferation and fibronectin biosynthesis by binding to AT1 receptors. *Kidney Int* 45:177-184, 1994.

129. Ihm CG, Park JK, Ahn JH, Lee TW, Kim MJ: Effect of angiotensin converting enzyme inhibitor on collagen production by cultured mesangial cells. *Korean J Intern Med* 9:9-13, 1994.

130. Sorbi D, Fadly M, Hicks R, Alexander S, Arbeit L: Captopril inhibits the 72 kDa and 92 kDa matrix metalloproteinases. *Kidney Int* 44:1266-1272, 1993.

131. Nakamura T, Takahashi T, Fukui M, Ebihara I, Osada S, Tomino Y, Koide H: Enalapril attenuates increased gene expression of extracellular matrix components in diabetic rats. *J Am Soc Nephrol* 5:1492-1497, 1995.

132. Horikoshi S, McCune BK, Ray PE, Kopp JB, Sporn MB, Klotman PE: Water deprivation stimulates transforming growth factor-beta 2 accumulation in the juxtaglomerular apparatus of mouse kidney. *J Clin Invest* 88:2117-2122, 1991.

133. Ray PE, McCune BK, Gomez RA, Horikoshi S, Kopp JB, Klotman PE: Renal vascular induction of TGF-beta 2 and renin by potassium depletion. *Kidney Int* 44:1006-1013, 1993.

134. Flyvbjerg A, Hill C, Grønbæk H, Logan A: Effect of ACE-inhibition on renal TGF-β type II receptor expression in experimental diabetes in rats (Abstract). *J Am Soc Nephrol* 10:679A, 1999.

135. Krolewski AS: Genetics of diabetic nephropathy: evidence for major and minor gene effects. *Kidney Int* 55:1582-1596, 1999.

136. Cowie CC, Port FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne VM: Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *N Engl J Med* 321:1074-1079, 1989.

137. Kalter-Leibovici O, Van Dyk DJ, Leibovici L, Loya N, Erman A, Kremer I, Boner G, Rosenfeld JB, Karp M, Laron Z: Risk factors for development of diabetic nephropathy and retinopathy in Jewish IDDM patients. *Diabetes* 40:204-210, 1991.

138. Allawi J, Rao PV, Gilbert R, Scott G, Jarrett RJ, Keen H, Viberti GC, Mather HM: Microalbuminuria in non-insulin-dependent diabetes: its prevalence in Indian compared with Europid patients. *Br Med J (Clin Res Ed)* 296:462-464, 1988.

139. Krolewski M, Eggers PW, Warram JH: Magnitude of end-stage renal disease in IDDM: a 35 year follow-up study. *Kidney Int* 50:2041-2046, 1996.

140. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161-1165, 1989.

141. Borch-Johnsen K, Norgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving HH: Is diabetic nephropathy an inherited complication? *Kidney Int* 41:719-722, 1992.

142. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39:940-945, 1996.

143. Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. *Am J Kidney Dis* 25:710-713, 1995.

144. Faronato PP, Maioli M, Tonolo G, Brocco E, Noventa F, Piarulli F, Abaterusso C, Modena F, de Bigontina G, Velussi M, Inchiostro S, Santeusanio F, Bueti A, Nosadini R: Clustering of albumin excretion rate abnormalities in Caucasian patients with NIDDM. The Italian NIDDM Nephropathy Study Group. *Diabetologia* 40:816-823, 1997.

145. Canani LH, Gerchman F, Gross JL: Familial clustering of diabetic nephropathy in Brazilian type 2 diabetic patients. *Diabetes* 48:909-913, 1999.

146. Fava S, Azzopardi J, Hattersley AT, Watkins PJ: Increased prevalence of proteinuria in diabetic sibs of proteinuric type 2 diabetic subjects. *Am J Kidney Dis* 35:708-712, 2000.

147. Viberti GC, Keen H, Wiseman MJ: Raised arterial pressure in parents of proteinuric insulin dependent diabetics. *Br Med J (Clin Res Ed)* 295:515-517, 1987.

148. Krolewski AS, Canessa M, Warram JH, Laffel LM, Christlieb AR, Knowler WC, Rand LI: Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318:140-145, 1988.

149. Barzilay J, Warram JH, Bak M, Laffel LM, Canessa M, Krolewski AS: Predisposition to hypertension: risk factor for nephropathy and hypertension in IDDM. *Kidney Int* 41:723-730, 1992.

150. Nelson RG, Pettitt DJ, Baird HR, Charles MA, Liu QZ, Bennett PH, Knowler WC: Pre-diabetic blood pressure predicts urinary albumin excretion after the onset of type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. *Diabetologia* 36:998-1001, 1993.

151. Nelson RG, Pettitt DJ, de Courten MP, Hanson RL, Knowler WC, Bennett PH: Parental hypertension and proteinuria in Pima Indians with NIDDM. *Diabetologia* 39:433-438, 1996.

152. Earle K, Walker J, Hill C, Viberti G: Familial clustering of cardiovascular disease in patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 326:673-677, 1992.

153. Freire MB, Ferreira SR, Vivolo MA, Oliveira JM, Zanella MT: Familial hypertension and albuminuria in normotensive type I diabetic patients. *Hypertension* 23:I256-258, 1994.

154. Adragna NC, Canessa ML, Solomon H, Slater E, Tosteson DC: Red cell lithium-sodium countertransport and sodium-potassium cotransport in patients with essential hypertension. *Hypertension* 4:795-804, 1982.

155. Boerwinkle E, Turner ST, Weinshilboum R, Johnson M, Richelson E, Sing CF: Analysis of the distribution of erythrocyte sodium lithium countertransport in a sample representative of the general population. *Genet Epidemiol* 3:365-378, 1986.

156. Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti G: Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 318:146-150, 1988.

157. Jones SL, Trevisan R, Tariq T, Semplicini A, Mattock M, Walker JD, Nosadini R, Viberti G: Sodium-lithium countertransport in microalbuminuric insulin-dependent diabetic patients. *Hypertension* 15:570-575, 1990.

158. Hardman TC, Dubrey SW, Leslie DG, Hafiz M, Noble MI, Lant AF: Erythrocyte sodium-lithium countertransport and blood pressure in identical twin pairs discordant for insulin dependent diabetes. *Br Med J* 305:215-219, 1992.

159. Monciotti CG, Semplicini A, Morocutti A, Maioli M, Cipollina MR, Barzon I, Palaro C, Brocco E, Trevisan M, Fioretto P, Crepaldi G, Nosadini R: Elevated sodium-lithium countertransport activity in erythrocytes is predictive of the development of microalbuminuria in IDDM. *Diabetologia* 40:654-661, 1997.

160. Mahnensmith RL, Aronson PS: The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. *Circ Res* 56:773-788, 1985.

161. Ng LL, Simmons D, Frighi V, Garrido MC, Bomford J, Hockaday TD: Leucocyte Na+/H+ antiport activity in type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia* 33:371-377, 1990.

162. Trevisan R, Li LK, Messent J, Tariq T, Earle K, Walker JD, Viberti G: Na+/H+ antiport activity and cell growth in cultured skin fibroblasts of IDDM patients with nephropathy. *Diabetes* 41:1239-1246, 1992.

163. Lurbe A, Fioretto P, Mauer M, LaPointe MS, Batlle D: Growth phenotype of cultured skin fibroblasts from IDDM patients with and without nephropathy and overactivity of the Na+/H+ antiporter. *Kidney Int* 50:1684-1693, 1996.

164. Trevisan R, Fioretto P, Barbosa J, Mauer M: Insulin-dependent diabetic sibling pairs are concordant for sodium-hydrogen antiport activity. *Kidney Int* 55:2383-2389, 1999.

165. Adler SG, Pahl M, Seldin MF: Deciphering diabetic nephropathy: progress using genetic strategies. *Curr Opin Nephrol Hypertens* 9:99-106, 2000.

166. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F: Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabetique (GENEDIAB) study group. *J Clin Invest* 99:1585-1595, 1997.

167. Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, Tsai JH: Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan. *Nephrol Dial Transplant* 15:1008-1013, 2000.

168. Doi Y, Yoshizumi H, Yoshinari M, Iino K, Yamamoto M, Ichikawa K, Iwase M, Fujishima M: Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complications in Japanese patients with NIDDM. *Diabetologia* 39:97-102, 1996.

169. Jeffers BW, Estacio RO, Raynolds MV, Schrier RW: Angiotensin-converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus and its relationship with diabetic nephropathy. *Kidney Int* 52:473-477, 1997.

170. Ohno T, Kawazu S, Tomono S: Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. *Metabolism* 45:218-222, 1996.

171. Hadjadj S, Belloum R, Bouhanick B, Gallois Y, Guilloteau G, Chatellier G, Alhenc-Gelas F, Marre M: Prognostic Value of Angiotensin-I Converting Enzyme I/D Polymorphism for Nephropathy in Type 1 Diabetes Mellitus: A Prospective Study. *J Am Soc Nephrol* 12:541-549, 2001.

172. Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, Ueda H, Shintani M, Fukuda M, Ogihara T: Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. *Diabetologia* 41:47-53, 1998.

173. Tarnow L, Gluud C, Parving HH: Diabetic nephropathy and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. *Nephrol Dial Transplant* 13:1125-1130, 1998.

174. Ringel J, Beige J, Kunz R, Distler A, Sharma AM: Genetic variants of the renin-angiotensin system, diabetic nephropathy and hypertension. *Diabetologia* 40:193-199, 1997.

175. Schmidt S, Schone N, Ritz E: Association of ACE gene polymorphism and diabetic nephropathy? The Diabetic Nephropathy Study Group. *Kidney Int* 47:1176-1181, 1995.

176. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving HH: Lack of relationship between an insertion/deletion polymorphism in the angiotensin I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes* 44:489-494, 1995.

177. De Cosmo S, Margaglione M, Tassi V, Garrubba M, Thomas S, Olivetti C, Piras GP, Trevisan R, Vedovato M, Cavallo Perin P, Bacci S, Colaizzo D, Cisternino C, Zucaro L, Di Minno G, Trischitta V, Viberti GC: ACE, PAI-1, decorin and Werner helicase genes are not associated with the development of renal disease in European patients with type 1 diabetes. *Diabetes Metab Res Rev* 15:247-253, 1999.

178. Powrie JK, Watts GF, Ingham JN, Taub NA, Talmud PJ, Shaw KM: Role of glycaemic control in development of microalbuminuria in patients with insulin dependent diabetes. *Br Med J* 309:1608-1612, 1994.

179. Chowdhury TA, Dronsfield MJ, Kumar S, Gough SL, Gibson SP, Khatoon A, MacDonald F, Rowe BR, Dunger DB, Dean JD, Davies SJ, Webber J, Smith PR, Mackin P, Marshall SM, Adu D, Morris PJ, Todd JA, Barnett AH, Boulton AJ, Bain SC: Examination of two genetic polymorphisms within the renin-angiotensin system: no evidence for an association with nephropathy in IDDM. *Diabetologia* 39:1108-1114, 1996.

180. Dudley CR, Keavney B, Stratton IM, Turner RC, Ratcliffe PJ: U.K. Prospective Diabetes Study. XV: Relationship of renin-angiotensin system gene polymorphisms with microalbuminuria in NIDDM. *Kidney Int* 48:1907-1911, 1995.

181. Bjorck S, Blohme G, Sylven C, Mulec H: Deletion insertion polymorphism of the angiotensin converting enzyme gene and progression of diabetic nephropathy. *Nephrol Dial Transplant* 12:67-70, 1997.

182. Oue T, Namba M, Nakajima H, Ono A, Horikawa Y, Yamamoto K, Hamaguchi T, Fujino-Kurihara H, Yamasaki T, Tomita K, Miyagawa J, Hanafusa T, Matsuzawa Y: Risk factors for the progression of microalbuminuria in Japanese type 2 diabetic patients--a 10 year follow-up study. *Diabetes Res Clin Pract* 46:47-55, 1999.

183. Parving HH, Jacobsen P, Tarnow L, Rossing P, Lecerf L, Poirier O, Cambien F: Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin converting enzyme: observational follow up study. *Br Med J* 313:591-594, 1996.

184. Penno G, Chaturvedi N, Talmud PJ, Cotroneo P, Manto A, Nannipieri M, Luong LA, Fuller JH: Effect of angiotensin-converting enzyme (ACE) gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial. EURODIAB Controlled Trial of Lisinopril in IDDM. *Diabetes* 47:1507-1511, 1998.

185. Solini A, Dalla Vestra M, Saller A, Bortoloso E, Velussi M, Cernigoi AM, Frigato F, Nosadini R, Fioretto P: Glomerular structure and angiotensin converting enzyme gene polymorphism in type 2 diabetic patients (Abstract). *Diabetes* 49 (suppl 1):A20, 2000.

186. Rogus JJ, Moczulski D, Freire MB, Yang Y, Warram JH, Krolewski AS: Diabetic nephropathy is associated with AGT polymorphism T235: results of a family-based study. *Hypertension* 31:627-631, 1998.

187. Freire MB, Ji L, Onuma T, Orban T, Warram JH, Krolewski AS: Gender-specific association of M235T polymorphism in angiotensinogen gene and diabetic nephropathy in NIDDM. *Hypertension* 31:896-899, 1998.

188. van Ittersum FJ, de Man AM, Thijssen S, de Knijff P, Slagboom E, Smulders Y, Tarnow L, Donker AJ, Bilo HJ, Stehouwer CD: Genetic polymorphisms of the renin-angiotensin system and complications of insulin-dependent diabetes mellitus. *Nephrol Dial Transplant* 15:1000-1007, 2000.

189. Doria A, Onuma T, Warram JH, Krolewski AS: Synergistic effect of angiotensin II type 1 receptor genotype and poor glycaemic control on risk of nephropathy in IDDM. *Diabetologia* 40:1293-1299, 1997.

190. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, Poirier O, Parving HH: Angiotensinogen gene polymorphisms in IDDM patients with diabetic nephropathy. *Diabetes* 45:367-369, 1996.

191. Schmidt S, Giessel R, Bergis KH, Strojek K, Grzeszczak W, Ganten D, Ritz E: Angiotensinogen gene M235T polymorphism is not associated with diabetic nephropathy. The Diabetic Nephropathy Study Group. *Nephrol Dial Transplant* 11:1755-1761, 1996.

192. Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R, Fagard R: M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *J Hypertens* 17:9-17, 1999.

193. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, Poirer O, Parving HH: Angiotensin-II type 1 receptor gene polymorphism and diabetic microangiopathy. *Nephrol Dial Transplant* 11:1019-1023, 1996.

194. Chowdhury TA, Dyer PH, Kumar S, Gough SC, Gibson SP, Rowe BR, Smith PR, Dronsfield MJ, Marshall SM, Mackin P, Dean JD, Morris PJ, Davies S, Dunger DB, Boulton AJ, Barnett AH, Bain SC: Lack of association of angiotensin II type 1 receptor gene polymorphism with diabetic nephropathy in insulin-dependent diabetes mellitus. *Diabet Med* 14:837-840, 1997.

195. Maeda S, Haneda M, Hayashi K, Koya D, Kikkawa R: (A-C)n dinucleotide repeat polymorphism at 5' end of matrix metaloproteinase 9 (MMP9) gene is associated with nephropathy in Japanese subjects with type 2 diabetes (Abstract). *J Am Soc Nephrol* 9:118A, 1998.

196. Pociot F, Hansen PM, Karlsen AE, Langdahl BL, Johannesen J, Nerup J: TGF-beta1 gene mutations in insulin-dependent diabetes mellitus and diabetic nephropathy. *J Am Soc Nephrol* 9:2302-2307, 1998.

197. Araki S, Antonellis A, Canani L, Warram JH, Krolewski AS: Polymorphism in protein kinase C ß (PKCß) gene and risk of diabetic nephropathy in type 1 diabetes (Abstract). *Diabetes* 49 (Ssuppl 1):A152, 2000.

198. Wong TY, Poon P, Szeto CC, Chan JC, Li PK: Association of plasminogen activator inhibitor-1 4G/4G genotype and type 2 diabetic nephropathy in Chinese patients. *Kidney Int* 57:632-638, 2000.

199. Chen JW, Hansen PM, Tarnow L, Hellgren A, Deckert T, Pociot F: Genetic variation of a collagen IV alpha 1-chain gene polymorphism in Danish insulin-dependent diabetes mellitus (IDDM) patients: lack of association to nephropathy and proliferative retinopathy. *Diabet Med* 14:143-147, 1997.

200. Johannesen J, Tarnow L, Parving HH, Nerup J, Pociot F: CCTTT-repeat polymorphism in the human NOS2-promoter confers low risk of diabetic nephropathy in type 1 diabetic patients. *Diabetes Care* 23:560-562, 2000.

201. Hansen PM, Chowdhury T, Deckert T, Hellgren A, Bain SC, Pociot F: Genetic variation of the heparan sulfate proteoglycan gene (perlecan gene). Association with urinary albumin excretion in IDDM patients. *Diabetes* 46:1658-1659, 1997.

202. Zanchi A, Moczulski DK, Hanna LS, Wantman M, Warram JH, Krolewski AS: Risk of advanced diabetic nephropathy in type 1 diabetes is associated with endothelial nitric oxide synthase gene polymorphism. *Kidney Int* 57:405-413, 2000.

203. Neugebauer S, Baba T, Watanabe T: Association of the nitric oxide synthase gene polymorphism with an increased risk for progression to diabetic nephropathy in type 2 diabetes. *Diabetes* 49:500-503, 2000.

204. Wang Y, Kikuchi S, Suzuki H, Nagase S, Koyama A: Endothelial nitric oxide synthase gene polymorphism in intron 4 affects the progression of renal failure in non-diabetic renal diseases. *Nephrol Dial Transplant* 14:2898-2902, 1999.

205. Fujita H, Narita T, Meguro H, Ishii T, Hanyu O, Suzuki K, Kamoi K, Ito S: Lack of association between an ecNOS gene polymorphism and diabetic nephropathy in type 2 diabetic patients with proliferative diabetic retinopathy. *Horm Metab Res* 32:80-83, 2000.

206. Shah VO, Scavini M, Nikolic J, Sun Y, Vai S, Griffith JK, Dorin RI, Stidley C, Yacoub M, Vander Jagt DL, Eaton RP, Zager PG: Z-2 microsatellite allele is linked to increased expression of the aldose reductase gene in diabetic nephropathy. *J Clin Endocrinol Metab* 83:2886-2891, 1998.

207. Heesom AE, Hibberd ML, Millward A, Demaine AG: Polymorphism in the 5'-end of the aldose reductase gene is strongly associated with the development of diabetic nephropathy in type I diabetes. *Diabetes* 46:287-291, 1997.

208. Moczulski DK, Scott L, Antonellis A, Rogus JJ, Rich SS, Warram JH, Krolewski AS: Aldose reductase gene polymorphisms and susceptibility to diabetic nephropathy in Type 1 diabetes mellitus. *Diabet Med* 17:111-118, 2000.

209. Maeda S, Haneda M, Yasuda H, Tachikawa T, Isshiki K, Koya D, Terada M, Hidaka H, Kashiwagi A, Kikkawa R: Diabetic nephropathy is not associated with the dinucleotide repeat polymorphism upstream of the aldose reductase (ALR2) gene but with erythrocyte aldose reductase content in Japanese subjects with type 2 diabetes. *Diabetes* 48:420-422, 1999.

210. Dyer PH, Chowdhury TA, Dronsfield MJ, Dunger D, Barnett AH, Bain SC: The 5'-end polymorphism of the aldose reductase gene is not associated with diabetic nephropathy in Caucasian type I diabetic patients. *Diabetologia* 42:1030-1031, 1999.

211. Moczulski DK, Burak W, Doria A, Zychma M, Zukowska-Szczechowska E, Warram JH, Grzeszczak W: The role of aldose reductase gene in the susceptibility to diabetic nephropathy in Type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 42:94-97, 1999.

212. Liu ZH, Guan TJ, Chen ZH, Li LS: Glucose transporter (GLUT1) allele (XbaI-) associated with nephropathy in non-insulin-dependent diabetes mellitus. *Kidney Int* 55:1843-1848, 1999.

213. Gutierrez C, Vendrell J, Pastor R, Broch M, Aguilar C, Llor C, Simon I, Richart C: GLUT1 gene polymorphism in non-insulin-dependent diabetes mellitus: genetic susceptibility relationship with cardiovascular risk factors and microangiopathic complications in a Mediterranean population. *Diabetes Res Clin Pract* 41:113-120, 1998.

214. Nair KS, Pabico RC, Truglia JA, McKenna BA, Statt M, Lockwood DH: Mechanism of glomerular hyperfiltration after a protein meal in humans. Role of hormones and amino acids. *Diabetes Care* 17:711-715, 1994.

215. Solling K, Christensen CK, Solling J, Christiansen JS, Mogensen CE: Effect on renal haemodynamics, glomerular filtration rate and albumin excretion of high oral protein load. *Scand J Clin Lab Invest* 46:351-357, 1986.

216. Krishna GG, Newell G, Miller E, Heeger P, Smith R, Polansky M, Kapoor S, Hoeldtke R: Protein-induced glomerular hyperfiltration: role of hormonal factors. *Kidney Int* 33:578-583, 1988.

217. Azevedo MJ, Padilha LM, Gross JL: A short-term low-protein diet reduces glomerular filtration rate in insulin-dependent diabetes mellitus patients. *Braz J Med Biol Res* 23:647-654, 1990.

218. Rudberg S, Dahlquist G, Aperia A, Persson B: Reduction of protein intake decreases glomerular filtration rate in young type 1 (insulin-dependent) diabetic patients mainly in hyperfiltering patients. *Diabetologia* 31:878-883, 1988.

219. Toeller M, Buyken A, Heitkamp G, Bramswig S, Mann J, Milne R, Gries FA, Keen H: Protein intake and urinary albumin excretion rates in the EURODIAB IDDM Complications Study. *Diabetologia* 40:1219-1226, 1997.

220. Riley MD, Dwyer T: Microalbuminuria is positively associated with usual dietary saturated fat intake and negatively associated with usual dietary protein intake in people with insulin-dependent diabetes mellitus. *Am J Clin Nutr* 67:50-57, 1998.

221. Pecis M, de Azevedo MJ, Gross JL: Chicken and fish diet reduces glomerular hyperfiltration in IDDM patients. *Diabetes Care* 17:665-672, 1994.

222. Mollsten AV, Dahlquist GG, Stattin EL, Rudberg S: Higher intakes of fish protein are related to a lower risk of microalbuminuria in young Swedish type 1 diabetic patients. *Diabetes Care* 24:805-810, 2001.

223. Coonrod BA, Ellis D, Becker DJ, Bunker CH, Kelsey SF, Lloyd CE, Drash AL, Kuller LH, Orchard TJ: Predictors of microalbuminuria in individuals with IDDM. Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 16:1376-1383, 1993.

224. Diamond JR: *The role of cholesterol in glomerular injury*, in *Contemporary issues in nephrology*, K Keane, Editor. 1991, Churchill Livingstone: New York. p. 109-126.

225. Sawicki PT, Didjurgeit U, Muhlhauser I, Bender R, Heinemann L, Berger M: Smoking is associated with progression of diabetic nephropathy. *Diabetes Care* 17:126-131, 1994.

226. Chase HP, Garg SK, Marshall G, Berg CL, Harris S, Jackson WE, Hamman RE: Cigarette smoking increases the risk of albuminuria among subjects with type I diabetes. *Jama* 265:614-617, 1991.

227. Microalbuminuria Collaborative Study Group, United Kingdom: Risk factors for development of microalbuminuria in insulin dependent diabetic patients: a cohort study. *Br Med J* 306:1235-1239, 1993.

228. Ferreira SR, Pinto FM: Factors associated with the development of renal complications of diabetes mellitus in Sao Paulo city. *Braz J Med Biol Res* 30:735-744, 1997.

229. Muhlhauser I: Smoking and diabetes. *Diabet Med* 7:10-15, 1990.

230. Groppelli A, Giorgi DM, Omboni S, Parati G, Mancia G: Persistent blood pressure increase induced by heavy smoking. *J Hypertens* 10:495-499, 1992.

OBJETIVOS

1. Analisar criticamente o valor da microalbuminúria como indicador do desenvolvimento de nefropatia diabética em pacientes com diabete melito. (Capítulo I)

2. Analisar as relações entre a estrutura glomerular e a função renal em pacientes com diabete melito tipo 1 de longa duração. (Capítulo II)

CAPÍTULO I

Marcadores de Risco para Nefropatia Diabética:

É a Avaliação da Excreção Urinária de Albumina Suficiente?

The Need for Early Predictors of Diabetic Nephropathy Risk: Is Albumin Excretion Rate Sufficient? *

* Caramori ML, Fioretto P, Mauer M. The need for early predictors of diabetic nephropathy risk. Is albumin excretion rate sufficient? *Diabetes* 49:1399-1408, 2000.

ABREVIATIONS

ACEI: angiotensin converting enzyme inhibitor

AER: albumin excretion rate

DCCT: Diabetes Control and Complications Trial

DN: diabetic nephropathy

ESRD: end-stage renal disease

GBM: glomerular basement membrane

GFR: glomerular filtration rate

HbA_{1c}: glycated hemoglobin

MA: microalbuminuria

NIH: National Institutes of Health

Vv(Mes/glom): mesangial fractional volume per glomerulus

SINOPSE

Os estudos iniciais, mostrando que cerca de 80% dos pacientes com diabete melito tipo 1 e microalbuminúria progrediam para proteinúria, levaram ao uso da microalbuminúria como um indicador de pacientes com risco aumentado para nefropatia diabética. Entretanto, alguns pacientes com microalbuminúria apresentam alterações de estrutura renal avançadas e a microalbuminúria pode, nestes casos, ser mais um marcador da presença de lesões glomerulares do que um indicador do desenvolvimento futuro de nefropatia diabética. Estudos mais recentes têm observado que apenas 30-45% dos pacientes microalbuminúricos com diabete melito tipo 1 progridem para proteinúria após 10 anos, enquanto que cerca de 30% deles retorna à faixa de normoalbuminúria e o restante permanece microalbuminúrico. O achado de que alguns pacientes microalbuminúricos apresentam lesões renais iniciais é consistente com um risco de progressão de microalbuminúria para proteinúria mais baixo do que originalmente estimado e com a noção de que alguns pacientes com microalbuminúria podem reverter para normoalbuminúria. Para aumentar a complexidade do cenário, alguns pacientes normoalbuminúricos com diabete melito tipo 1 de longa duração apresentam lesões renais marcadas e aproximadamente 40% de todos os pacientes destinados a desenvolver proteinúria são normoalbuminúricos na avaliação inicial, apesar de apresentarem diabete melito por vários anos. Os achados em pacientes com diabete melito tipo 2 são similares, entretanto há menos estudos

nesta área. Em conclusão, a microalbuminúria indica pacientes que irão progredir para proteinúria na próxima década de forma menos precisa do que originalmente descrito. Não está claro se estas diferenças foram causadas por mudanças no curso clínico da nefropatia diabética, devido ao melhor controle da glicemia e da pressão arterial, ou se os estudos iniciais, devido ao pequeno número de pacientes avaliados, à escolha de valores críticos para a excreção urinária de albumina *a posteriori* e ao uso de definições variadas de microalbuminúria, superestimaram o risco de progressão para nefropatia diabética. Apesar disto, a excreção urinária de albumina ainda é o melhor indicador não-invasivo de pacientes em risco para nefropatia diabética e deve ser pesquisada de acordo com as normas vigentes. Entretanto, talvez a excreção urinária de albumina não defina pacientes que irão, ou que não irão, desenvolver nefropatia diabética com a acurácia necessária para que decisões clínicas sejam tomadas e para o delineamento de estudos clínicos. É necessário que novos indicadores de risco sejam avaliados. Estes indicadores poderiam ser usados isoladamente ou em associação a outros parâmetros disponíveis.

ABSTRACT

The initial studies showing approximately an 80% rate of progression from microalbuminuria (MA) to proteinuria in type 1 diabetic patients, led to the broad acceptance of MA as a useful clinical predictor of increased diabetic nephropathy (DN) risk. Some MA patients however, have quite advanced renal structural changes, and MA may, in these cases, be a marker, rather than a predictor of DN. More recent studies have observed only about a 30-45% risk of progression of MA to proteinuria over 10 years, while about 30% of MA type 1 diabetic patients became normoalbuminuric and the rest remained MA. The finding that some MA patients have only mild diabetic renal lesions is consistent with the lower than originally estimated risk of progression from MA to proteinuria and with the concept that some MA patients revert to normoalbuminuria. To increase the complexity of the scenario, some normoalbuminuric longstanding type 1 diabetic patients have well established DN lesions and about 40% of all patients destined to progress to proteinuria are normoalbuminuric at initial screening, despite many years of diabetes. A similar picture is emerging in type 2 diabetic patients, although fewer studies have been conducted. Thus, the predictive precision for MA to progress to overt nephropathy over the subsequent decade or so is considerably less than originally described. It is unclear whether this is due to changes in the natural history of DN resulting from improved glycemia and blood pressure control, or whether there were over estimates

of risk in the original studies due to the small sample sizes, *post hoc* analyses and variable MA definitions. Albumin excretion rate (AER) remains the best available non-invasive predictor of DN risk and should be regularly measured following established guidelines. However, AER may be unable to define patients who are safe from or at risk of DN with an accuracy which is adequate for optimal clinical decision making or for the design of certain clinical trials. Investigations into new risk markers or into the combined use of several currently available predictive parameters are needed.

INTRODUCTION

The proportion of patients with end stage renal disease (ESRD) caused by diabetes has progressively increased in the last few decades and diabetic nephropathy (DN) is now the single most common cause of ESRD in the Western world. In fact, in 1997, 44% of all new cases of ESRD in the USA were diabetic, over 80% of whom have type 2 diabetes (1). Although a recent study from Sweden (2), where patients were maintained under strict glycemic control, reported a decrease in the incidence of DN in type 1 diabetic patients, this has not been confirmed (3).

It had generally been considered, based on studies in type 1 diabetes, that once overt DN, manifesting as persistent proteinuria is present, it was only possible to slow, but not halt progression towards ESRD (4-6). This led investigators in the early 1980's to search for early predictors of DN through measures of low concentrations of albumin in the urine. Some diabetic patients were found to have increased urinary albumin excretion rates (AER) not detectable by standard laboratory methods and termed this microalbuminuria (MA). Initial retrospective studies in type 1 diabetic patients (7-9) observed a risk of progression from MA to proteinuria of approximately 80% over the subsequent 6-14 years. These early studies, each of which used different AER criteria for MA, led to a consensus conference where general agreement was reached on the definition of MA (AER: 20-200 μg/min) (10). Since then, there has been broad acceptance of MA as a marker of increased DN risk. However,

concern has been raised that there is a wide range of underlying diabetic glomerular lesions among long-standing type 1 diabetic patients (11,12). For some patients with persistent MA, renal lesions are quite advanced (11-13) and treatment for these patients could be less effective than at earlier stages of disease. Thus, for these patients, MA may be a marker, rather than a predictor, of advanced renal structural changes. It is not surprising, therefore, that patients with MA may progress to proteinuria despite the institution of strict glycemic control (14) and effective antihypertensive treatment. Thus it would make sense to try to identify normoalbuminuric patients at increased DN risk in order to select those at early stages still amenable to aggressive intervention strategies such as strict glycemic control.

Although MA remains the best available marker for DN risk, we will review more recent studies suggesting that the percentage of MA patients progressing to proteinuria over approximately 10 years is 30-45%, much less than the initial reports of about 80% (7-9). Also, some MA patients may revert to normoalbuminuria. Although these differences may represent changes in disease natural history with better treatments, still MA is a less precise predictor of DN risk than originally suggested. In fact, MA patients often have only mild diabetic renal injury (11,12), a finding consistent with a lower risk of progression from MA to proteinuria.

The presence of normal AER in long-standing diabetic patients has been said to identify patients at low risk of DN. However, a significant proportion of normoalbuminuric long-standing diabetic patients have well established DN lesions (11, 12) and about 40% of those who are ultimately at risk of progression to

proteinuria are normoalbuminuric despite many years of diabetes. Thus, it will be argued that AER, albeit the best currently available non-invasive predictor of DN risk, is unable to define patients who are safe from DN with an accuracy optimal for clinical decision making or for the design of certain clinical trials. For these reasons, it is suggested that investigations into new risk markers or into the combined use of several currently available markers may lead to important advances in this field.

METHODS

A predictor of DN, in order to be optimally useful, should identify individuals at increased risk of the development of serious diabetic renal disease early enough in the natural history of the disorder that the evolution of the process can be influenced by intervention strategies. MA is uncommon in the first decade of type 1 diabetes, especially in the first 5 years (15-20) and by 20-25 years much of the natural history of the disorder has already declared itself within a patient population. Therefore, we used data derived from patients with 10-15 years of type 1 diabetes duration to determine the prevalence of normoalbuminuria, MA and proteinuria in cross-sectional studies. Since duration of type 2 diabetes is usually not accurately known, diabetes duration was not considered in the selection of prevalence data for our calculations. Longitudinal studies utilizing AER as a predictor of the subsequent development of proteinuria and studies where such information could be extracted (e.g., control populations in clinical trials) were also reviewed. The authors attempted to review all

pertinent published articles in this area but, rather than performing meta-analyses, we selected those longitudinal studies for review which met the criteria outlined below. Omitted were papers with unorthodox definitions of MA, short follow-up times or inadequate descriptions of the methods. It was considered important that patients be followed for at least 5 years from the baseline evaluation. This long follow-up was selected in order to improve the likelihood that the patient's final outcome would be reflected by the follow-up data. Shorter durations of follow-up were not extrapolated to longer follow-up times since data on patterns of progression of MA patients over time (linear, log linear, etc.) are not currently available. We divided these studies into 3 groups. Group A consisted of studies that adopted the consensus (10) definition of MA (AER of 20-200 μg/min in at least 2 of 3 sequential timed urine collections performed over 1-6 months) where only patients with 5 or more years of follow-up were included. In Group B studies, baseline AER was defined on the basis of a single urine sample and/or the follow-up was a mean or median of 5 years. Studies that would have been in Group A or Group B but that used different AER criteria to define MA were put in Group C. Studies with a mean or median follow-up of less than 5 years were not included. Studies in type 1 diabetic patients were included only if baseline diabetes duration for all patients in these longitudinal studies was at least 7 years. We considered type 1 and type 2 diabetes studies separately.

TYPE 1 DIABETES

Diabetic Nephropathy Risk in Normoalbuminuric Type 1 Diabetic Patients

Three landmark studies placed the issue of AER measurements in diabetic patients at center stage. Two of these met the criteria for inclusion in this review (7, 9) while one included normoalbuminuric patients with as little as 1 year of diabetes duration at baseline (8). The two included studies were in Group C (Table 1). One study found progression from normoalbuminuria to MA (defined as AER 15-150 μg/min) in approximately 14% (9) and one found progression to proteinuria in approximately 12% of patients (7).

Three studies were included in Group A. Forsblom et al (21), in a small well designed study, found that about 7% of normoalbuminuric patients progressed to proteinuria and 14% to MA over 10 years of follow-up. Drs. Peter Rossing and Hans-Henrik Parving (personal communication), at our request, kindly reanalyzed their extensive data based on criteria we imposed for Group A studies. This study, the largest to date by far, found progression rates similar to Forsblom et al (Table 1). Moreover, there was no difference in diabetes duration at baseline in the patients remaining normoalbuminuric compared to those progressing to MA or proteinuria at follow-up. Mathiesen et al (22) found somewhat lower progression rates (Table 1), but excluded some hypertensive patients. Interestingly, this paper noted that the rate of progression from normoalbuminuria to MA and proteinuria was almost constant

throughout the 10 years of the study. The progression risk was somewhat lower in the normoalbuminuric younger patients of Chiarelli et al (23; Table1).

Drs. Michael Steffes and William Thomas kindly facilitated our access to Diabetes Control and Complications Trial (DCCT) data. Normoalbuminuric patients randomized to conventional insulin treatment with at least 5 years of follow up were selected for Group B studies (DCCT, unpublished observations). The DCCT used a single baseline urine sample for initial classification (14). Nonetheless, the DCCT results are identical to those of Rossing and Parving (Table 1). The study of Rudberg et al (24) in children and adolescents showed somewhat higher rates of progression to MA (Table 1).

Based on these studies, we estimate that 5% of normoalbuminuric patients with at least 7 years of type 1 diabetes will progress to proteinuria over the next 5-10 years while 17% will progress to MA. Progression from normoalbuminuria to proteinuria presumes at least the transient presence of MA. Thus, careful follow-up and repeated measures of AER are necessary to detect increasing AER in these patients. In fact, studies show that AER values in the higher range of normoalbuminuria indicate greater risk of progression to MA (25) and these findings should be considered in clinical and clinical research settings.

Glomerular Structure in Normoalbuminuric Type 1 Diabetic Patients

Glomerular structure is normal at onset of diabetes and changes can be detected by morphometric measurements within 1.5-2.5 years after onset (26). However, since

the normal range for glomerular structures, such as glomerular basement membrane (GBM) width or mesangial fractional volume [Vv(Mes/glom)] is quite wide, it may take some time for some individuals to emerge from the normal to the abnormal range. However, glomerular changes of long-standing diabetes are always discernible as evidenced by direct comparison with measures from the patient's non-diabetic identical twin (27). Thus, all patients with type 1 diabetes appear to be developing glomerular structural changes of diabetes, albeit, some at very slow rates. Others develop lesions so fast as to result in overt DN in as little as 10 years. It is not surprising, therefore, that long-standing normoalbuminuric type 1 diabetic patients have increased GBM width and Vv(Mes/glom) compared to age and gender matched non-diabetic normal controls. In the largest study performed so far (12), 66 non-proteinuric patients were divided into 4 groups on the basis of their AER as follows: I) normoalbuminuric with AER <15 μg/min; N=33, II) low level MA with AER 15-30 μg/min; N=11, III) MA with AER 31-70 μg/min; N=13, IV) MA with AER 71-150 μg/min; N=9. Glomerular structural parameters were compared to 52 age- and gender-matched normal controls. Since MA is uncommon in the first decade of diabetes and the degree of glomerulopathy is directly related to the duration of diabetes (28), only patients with diabetes duration of at least 10 years were included. All parameters of glomerulopathy were abnormal in the normoalbuminuric group although approximately half of the patients fell into the normal range. Fig 1 shows data on Vv(Mes/glom) in these patients, but similar results were obtained for GBM width. Note that in many of group I (normoalbuminuric) patients, Vv(Mes/glom), the

structural parameter most closely related to renal functional disturbances in diabetes (29), overlapped with values in patients in the MA groups (Groups III and IV) and, in some instances, approached levels regularly associated with overt DN. Note also that several of the normoalbuminuric patients (Group I) with Vv(Mes/glom) above the normal range had reduced glomerular filtration rate (GFR) (<90ml/min/1.73m^2), hypertension, or both (Fig. 1). The combination of normoalbuminuria and reduced GFR is more likely to occur in type 1 diabetic women (30, 31) and may be related to a self-selected low-protein diet. Whether some of these patients would have been MA on a normal-protein diet is not known.

Other studies have disputed that significant glomerular lesions can be present in normoalbuminuric patients. Berg et al (32) found that 36 normoalbuminuric adolescents (median diabetes duration 10.8 years, range 7.5–19.2) had greater GBM width and mesangial matrix fractional volume than normal controls subjects, but Berg et al did not report an increase in Vv(Mes/glom) in these patients with about half of the diabetes duration of our cohort (12). Using pooled data from several studies from her laboratories (28), Østerby described an increase in GBM width in normoalbuminuric type 1 diabetic patients; while Vv(Mes/glom) was similar in controls and normoalbuminuric patients. However, the differences in ours (12) compared to Østerby's (28) results are best explained by the marked differences in duration of diabetes in the 2 groups of normoalbuminuric patients (12 years in the Aarhus cohort vs. 21 years in the Minnesota cohort). It should also be pointed out that the results of the normoalbuminuric patients shown in Fig. 1 were confirmatory of our

earlier study (11). However, different structural definitions were used in our earlier study (11), and this has led to some confusion in the interpretation of our results (32a). In fact, our findings of advanced lesions in some normoalbuminuric patients are also entirely consistent with the natural history data described above. Thus, it is not surprising that some patients, who are normoalbuminuric after many years of type 1 diabetes, but who have advanced glomerular lesions may progress to MA and proteinuria. In fact, in a preliminary 5-17 years follow-up study of normoalbuminuric patients with long-standing diabetes, we found that those progressing to MA or proteinuria had worse glomerular lesions at baseline than those who remained normoalbuminuric (33). The increase in AER at early clinical stages is related primarily to increasing Vv(Mes/glom). Thus, we previously showed that changes in AER over 5 years correlated with changes in Vv(Mes/glom) over this time, but not with other structural variables (34).

Diabetic Nephropathy Risk in Microalbuminuric Type 1 Diabetic Patients

The three original papers (7-9) on this subject studied a total of 30 patients, used 3 different ranges of AER to define MA, may have used *post hoc* methods to select those ranges, and included some patients whose baseline status was defined by a single urine sample (7, 8). Progression to proteinuria from MA over 6-14 years occurred in about 80% of these patients (Table 2, Group C).

The prospective study of Forsblom et al (21) suggested that these 3 initial studies may have over estimated the risk of progression from MA to proteinuria. This study evaluated 20 MA type 1 diabetic patients with 16-36 years of diabetes duration using

Group A criteria (Table 2) and found progression to proteinuria 10 years later in only 25% while 35% reverted to normoalbuminuria and 40% remained MA. One argument that could be raised against the conclusions of this study is that by selecting patients with at least 15 years duration, the study was biased towards patients less likely to progress, since most patients destined to develop proteinuria will do so before 20 years duration. Drs. Peter Rossing and Hans-Henrik Parving (personal communication) kindly performed analyses we requested on their extensive patient population and permitted the use of the data for this review (Table 2). Using Group A criteria, they followed 132 MA patients with 20.3 ± 8.7 (7-40) years duration for a mean of 9.1 years. Thirty percent had developed proteinuria, 20% became normoalbuminuric, and 50% remained MA at follow-up. Duration of diabetes was, in fact, shorter at baseline in those MA progressing to proteinuria (17 ± 8 years) than those remaining MA (22 ± 9 years; $p<0.005$) but not different from those becoming normoalbuminurics (20 ± 9). This is consistent with an earlier abstract from Rossing et al (35) indicating a 45% risk of progression to proteinuria in MA patients with <15 years diabetes duration vs. 26% progression rate in patients with >15 years duration. Indeed, these studies confirmed Forsblom et al (21) observations of a 25% risk of progression of patients with 15 years or more of diabetes duration. Interestingly, data extracted from conventionally treated MA DCCT patients (Table 2, Group B) revealed progression rates to proteinuria similar to those of the Group A studies (Table 2). However, duration of diabetes in this DCCT cohort was 7-15 years and progression to proteinuria was only 23%. This was less than the 45% progression rate in the similar

but much larger cohort of Rossing et al (35). These differences could be due to the less rigorous definition of MA at baseline in the DCCT study or could represent true population differences. Rudberg et al (24) found an even lower progression rate (18.2%, Table 2) in MA children and adolescents and the reason for this is not clear, but it may be age related [see also Chiarelli et al (23), Table 1] or a consequence of the small sample size.

Based on these more recent studies, we estimate the rate of progression from MA to proteinuria over 5-10 years to be approximately 30%, perhaps 15% higher in patients with <15 years diabetes duration, but considerably lower than originally estimated. It is possible, of course, that with follow-up longer than 10 years, more MA patients would progress to proteinuria. However, it is also possible that more progression would be seen with longer follow-up in the normoalbuminuric patients. These long-term data are needed but are currently not available. One hypothesis which could explain these reduced progression rates is recent changes in the natural history of this disorder based on newer treatment strategies such as improved systemic blood pressure control. Although not answerable with currently available data, Drs. Rossing and Parving did not find a different rate of return to normoalbuminuria from MA in patients treated or not treated with antihypertensive medications (P. Rossing and H-H. Parving, personal communication), including angiotensin converting enzyme inhibitors (ACEI). Another suggestion is that, overall, management of glycemia has improved since the original observations. There are no studies with adequate statistical power to answer this question with confidence. Nonetheless, the DCCT

could not demonstrate a benefit of improved glycemia on risk of progression from MA to proteinuria (14).

Glomerular Structure in Microalbuminuric Type 1 Diabetic Patients

Unlike the controversies regarding normoalbuminuric patients, there is general consensus that, on average, MA patients have increased GBM width and Vv(Mes/glom) compared to normoalbuminuric patients (11-13, 36) and controls (11-13). However, all studies have shown wide ranges of glomerular structure among type 1 MA patients. Thus, GBM width ranges from the upper limits of normal to markedly increased. Moreover, there is no significant increase in GBM width in patients with different levels of MA (12). The same is true for Vv(Mes/glom) (Fig. 1) where values in MA patients in Groups III and IV ranged from the upper limits of normal (12) to levels which overlapped with patients with proteinuria (P.F., M.M.). The values in the patients in Groups III and IV were greater than in patients with lower levels of increased AER (15-30 μg/min; Group II) and normoalbuminuric patients (Group I), while Group I and II overlapped completely (12). Østerby (28) found some MA patients with Vv(Mes/glom) in the normal range. We also found this to be true when patients with AER in the range of 15-30 μg/min were examined (Group II, Fig. 1). However, at higher levels of MA, Vv(Mes/glom) was increased in virtually all patients. Also, we found that patients with AER above 30 μg/min had a relatively high incidence of hypertension, decreased GFR (<90 ml/min/1.73m^2), or both (12). Nonetheless, even among these patients, the range of Vv(Mes/glom) was quite wide and the values in these MA patients overlapped with those of normoalbuminuric

patients (Fig. 1). In longitudinal studies of MA patients Bangstad et al (37) found that GBM width at baseline biopsy was predictive (r^2=0.67; p<0.0001) of AER after 6 years of follow-up whereas Vv(Mes/glom) was a significant but less precise predictor.

In summary, the presence of serious diabetic glomerular lesions in some normoalbuminuric patients suggests that altered glomerular permeability to proteins is not a necessary precondition for the development of these lesions. It is unlikely that established diabetic glomerular lesions are of little prognostic value in normoalbuminuric patients. On the contrary, preliminary studies indicate a greater risk of progression in normoalbuminuric patients with more advanced lesions (33). The risk of progression to proteinuria over the next decade of long-standing type 1 diabetic patients with persistent MA is less than originally estimated. Moreover, about 1/3 of MA patients will return to normoalbuminuria. On the other hand, since 5% of long-standing normoalbuminuric patients will be proteinuric and 17% will be MA after 5-10 years follow-up, and since about 75% of long-standing type 1 diabetic patients will be normoalbuminuric at initial evaluation (15-19), one can estimate that 40% of those patients at risk of DN will be normoalbuminuric at baseline. This variable outcome could reflect the wide range of glomerular structural measures seen among normoalbuminuric and MA patients; however, this hypothesis has not been adequately tested. Finally, these observations should be taken into account in discussing prognosis with individual patients and, perhaps, in making decisions about treatment. Certainly these data need to be carefully considered in designing intervention trials for MA patients.

TYPE 2 DIABETES

Diabetic Nephropathy Risk in Normoalbuminuric Type 2 Diabetic Patients

Mogensen (38) first studied the prognostic value of AER in type 2 diabetic patients (Table 3). AER was measured on spot urines and on single samples in 32% of cases. In this 10-year retrospective study, MA was defined as urinary albumin concentration of 30-140 μg/ml. Progression from normoalbuminuria to proteinuria occurred in 5.5% of these patients. The risk of progression to MA was not stated. The 48% death rate in these initially normoalbuminuric patients was remarkably high. Thus, the data from this study could not be used to estimate the risk of renal progression among normoalbuminuric type 2 diabetic patients.

For this review, we used the 3 published reports which met the Group A studies' criteria other than duration, which cannot be accurately determined in type 2 diabetic patients (Table 3). These studies (39-41) followed patients for 6-9 years from the baseline AER measurement and found incidence of progression from normoalbuminuria to MA of 15-30% and from normoalbuminuria to proteinuria from 0-8%. Table 3 excludes one study with similar outcomes where more than 20% of cases were lost to follow-up (42).

Two papers were categorized in Group B. One produced similar (43) and one produced higher values (44) of progression to proteinuria than the Group A studies (Table 3). Five papers (38, 45-48) were in Group C because of the MA definition used

(Table 3). Progression from normoalbuminuria to MA varied markedly from 5.9 to 57.6% while progression from normoalbuminuria to proteinuria varied from 0-11.8%. Only the data from Group A studies were used for the risk calculation.

Glomerular Structure in Normoalbuminuric Type 2 Diabetic Patients

There are few published papers on renal structure in normoalbuminuric type 2 diabetic patients compared to controls, so information was also extracted from published abstracts.

The rate of development of DN lesions is less clear in type 2 compared with type 1 diabetic patients since, with the exception of the Pima Indian studies (49), duration is usually not precisely established in these patients. Nonetheless, GBM width and Vv(Mes/glom) are increased in normoalbuminuric Caucasian (50), Pima Indian (49), and Japanese (51) long-term type 2 diabetic patients. As in type 1 diabetic patients, there is considerable overlap with normal controls and some normoalbuminuric type 2 diabetic patients have relatively advanced glomerular lesions (50, 51). Thus, as is true for type 1 diabetic patients, there is a structural basis for explaining the progression to MA and proteinuria among some normoalbuminuric type 2 diabetic patients. Whether normoalbuminuric type 2 diabetic patients with more advanced diabetic renal lesions are at greater risk of progression needs to be determined. In Pima Indians, glomerular structure was not different in MA compared with normoalbuminuric patients with long diabetes duration, while MA had more advanced glomerulopathy than normoalbuminuric patients with short duration. These results might explain the observation that some long-term normoalbuminuric patients

are at high risk of progression. Further, as discussed below, there are more varied renal structural patterns and patterns of functional progression among MA, and also proteinuric, type 2 diabetic patients compared with type 1 diabetic patients, and the final outcome of these patients remains to be fully established.

Diabetic Nephropathy Risk in Microalbuminuric Type 2 Diabetic Patients

For reasons outlined above, the initial retrospective examination of outcomes in 76 MA type 2 diabetic patients by Mogensen (38) was a Group C study (Table 4). MA patients in this study had a 77.6% 10 year mortality mostly from cardiovascular disease while 22% progressed to proteinuria.

Subsequently, three prospective Group A studies (40, 52, 53) with much lower mortality rates among MA type 2 diabetic patients have been published. These studies included a total of 159 MA patients followed for 5-6 years with an average risk of progression to proteinuria of about 40% (Table 4). The risk of proteinuria over a longer term follow-up is not known but is presumably greater. One of these studies (40) reported that no patients were normoalbuminuric at follow-up (Table 4). The other 2 studies (52, 53) did not provide these data.

One Group B study evaluating 86 MA patients (Mari-Anne Gall and Hans-Henrik Parving, personal communication) observed, after 5 years of follow-up, approximately the same progression rate to proteinuria as seen in Group A studies, but return to normoalbuminuria was not defined in this cohort (Table 4). Five studies were classified as Group C because of MA definitions (38, 45, 47, 48, 54; Table 4). In

these studies the progression rates from MA to proteinuria ranged from 0-40% and these papers were not used in the risk calculations.

Glomerular Structure in Microalbuminuric Type 2 Diabetic Patients

Caucasian MA type 2 diabetic patients have more complex patterns of renal structural changes than MA type 1 diabetic patients. A light microscopic study of 34 unselected MA type 2 diabetic patients described that 10 (29.4%) had normal or near normal renal structure (55), a finding uncommon in type 1 diabetes. Ten patients had renal structural changes typical of those seen in type 1 diabetic patients with more or less balanced severity of glomerular, tubulointerstitial, vascular and global glomerulosclerosis lesions. However 14 (41.2%) had atypical patterns of renal injury with absent or only mild diabetic glomerular changes associated with other disproportionately severe renal structural changes, including important tubulointerstitial lesions with or without arteriolar hyalinosis and with or without increased global glomerular sclerosis. Patients with proliferative retinopathy all had typical and well-established glomerulopathy lesions. None of the patients without retinopathy had typical lesions. However, background retinopathy could be associated with any of the three structural categories defined above. These studies were confirmed by electron microscopic observations (56) showing that MA type 2 diabetic patients more frequently had electron microscopic morphometric glomerular structural measures in the normal range and, as a group, had less severe lesions than MA type 1 diabetic patients. Many of these observations have been confirmed in Japanese type 2 diabetic patients (51). On the other hand, Pima Indian type 2 diabetic patients at very

high risk of ESRD from diabetes, appear to have lesions more similar to those seen in type 1 diabetic patients. One study has argued that the underlying pattern of renal injury does not predict the rate of GFR decline among a Caucasian cohort of already proteinuric type 2 diabetic patients (57). In contrast, a large 4.3 years follow-up study of ACEI treated Caucasian type 2 diabetic patients with MA or proteinuria observed that patients with more rapid GFR decline had greater GBM width and Vv(Mes/glom) at baseline (58).

In summary, assuming the risk of progression from MA to proteinuria in type 2 diabetic patients to be approximately 40% (Table 4), then the risk of developing proteinuria over the next 10-15 years in normoalbuminuric type 2 diabetic patients would be about 12% (Table 3). Based on studies of more than 6,000 patients, approximately 70% of screened type 2 diabetic patients are normoalbuminuric (42, 59-67). It can then be estimated that about 40% of the dipstick negative type 2 diabetic patients who are ultimately destined to develop proteinuria will be normoalbuminuric at initial screening while about 60% will be MA. Thus, the predictive value of AER below the range of overt proteinuria appears to be similar among type 1 and type 2 diabetic patients. This similarity in prognostic value of AER emerges despite that knowledge of duration in type 2 diabetes is less precise than in type 1 diabetes, and despite that follow-up in the type 2 diabetes studies has tended to be shorter than in type 1 studies. Whether risk of progression in type 2 diabetic patients would be even greater if follow-up were extended is an important but unanswered question. The picture in type 2 diabetes is further complicated by the findings of greater renal

structural heterogeneity among type 2 than type 1 MA and proteinuric patients (50, 51, 56, 68). The regularity with which type 2 patients with proteinuria progress to ESRD is less well known than for type 1 patients. Nelson et al (69) suggested that the rate of decline of GFR among type 2 diabetic Pima Indian patients is similar to that of Caucasian type 1 diabetic patients. However, in contrast to Pima Indians and type 1 diabetic patients, some proteinuric Caucasian and Japanese type 2 diabetic patients have normal or near normal glomerular structure and they seem not to progress towards ESRD at the same rate as patients with advanced lesions (58). At any rate, the prognostic value of proteinuria is less clear in type 2 *versus* type 1 diabetes and, consequently, so is the meaning of progression from MA to proteinuria in these patients. The higher cardiovascular death rate among MA type 2 diabetic patients may further obscure nephropathy risk. There is also a higher incidence of hypertension among normoalbuminuric and MA type 2 patients compared to type 1 patients. On one hand, left untreated, this could superimpose hypertensive renal injury on the diabetic nephropathy lesions. Theoretically, hypertension could also accelerate diabetic lesions. Further, hypertension per se could be associated with increased urinary AER (70-72). Thus, the greater incidence of hypertension in type 2 patients could complicate the predictive value of AER for DN risk in these patients. On the other hand, antihypertensive treatment with drugs, such as ACEI, could directly influence AER (73) obscuring outcomes defined by this measure. Finally, racial factors may have greater influence in nephropathy risk in type 2 than in type 1 diabetes (74). Perhaps even more than it is true for type 1 diabetes, examination of renal

structure may provide a substantial basis for understanding the heterogeneity in outcome among MA type 2 diabetic patients. For these reasons, it is considered vital that well designed large longitudinal natural history and renal biopsy studies be carried out among various ethnic and racial groups with type 2 diabetes. It is worth reiterating that more than 35% of all new ESRD patients in the USA have type 2 diabetes yet their underlying renal disease is still poorly understood.

THE NEED FOR NEW MARKERS AND PREDICTORS OF DIABETIC NEPHROPATHY RISK

DN has rapidly risen to the level of an important public health problem. Early detection of risk leading to the possibility of intervention before advanced renal damage has occurred is an obviously important goal. This goal is made difficult because so much important diabetic renal structural injury can occur in absolute clinical silence. It may not to be practical to treat all diabetic patients with all potentially useful therapies, e.g., strict glycemic control, antihypertensive medications, etc. because of issues of cost and inadequate health care infrastructure, and because those without risk of renal complications would be needlessly exposed to the risk of these treatments. It would be far better to focus the available health care resources on those most likely to benefit. Measurement of AER in the subproteinuric range has been a very important advance in this field. This review confirms that AER is the strongest broadly available marker or predictor of DN risk. However, we need

improved markers and predictors of DN risk. These will be addressed in two general categories: (1) better use of existing methods; and (2) development of new technologies.

Existing Methods

Longitudinal studies are indicated in type 1 and type 2 diabetes which would examine the potential value of using repeated measures of AER over time, different set points for the definition of MA or both. In addition, the combination of measures of AER with multiple clinical and renal structural parameters may lead to the development of more precise risk estimates for DN. These additional variables could include age, diabetes duration, blood pressure (including 24 hr blood pressure monitoring), GFR, glycosylated hemoglobin (HbA_{1c}), retinopathy, and renal biopsy measurements. Prospective studies in type 1 and type 2 diabetic patients generally support the concept that normoalbuminuric and MA patients that progress have significantly higher baseline levels of blood pressure (25, 39, 43, 44, 47, 75-77) and HbA_{1c} (14, 21, 22, 25, 39, 40, 43-45, 47, 77-79) compared to patients that do not progress. However, there is still controversy as to whether increased baseline GFR is a predictor of progression (9, 24, 80-85). Preliminary results of our prospective study in normoalbuminuric type 1 diabetic patients have shown that patients who progress to MA or proteinuria have worse baseline glomerular lesions, lower GFR and are more frequently hypertensive than patients remaining normoalbuminuric (33). Other variables, in a list by no means meant to be exhaustive, could include plasma prorenin (86, 87), erythrocyte sodium/lithium countertransport activity (23), lipid levels,

smoking history, and family history of cardiovascular disease and DN. From such studies could emerge a multivariate risk assessment scheme far more exact than AER alone.

Development of New Technologies

New tests are needed to provide accurate DN risk estimates before renal functional disturbances are well established (88). Initially these tests will need to be validated, at least in part, by their association with important renal lesions as ascertained in renal biopsies. If sufficiently precise, these early predictors could obviate the need for renal biopsy except as a research tool. There are many possibilities for such new approaches to this problem and only a few are suggested here:

- Identification of genes associated with increased or decreased DN risk.

- Measures of substances in blood or urine, such as extracellular matrix molecules, products of glycation or growth factors.

- Measurements of tubular function.

- Measurement of cellular functions (e.g., in cultured skin fibroblasts) which may be associated with DN risk, including extracellular matrix molecules and growth factors.

- Less invasive methods such as fine needle aspiration to sample renal tissues for structural or biochemical changes associated with nephropathy risk.

- Development and application of new imaging technologies (e.g., positron emission tomography, magnetic resonance imaging) as tools to detect early renal diabetic biochemical or structural changes.

CONCLUSIONS

The measurements of urinary AER has led to very important advances in the field of DN. AER is currently the best available non-invasive means of following the course of kidney disease in non-proteinuric diabetic patients, therefore this review strongly supports the current recommendation that urinary AER should be monitored on a regular basis, in accordance with accepted protocols and procedures (89, 90). Moreover, given their increased risk of progression, patients with persistent MA should be considered for antihypertensive therapy and improved glycemic control. However, concerns have been previously raised (39, 51, 91) and this review concurs that AER does not predict DN risk with the accuracy suggested by the original studies in this field (7-9) and that changes in the natural history of this disease may not fully explain these discrepancies. Moreover, AER as a predictor in non-proteinuric diabetic patients may not be sufficient for optimal clinical decision making, clinical research design or public health policy development. Improved predictors could come from existing methodologies or from technologies not yet fully developed. The growing magnitude of the DN problem and its huge human and social costs mandate that we commit far greater basic and clinical research resources to this problem. The value of

long-term continuous research support can be seen in the NIH's use of intramural funding studies of the Pima Indian population and this concept should be expanded to the study of other important patient groups. This need is dictated by the very long and largely silent natural history of DN and this natural history will not be changed by wishful thinking.

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TABLE 1
Risk of progression from normoalbuminuria to microalbuminuria or proteinuria in type 1 diabetic patients

Group	Authors	N	Diabetes* Duration in years	Observation* Period in years	Cumulative Incidence of Proteinuria (%)	Cumulative Incidence of Microalbuminuria (%)
Group A	Forsblom et al. (21)	29	22.1 ± 5.4 (15-38)	10	6.9	13.8
	Rossing and Parving (personal communication)	453	19.7 ± 9.3 (7-40)	9.0 ± 1.3 (5-10)	5	17
	Mathiesen et al. (22)	209	17 ± 5 (10-30)	10	3.8	10
	Chiarelli et al. (23)	170	≈9.3 (7.1-23.2)	≈8 (8.1-9.3)	0	10.6
Group B	DCCT (unpublished data)	204	10.6 ± 2.3 (7-15)	6.8 ± 1.5 (5-9)	5	17
	Rudberg et al. (24)	53	≈11.5 >8	8	5.7	28.3
Group C	Mogensen and Christensen (9)	29	— (7-19)	— (7-14)	0	13.8
	Parving et al. (7)	17	15 ± 4 (10-24)	6	11.8	0

*Mean ± SD (range); —:data not provided; N=number.

TABLE 2
Risk of progression from microalbuminuria to proteinuria in type 1 diabetic patients

Group	Authors	N	Diabetes* Duration in years	Observation* Period in years	Cumulative Incidence of Proteinuria (%)	Cumulative Incidence of Normoalbuminuria (%)
Group A	Forsblom et al. (21)	20	25.7 ± 5.7 (16-36)	10	25	35
	Rossing and Parving (personal communication)	132	20.3 ± 8.7 (7-40)	9.1 ± 1.3 (5-10)	30	20
Group B	DCCT (unpublished data)	30	11.4 ± 2.3 (7-15)	7.3 ± 1.6 (5-9)	23	43
	Rudberg et al. (24)	11	≈11.5 >8	8	18.2	36.4
Group C	Mogensen and Christensen (9)	14	— (7-19)	≈10 (7-14)	85.7	7.1
	Viberti et al. (8)	8	14.1 ± 2.9 (7-33)	14	87.5	0
	Parving et al. (7)	8	19 ± 5 (13-25)	6	62.5	25

*Mean ± SD (range); —: data not provided; N= number.

TABLE 3
Risk of progression from normoalbuminuria to microalbuminuria or proteinuria in type 2 diabetic patients

Group	Authors	N	Age* in years	Observation* Period in years	Cumulative Incidence of Proteinuria (%)	Cumulative Incidence of Microalbuminuria (%)
Group A	Forsblom et al (39)	108	≈ 58 (35-70)	9	8.3	20.4
	Tanaka et al (40)	74	≈ 61 (60-75)	6	0	32.4
	Ravid et al (41)	97	54.4 ± 2.9 (38-59)	6	0	15.5
Group B	Gall et al (43)	191	55 (20-65)	5.8 (1.5-6.0)	2.6	18.8
	Ravid et al (44)	621	47.7 ± 4.5 (40-60)	7.8 ± 0.9 (2-9)	14.5	17.9
Group C	Mogensen (38)	128	≈ 66 50-75	10	5.5	—
	Kawazu et al (45)	33	≈ 54	8	0	57.6
	Jerums et al (46)	51	57 ± 7.1	6.4 ± 2.1 (3-10.3)	11.8	5.9
	Haneda et al (47)	34	≈ 57	5	2.9	29.4
	Niskanen et al (48)	92	≈ 56 (45-64)	5	0	10.9

*Mean ± SD (range); —:data not provided; N=number.

TABLE 4
Risk of progression from microalbuminuria to proteinuria in type 2 diabetic patients

Group	Authors	N	Age* in years	Observation Period in years	Cumulative Incidence of Proteinuria (%)	Cumulative Incidence of Normoalbuminuria (%)
Group A	Tanaka et al (40)	49	≈ 65 (60-75)	6	53.1	0
	Ravid et al (52)	52	44.8 ± 3.5 (36-49)	5	36.5	—
	Ahmad et al (53)	58	50.3 ± 2.1 (45-55)	5	20.7	—
Group B	Gall and Parving (personal communication)	86	58 (28-65)	5	34.8	—
Group C	Mogensen (38)	76	≈ 66 50-75	10	22.4	—
	Yajima et al (54)	59	—	9	35.6	—
	Kawazu et al (45)	15	≈ 56	8	40	0
	Haneda et al (47)	18	—	5	33.3	0
	Niskanen et al (48)	21	≈ 56 (45-64)	5	0	42.9

*Mean ± SD (range); —: data not provided; N=number.

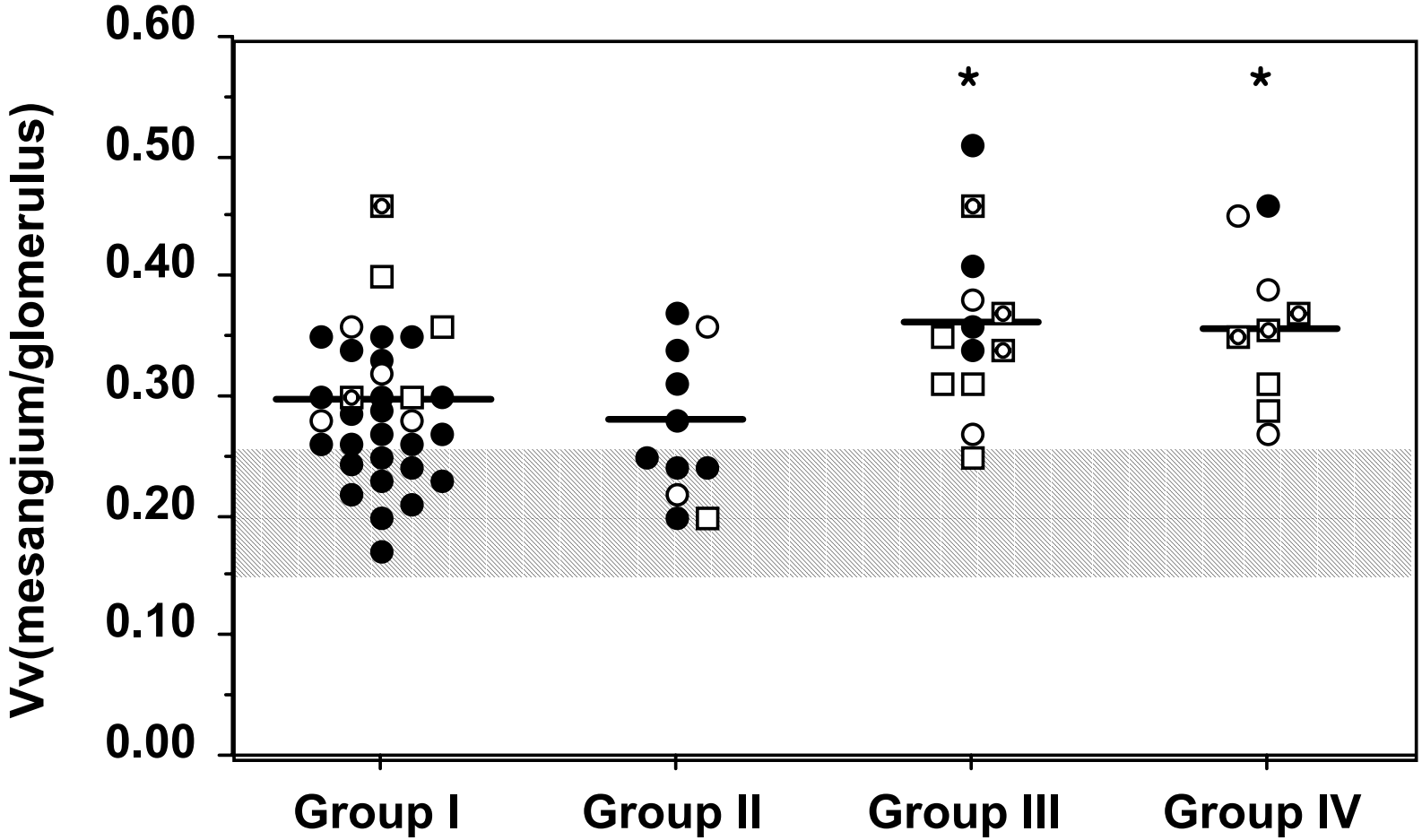

Figure 1. Mesangial fractional volume [Vv (mesangium/glomerulus)] in the four groups of patients. The shaded area represents mean ± 2 SD in a group of 52 age-matched normal control subjects. ∀ Normal BP and GFR; (, reduced GFR (<90ml/min/1.73m^2); □ , hypertension (≥140/85); (□ , reduced GFR and hypertension. *p<0.005 *vs*. groups I and II (12).

REFERENCES

1. 1999 United States Renal Data System Annual Report. National Technical Information Service. US Department of Health and Human Services, Springfield, VA.

2. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J. Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* 330:15-18, 1994.

3. Rossing P, Rossing K, Jacobsen P, Parving HH. Unchanged incidence of diabetic nephropathy in IDDM patients. *Diabetes* 44:739-743, 1995.

4. Mogensen CE. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *Br Med J* 285:685-688, 1982.

5. Parving H-H, Andersen AR, Smidt UM, Svendensen PA. Early and aggressive antihypertensive treatment reduces the rate of decline in kidney function in diabetic nephropathy. *Lancet* 1:1175-1179, 1983.

6. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 329:1456-1462, 1993.

7. Parving H-H, Oxenbøll B, Svendsen PA, Christiansen JS, Andersen AR. Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *Acta Endocrinol (Copenh)* 100:550-555, 1982.

8. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1:1430-1432, 1982.

9. Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89-93, 1984.

10. Mogensen CE, Chachati A, Christensen CK, Close CF, Deckert T, Hommel E, Kastrup J, Lefebvre P, Mathiesen ER, Feldt-Rasmussen B, Schmitz A, Viberti GC. Microalbuminuria: An early marker of renal involvement in diabetes. *Uremia Invest* 9:85-95, 1985-1986.

11. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM. Glomerular lesions and urinary albumin excretion in type I diabetic patients without overt proteinuria. *N Engl J Med* 320:966-970, 1989.

12. Fioretto P, Steffes MW, Mauer SM. Glomerular structure in non-proteinuric insulin-dependent diabetic patients with various levels of albuminuria. *Diabetes* 43:1358-1364, 1994.

13. Bangstad HJ, Østerby R, Dahl-Jorgensen K, Berg KJ, Hartmann A, Nyberg G, Frahm Bjorn S, Hanssen KF. Early glomerulopathy is present in young, type 1 (insulin-dependent) diabetic patients with microalbuminuria. *Diabetologia* 36:523-529, 1993.

14. The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney Int* 47:1703-1720, 1995.

15. Parving H-H, Hommel E, Mathiesen E, Skøtt P, Edsberg B, Bahnsen M, Lauritzen M, Hougaard P, Lauritizen E. Prevalence of microalbuminuria, arterial hypertension, retinopathy and neuropathy in patients with insulin dependent diabetes. *Br Med J* 296: 156-160, 1988.

16. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH. Prevalence of complications in IDDM by sex and duration. Pittsburgh epidemiology of diabetes complications study II. *Diabetes* 39:1116-1124, 1990.

17. Mangili R, Deferrari G, DiMario U, Giampietro O, Navalesi R, Nosadini R, Rigamonti G, Spezia R, Crepaldi G. Arterial hypertension and microalbuminuria in IDDM. The Italian microalbuminuria study. *Diabetologia* 37:1015-1024, 1994.

18. Olsen BS, Johannesen J, Sjølie AK, Borch-Johnsen K, Hougaard P, Thorsteinsson B, Pramming S, Marinelli K, Mortensen HB and the Danish study group of diabetes in childhood. Metabolic control and prevalence of microvascular complications in young patients with type 1 diabetes mellitus. *Diabet Med* 16:79-85, 1999.

19. Warran JH, Gearin G, Laffel L, Krolewski AS. Effect of duration of type 1 diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol* 7:930-937, 1996.

20. Microalbuminuria Collaborative Study Group. Microalbuminuria in type 1 diabetic patients. Prevalence and Clinical Characteristics. *Diabetes Care* 15:495-501, 1992.

21. Forsblom CM, Groop P-H, Ekstrand A, Groop LC. Predictive value of microalbuminuria in patients with insulin dependent diabetes of long duration. *Br Med J* 305:1051-1053, 1992.

22. Mathiesen ER, Rønn B, Storm B, Foght H, Deckert T. The natural course of microalbuminuria in insulin dependent diabetes: A 10-year prospective study. *Diabet Med* 12:482-487, 1995.

23. Chiarelli F, Catino M, Tumini S, Martino M, Mezzetti A, Varrotti A, Vanelli M. Increased Na^+/Li^+ countertransport activity may help to identify type 1 diabetic adolescents and young adults at risk for developing persistent microalbuminuria. *Diabetes Care* 22:1158-1164, 1999.

24. Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration rate as a predictor of diabetic nephropathy-an 8-year prospective study. *Kidney Int* 41:822-828, 1992.

25. Microalbuminuria Collaborative Study Group. Predictors of the development of microalbuminuria in patients with type 1 diabetes mellitus: a seven-year prospective study. *Diabet Med* 16:918-925, 1999.

26. Østerby R. Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes: I. Development of initial basement membrane thickening. *Diabetologia* 8:84-92, 1972.

27. Steffes MW, Sutherland DER, Goetz FC, Rich SS, Mauer SM. Studies of kidney and muscle biopsy specimens from identical twins discordant for type I diabetes mellitus. *N Engl J Med* 312:1282-1287, 1985.

28. Østerby R. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia* 35:803-812, 1992.

29. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143-1155, 1984.

30. Lane PH, Steffes MW, Mauer M. Glomerular structure in IDDM women with low glomerular filtration rate and normal urinary albumin excretion. *Diabetes* 41:581-586, 1992.

31. Tsalamandris C, Allen TJ, Gilbert RE, Sinha A, Panagiotopoulos S, Cooper ME, Jerums G. Progressive decline in renal function in diabetic patients with and without albuminuria. *Diabetes* 43:649-655, 1994.

32. Berg UB, Torbjörnsdotter TB, Jaremko G, Thalme B. Kidney morphological changes in relation to long-term renal function and metabolic control in adolescents with IDDM. *Diabetologia* 41:1047-1056, 1998.

32a. Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. *Diabetologia* 42:263-285, 1999.

33. Caramori ML, Fioretto P, Mauer M. Long-term follow-up of normoalbuminuric longstanding type 1 diabetic patients: Progression is associated with worse baseline glomerular lesions and lower glomerular filtration rate (Abstract). *J Am Soc Nephr* 10:126A, 1999.

34. Fioretto P, Steffes MW, Sutherland DER, Mauer M. Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. *Kidney Int* 48:1929-1935, 1995.

35. Rossing P, Hougaard P, Borch-Johnsen K, Parving H-H. Progression from microalbuminuria to diabetic nephropathy in IDDM (Abstract). *J Am Soc Nephr* 8:117A, 1997.

36. Walker JD, Close CF, Jones SH, Rafftery M, Keen H, Viberti GC, Østerby R. Glomerular structure in type 1 (insulin-dependent) diabetic patients with normo-and microalbuminuria. *Kidney Int* 41:741-748, 1992.

37. Bangstad HJ, Østerby R, Hartmann A, Berg TJ, Hanssen KF. Severity of glomerulopathy predicts long-term urinary albumin excretion rate in patients with type 1 diabetes and microalbuminuria. *Diabetes Care* 22:314-319, 1999.

38. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 310:356-360, 1984.

39. Forsblom CM, Groop P-H, Ekstrand A, Totterman KJ, Sane T, Saloranta C, Groop L. Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. *Diabetes Care* 21:1932-1938, 1998.

40. Tanaka Y, Atsumi Y, Matsuoka K, Onuma T, Tohjima T, Kawamori R. Role of glycemic control and blood pressure in the development and progression of nephropathy in elderly Japanese NIDDM patients. *Diabetes Care* 21:116-120, 1998.

41. Ravid M, Brosh D, Levi Z, Bar-Dayan Y, Ravid D, Rachmani R. Use of enalapril to attenuate decline in renal function in normotensive, normoalbuminuric patients with type 2 diabetes mellitus. A randomized, controlled trial. *Ann Intern Med* 128:982-988, 1998.

42. John L, Sunder Rao PSS, Kanagasabapathy AS. Rate of progression of albuminuria in type II diabetes. Five-year prospective study from South India. *Diabetes Care* 17:888-890, 1994.

43. Gall MA, Hougaard P, Borch-Johsen K, Parving H-H. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. *Br Medical J* 314:783-788, 1997.

44. Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R. Main risk factor for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med* 158:998-1004, 1998.

45. Kawasu S, Tomono S, Shimizu M, Kato N, Ohno T, Ishii C, Murata K, Watanabe T, Negishi K, Suzuki M, Takahashi M, Ishii J. The relationship between early diabetic nephropathy and control of plasma glucose in non-insulin-dependent diabetes mellitus. *J Diabetes Complications* 8:13-17, 1994.

46. Jerums G, Cooper ME, Seeman E, Murray RML, Mcneil J. Spectrum of proteinuria in type I and type II diabetes. *Diabetes Care* 10:419-427, 1987.

47. Haneda M, Kikkawa R, Togawa M, Koya D, Kajiwara N, Uzu T, Shigeta Y. High blood pressure is a risk factor for the development of microalbuminuria in Japanese subjects with non-insulin-dependent diabetes mellitus. *J Diabetes Complications* 6:181-185, 1992.

48. Niskanen L, Uusitupa M, Siitonen O, Voutilainen E, Penttilä I, Pyörälä K. Microalbuminuria predicts the development of serum lipoprotein abnormalities favoring atherogenesis in newly diagnosed type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 33:237-243, 1990.

49. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW. Podocyte loss and progressive glomerular injury in type II diabetes. *J Clin Invest* 99:342-348, 1997.

50. Fioretto P, Mauer M, Velussi M, Carraro A, Muollo B, Baggio B, Crepaldi G, Nosadini R. Ultrastructural measures of glomerular extracellular matrix accumulation in non-proteinuric type 2 diabetic patients (Abstract). *J Am Soc Nephrol* 7:1356-1357, 1996.

51. Moriya T, Moryia R, Yajima Y, Steffes MW, Mauer M. Urinary albumin is a weaker predictor of diabetic nephropathy lesions in Japanese NIDDM patients than in Caucasians IDDM patients (Abstract). *J Am Soc Nephrol* 8:116A, 1997.

52. Ravid M, Savin H, Jutrin I, Bental T, Katz B, Lishner M. Long-term stabilizing effect of angiotensin-converting enzyme inhibition on plasma creatinine and on proteinuria in normotensive type II diabetic patients. *Ann Intern Med* 118:577-581, 1993.

53. Ahmad J, Siddiqui MA, Ahmad H. Effective postponement of diabetic nephropathy with enalapril in normotensive type 2 diabetic patients with microalbuminuria. *Diabetes Care* 20:1576-1581, 1997.

54. Yajima Y, Jim Y, Moriya T, Matoba K. Progression from microalbuminuria to overt nephropathy in NIDDM: The receiver operating characteristics curve analysis. *Diabetologia* 41(suppl 1):A287, 1998.

55. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R. Patterns of renal injury in NIDDM patients with microalbuminuria. *Diabetologia* 39: 1569-1576, 1996.

56. Fioretto P, Mauer M, Bortoloso E, Barzon I, Saller A, Dalla Vestra M, Abaterusso C, Baggio B, Nosadini R. Glomerular ultrastructure in type 2 diabetes (Abstract). *J Am Soc Nephrol* 9:114A, 1998.

57. Ruggenenti P, Gambara V, Perna A, Bertani T, Remuzzi G. The nephropathy of non-insulin-dependent diabetes: Predictors of outcome relative to diverse patterns of renal injury. *J Am Soc Nephrol* 9:2336-2343, 1998.

58. Nosadini R, Velussi M, Brocco E, Bruseguin M, Abaterusso C, Saller A, Della Vestra M, Carraro A, Bortoloso E, Sambataro M, Barzon I, Frigato F, Muollo B, Chiesura-Corona M, Pacini G, Baggio B, Piarulli F, Sfriso A, Fioretto P. Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. *Diabetes* 49:476-484, 2000.

59. Verthoeven S, van Ballegooie E, Casparie AF. Impact of late complications in type 2 diabetes in a Dutch population. *Diabet Med* 8:435-438, 1991.

60. Bruno G, Cavallo Perin P, Bargero G, Borra M, Calvi V, Derrico N, Deambrogio P, Pagano G. Prevalence and risk factors for micro- and macroalbuminuria in an Italian population-based cohort of NIDDM subjects. *Diabetes Care* 19: 43-47, 1996.

61. Piehlmeier W, Renner R, Schramm W, Kimmerling T, Garbe S, Proetzch R, Fahn J, Piwernetz K, Landgraf R. Screening of diabetic patients for microalbuminuria in primary care-The PROSIT-Project Proteinuria Screening and Intervention. *Exp Clin Endocrinol Diabetes* 107:244-251, 1999.

62. Klein R, Klein BE, Moss SE. Prevalence of microalbuminuria in older-onset diabetes. *Diabetes Care* 16:1325-1330, 1993.

63. Gall MA, Rossing P, Sk‡tt P, Damsbo P, Vaag A, Bech K, Dejgaard A, Lauritzen M, Lauritzen E, Hougaard P, Beck-Nielsen H, Parving H-H. Prevalence of micro- and macroalbuminuria, arterial hypertension, retinopathy and large vessel disease in European type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 34:655-661, 1991.

64. Schmitz A, Vaeth M. Microalbuminuria: a major risk factor in non-insulin-dependent diabetes. A 10-year follow-up study of 503 patients. *Diabet Med* 5:126-134, 1988.

65. Esmatjes E, Castell C, Gonzales T, Tresseras R, Lloveras G. Epidemiology of renal involvement in type II diabetics (NIDDM) in Catalonia. The Catalan Diabetic Nephropathy Study Group. *Diabetes Res Clin Pract* 32:157-163, 1996.

66. Delcourt C, Vauzelle Kervroedan F, Cathelineau G, Papoz L. Low prevalence of long-term complications in non-insulin dependent diabetes mellitus in France: a multicenter study. CODIAB-INSERM-ZENECA Pharma Study Group. *J Diabetes Complications* 12:88-95, 1998.

67. Torffvit O, Agardh E, Agardh CD. Albuminuria and associated medical risk factors: a cross-sectional study in 451 type II (non-insulin-dependent) diabetic patients. Part 2. *J Diabetes Complications* 5:29-34, 1991.

68. Østerby R, Gall M-A, Schmitz A, Nielsen FS, Nyberg G, Parving H-H. Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:1064-1070, 1993.

69. Nelson RG, Meyer TW, Myers BD, Bennett PH. Course of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. *Kidney Int* 52 (suppl 63):S45-S48, 1997.

70. Metcalf P, Baker J, Scott A, Wild C, Scragg R, Dryson E. Albuminuria in people at least 40 years old: Effect of Obesity, Hypertension, and Hyperlipidaemia. *Clin Chem* 38:1802-1808, 1992.

71. Bigazzi R, Bianchi S, Campese VM, Baldari G. Prevalence of microalbuminuria in a large population of patients with mild to moderate essential hypertension. *Nephron* 61:94-97, 1992.

72. Rambausek M, Fliser D, Ritz E. Albuminuria of hypertensive patients. *Clin Nephrol* 38(suppl 1):S40-S45, 1992.

73. United Kingdom Prospective Diabetes Study Group. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: United Kingdom Prospective Diabetes Study 39. *Br Med J* 317:713-720, 1998.

74. Cowie CC, Port FK, Wolfe RA, Savage PA, Moll PA, Hawthorne VM. Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *N Engl J Med* 321:1074-1079, 1989.

75. Poulsen PL, Hansen KW, Mogensen CE. Ambulatory blood pressure in the transition from normo- to microalbuminuria. A longitudinal study in IDDM patients. *Diabetes* 43:1248-1253, 1996.

76. United Kingdom Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *Br Med J* 317:703-713, 1998.

77. Warram JH, Scott LJ, Hanna LS, Wantman M, Cohen SE, Laffel LMB, Ryan L, Krolewski AS. Progression of microalbuminuria to proteinuria in type 1 diabetes. Nonlinear relationship with hyperglycemia. *Diabetes*, 49:94-100,2000.

78. United Kingdom Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-853, 1998.

79. United Kingdom Prospective Diabetes Study Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 352:854-865, 1998.

80. Mogensen CE. Early glomerular hyperfiltration in insulin-dependent diabetes and late nephropathy. *Scand J Clin Lab Invest* 46:201-206, 1986.

81. Chiarelli F, Verrotti A, Morgese E. Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. *Pediatr Nephrol* 9:154-158, 1995.

82. Caramori MLA, Pecis M, Gross JL, de Azevedo MJ. Glomerular filtration rate, urinary albumin excretion rate, and blood pressure changes in normoalbuminuric normotensive type 1 diabetic patients: An 8-year follow-up study. *Diabetes Care* 22:1512-1516, 1999.

83. Yip JW, Jones SL, Wiseman MJ, Hill C, Viberti GC. Glomerular hyperfiltration in the prediction of nephropathy in IDDM: A 10-year follow-up study. *Diabetes* 45:1729-1733, 1996.

84. Bognetti E, Meschi F, Bonfanti R, Gianolli L, Chiumello G. Decrease of glomerular hyperfiltration in short-term diabetic adolescents without microalbuminuria. *Diabetes Care* 16:120-124, 1993.

85. Lervang H-H, Jensen S, Brøchner-Mortensen J, Ditzel J. Early glomerular hyperfiltration and the development of late nephropathy in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 31:723-729, 1988.

86. Allen TJ, Cooper ME, Gilbert RE, Winikoff J, Skinner SJ, Jerums G. Serum total renin is increased before microalbuminuria in diabetes. *Kidney Int* 50:902-907, 1996.

87. Deinum J, Rønn B, Mathiesen E, Derkx FHM, Hop WCP, Schalekamp MADH. Increase in serum prorenin precedes onset of microalbuminuria in patients with insulin-dependent diabetes mellitus. *Diabetologia* 42:1006-1010, 1999.

88. Conquering Diabetes. A Report of the Congressionally-Established Diabetes Research Working Group 1999. NIH publication N°. 99-4398. National Diabetes Information Clearinghouse, Bethesda, MD.

89. American Diabetes Association. Diabetic nephropathy. *Diabetes Care* 22 (suppl 1):S66-S69, 1999.

90. Meltzer S, Leiter L, Daneman D, Gerstein HC, Lau D, Ludwig S, Yale JF, Zinman B, Lillie D. 1998 Clinical practice guidelines for the management of diabetes in Canada. Canadian Diabetes Association. *Can Med Assoc J* 159 (suppl 8):S1-S29, 1998.

91. Danne T, Kordonouri O, Hövener G, Weber B. Diabetic angiopathy in children. *Diabet Med* 14:1012-1025, 1997.

CAPÍTULO II

Relação entre a Estrutura Glomerular e a Função Renal em Pacientes com Diabete Melito Tipo 1.

Renal Structural-Functional Relationships in Long-standing Type 1 Diabetic Patients.

* Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, Russel G, Mauer M. *Diabetes*, in press.

ABBREVIATIONS

ACEI: angiotensin converting enzyme inhibitor

AER: albumin excretion rate

AIIRB: angiotensin II type 1 receptor blocker

ANOVA: analysis of variance

DBP: diastolic blood pressure

GBM: glomerular basement membrane

GFR: glomerular filtration rate

HbA_{1c}: glycated hemoglobin

MBP: mean blood pressure

MES: mesangium expansion score

RAS: renin-angiotensin system

Vv(Mes/glom): mesangial fractional volume per glomerulus

Vv(MC/glom): mesangial cell fractional volume per glomerulus

Vv(MM/glom): mesangial matrix fractional volume per glomerulus

Vv[MM/(MM+MC)]: mesangial matrix fractional volume per mesangium

SBP: systolic blood pressure

Sv(PGBM/glom): surface density of peripheral glomerular basement membrane per glomerulus

SINOPSE

Este estudo tem como objetivo avaliar as relações entre a estrutura glomerular e a função renal e elucidar os fatores associados à nefropatia diabética em pacientes com diabete melito tipo 1 de longa duração classificados de acordo com a estrutura glomerular renal. Foram considerados elegíveis para este estudo pacientes que apresentassem pelo menos 8 anos de duração de diabete melito tipo 1, taxa de filtração glomerular >30ml/min/1,73m^2 e material de biópsia renal e cutânea adequados. Os estudos da função renal incluíram avaliação das taxas de excreção urinária de albumina e de filtração glomerular. A estrutura glomerular foi avaliada por técnicas de morfometria aplicadas a imagens obtidas por microscopia eletrônica. Os pacientes foram classificados de acordo com a velocidade de progressão da expansão mesangial em "fast-track" (pacientes com mais rápida progressão de lesões, ou seja, pacientes com valores de expansão mesangial dentro do quintil mais elevado) ou "slow-track" (pacientes com lento desenvolvimento de lesões glomerulares, com valores de expansão mesangial dentro do quintil mais baixo).

Foram avaliados 125 pacientes com idade de 37,6 ± 9,3 anos e duração de diabete melito de 22,5 ± 10,0 anos. Oitenta e oito pacientes eram normoalbuminúricos, 17 microalbuminúricos, 19 proteinúricos e 1 não pode ser classificado. Comparados a valores de indivíduos controles, cada um dos três grupos de pacientes (normoalbuminúricos, microalbuminúricos e proteinúricos) apresentava

aumento na espessura da membrana basal glomerular e no volume fracional do mesângio. A gravidade das lesões aumentou com o avanço na classe de excreção urinária de albumina, entretanto houve sobreposição na estrutura glomerular entre os grupos. Nestes pacientes, foram observadas correlações entre a estrutura glomerular e a função renal. O volume fracional do mesângio ($r=0,75$; $p<0,001$) e a espessura da membrana basal glomerular ($r=0,48$; $p<0,001$) estavam correlacionadas com a excreção urinária de albumina, enquanto que a superfície de filtração por glomérulo ($r=0,48$; $p<0,001$) e o volume fracional do mesângio ($r= -0,49$; $p<0,001$) estavam correlacionados com a filtração glomerular. A análise de regressão linear múltipla revelou que o volume fracional do mesângio por glomérulo e a espessura da membrana basal glomerular explicavam 59% da variabilidade da excreção urinária de albumina. Por outro lado, a superfície de filtração glomerular, a excreção urinária de albumina e o sexo explicaram parte da variabilidade da filtração glomerular. Quando os pacientes foram analisados de acordo com a classificação como “fast-track” ou “slow-track”, os 25 pacientes “fast-track” apresentaram pior controle glicêmico, níveis mais elevados de excreção urinária de albumina e filtração glomerular mais baixa do que os 25 pacientes “slow-track”. Os pacientes “fast-track” também apresentavam mais hipertensão e retinopatia e, como esperado, lesões glomerulares mais graves do que os pacientes “slow-track”.

Em conclusão, há forte correlação entre a estrutura glomerular e a função renal, contudo, há considerável sobreposição destes parâmetros entre as classes de excreção urinária de albumina. Estudos longitudinais são necessários para verificar se a

avaliação da estrutura glomerular em pacientes normoalbuminúricos e microalbuminúricos com diabete melito tipo 1 de longa duração oferece informação adicional sobre o risco de progressão para nefropatia clínica.

ABSTRACT

This study is designed to evaluate the structural-functional relationships and elucidate the factors associated to diabetic nephropathy in type 1 diabetic patients. Patients were classified, according to their rate of development of mesangial expansion in "fast-track"or "slow-track". Entry criteria included 8 or more years of diabetes duration, glomerular filtration rate (GFR) ≥30 ml/min/1.73m^2 and kidney and skin biopsies material adequate for these studies. Renal functional studies included albumin excretion rate (AER) and GFR. Glomerular structure was evaluated by electron microscopy morphometry. Patients in the upper quintile of the distribution of the rate of development of mesangial expansion, i.e., patients with rapid development of diabetic nephropathy lesions, were classified as "fast-track" and patients in the lower quintile, i. e., patients with slow development of lesions, as "slow-track".

One hundred twenty-five long-standing type 1 diabetic patients (37.6 ± 9.3 years old) with average diabetes duration of 22.5 years were evaluated. Eighty eight patients were normoalbuminuric, 17 microalbuminuric, 19 proteinuric and 1 unclassifiable. All 3 groups had increased glomerular basement membrane (GBM) width and mesangial fractional volume [Vv(Mes/glom)], with increasing severity of lesions from normoalbuminuria to microalbuminuria to proteinuria, but with considerable overlap among these 3 groups. Strong structural-functional relationships were observed in these patients. Vv(Mes/glom) ($r=0.75$; $p<0.001$) and GBM width ($r=0.63$; $p<0.001$)

correlated with AER, while surface density of peripheral GBM per glomerulus [Sv(PGBM/glom)] ($r=0.48$; $p<0.001$) and Vv(Mes/glom) ($r=-0.49$; $p<0.001$) correlated with GFR. Multiple linear regression analysis revealed that Vv(Mes/glom) and GBM width explained 59% of AER variability. GFR was predicted by Sv(PGBM/glom), AER and gender. "Fast-track" patients had worse glycemic control, higher AER and lower GFR than "slow-track" patients. "Fast-track" patients also had more hypertension and retinopathy and, as expected, worse glomerular lesions compared to "slow-track" patients.

In summary, there are strong relationships between glomerular structure and renal function across the broad spectrum ranging from normoalbuminuria to proteinuria, but there is considerable structural overlap among AER categories. The study of glomerular structure in long-standing non-proteinuric type 1 diabetic patients may offer additional information on the risk of progression to proteinuria. This hypothesis need to be evaluated in longitudinal studies.

INTRODUCTION

The central abnormality in diabetic nephropathy is renal extracellular matrix accumulation, especially in the mesangium (1). Increase in glomerular (GBM) and tubular basement membrane width and mesangial matrix expansion, all representing extracellular matrix accumulation, are hallmarks of diabetic nephropathy. Since renal lesions can develop for years, despite normal kidney function (2-4), kidney biopsy is important in understanding early factors in diabetic nephropathy risk. At later stages, when lesions are far advanced (5), forces driving renal functional decline may be separated from factors responsible for earlier diabetic lesions (6-8).

This study aims to evaluate the clinical and glomerular structural characteristics and the renal structural-functional relationships in a large cohort of long-standing type 1 diabetic patients. The final goal is to detect cellular markers of diabetic nephropathy risk in skin fibroblasts derived from these patients, identifying candidate diabetic nephropathy genes (9) and thereby formulating pathogenetic hypotheses for the mechanisms involved in extracellular matrix accumulation.

METHODS

Patients

One hundred twenty-five long-standing type 1 diabetic patients that had kidney biopsies performed either as part of their evaluation for pancreas transplantation or as members of a study of renal structure and function in type 1 diabetic sibling pairs were included in this study. To be eligible, patients needed to have adequate tissue from kidney and skin biopsies. Additional eligibility criteria were diabetes duration of at least 8 years, so that duration was adequate to access the rate of development of diabetic nephropathy lesions, serum creatinine <2.0 mg/dL, and glomerular filtration rate (GFR) >30 mL/min/1.73m^2, to avoid the study of end-stage diabetic nephropathy. Patients with other kidney diseases were excluded. In addition, electron microscopy morphometry was carried out by currently used methods (2) and not by those initially utilized in our laboratory (5). These studies were approved by the committee for the Use of Human Subjects in Research of the University of Minnesota. Informed consent was obtained from all participants before each study. The patients were admitted in the General Clinical Research Center at the University of Minnesota where renal function studies, percutaneous kidney biopsy and skin biopsy were performed.

Kidney Function Studies

Blood pressure levels were assessed while the patients were in the General Clinical Research Center by trained observers using an oscillometric automatic

monitor. The mean value of multiple measurements was used to calculate systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure. Hypertension was defined as blood pressure levels ≥130/85 (10) or use of antihypertensive medication. Glycated hemoglobin (HbA_{1c}; reference values 4.3-6.0%) was measured by high-performance liquid chromatography. Serum and urinary creatinine were measure by an automated method using the Jaffe reaction. Albumin excretion rate (AER) was assessed from three 24 hour sterile urine collections by a fluorimetric assay (11). Patients were classified as normoalbuminuric (AER <20 μg/min), microalbuminuric (AER 20-200 μg/min), or proteinuric (>200 μg/min) depending on at least 2 out of 3 AER measurements being in the same range. Patients known to be microalbuminuric or proteinuric before antihypertensive treatment was begun, were classified according to their pre-treatment AER values. The median AER value for each patient was used for the analyses. AER data was not available in one patient. GFR was estimated by iothalamate clearance using 4 timed urine and blood collections or by Iohexol plasma clearance. These two methods of GFR determination are highly correlated (12). The mean of three 24 hour creatinine clearances carefully performed by the General Clinical Research Center nursing staff was taken as a GFR estimate in patients studied several years ago. We have previously demonstrated that General Clinical Research Center creatinine clearances are highly correlated with classical inulin clearances ($r=0.92$; $p<0.001$) (13).

Retinal Studies

Retinopathy was assessed by indirect fundoscopy and classified as none, background, or proliferative. Retinopathy was not evaluated in 2 patients.

Renal structural studies

Percutaneous kidney biopsy was performed with ultrasound guidance under local anesthesia. Renal tissue was processed for light and electron microscopy.

Morphometric analyses:

a. Tissue processing. Electron microscopy tissues were processed as detailed elsewhere (2). Briefly, kidney tissue was fixed in 2.5% glutaraldehyde in Millonig's buffer and embedded in Polybed 812®. Ultrathin sections were examined with a JEOL 100CX electron microscope (Tokyo, Japan). A calibration grid was photographed with each glomerulus. Ten-twenty evenly spaced micrographs were obtained 11,000x for measurement of GBM width and for mesangial composition. Micrographs at 3,900x were constructed into a montage of the entire glomerular profile for measurements of the fractional volume of glomeruli occupied by mesangium [Vv(Mes/glom)] and the surface density of the peripheral glomerular basement membrane per glomerulus [Sv(PGBM/glom)].

b. Electron Microscopy Measurements. At least two non-sclerotic glomeruli per biopsy in the electron microscopy blocks was an entry criterion for this study. Glomeruli were evaluated for: (i) GBM width, by the orthogonal intercept method (14); (ii) Vv(Mes/glom), on the low magnification montages by point counting (2);

(iii) Mesangial Components using a grid over the high magnification micrographs, where points falling on mesangial matrix and mesangial cells were noted, and percentage of glomerulus occupied by mesangial matrix [Vv(MM/glom)] and mesangial cell [Vv(MC/glom)] (1), as well as the percentage of mesangium occupied by mesangial matrix {Vv[MM/(MM+MC)]}, were calculated; and (iv) Sv(PGBM/glom), estimated on the low magnification montages using intercept counting (2). The rate of development of diabetic nephropathy was determined by the estimated rate of mesangial expansion, using MES, defined as:

$$\frac{\text{measured Vv(Mes/glom)} - \text{mean normal Vv(Mes/glom)}}{\text{diabetes duration in years X 100}}$$

Patients were ranked according to their estimated MES and polarized into two groups: the upper quintile, with rapid, and the lower quintile, with slow development of diabetic nephropathy lesions, chosen to maximize the ability to detect cellular variables associated with diabetic nephropathy risk. Diabetic patients with rapid or slow development of diabetic nephropathy lesions were designated "fast-track" or "slow-track", respectively. Thus, this study of highly characterized type 1 diabetic patients, while also evaluating renal functional parameters, uses renal structure factored for diabetes duration as the key determinant for the categorization of the diabetic nephropathy risk. This strategy allows uniform classification despite variations in diabetes duration, and could allow earlier detection of high-risk patients.

Reference values for glomerular structural parameters were derived from 76 age and gender-matched normal living kidney transplant donors. These subjects (33

males) were 37.6 ± 12.1 (19-64) years old. The mean ± 2 standard deviation (SD) of the measurements obtained in these patients were used to define the reference range for glomerular structure.

Statistical Analyses

Based on preliminary studies, it was estimated that 25 patients per group, ("fast-track" patients, "slow-track" patients and controls) would provide 80% power (assuming a 0.05 two-sided probability of type I error) of detecting group differences in the skin fibroblasts mRNA expression levels of genes which could be related to diabetic nephropathy. To obtain 25 "fast-track" and 25 "slow-track" patients in the upper and lower quintiles, 125 type 1 diabetic patients needed to be evaluated.

Results are presented as mean ± SD. AER, not normally distributed, is presented as median and range. Values for AER were logarithmically transformed before analyses. Patients were classified according to their AER category. Analysis of variance (ANOVA) was used to compare continuous variables between controls, normoalbuminuric, microalbuminuric and proteinuric patients. Bonferroni's procedure was used in *post hoc* analyses. Discrete variables were compared by chi-square. Pearson's correlation coefficient was used to evaluate the relationships between the variables studied. Multiple linear regression analyses were used to identify the predictors of AER, GFR, Vv(Mes/glom) and MES. Unpaired *t*-test and chi-square were used to compare clinical and structural parameters between "fast-track" and "slow-track" patients. Values for $p<0.05$ were considered significant.

RESULTS

Total Cohort of Long-standing Type 1 Diabetic Patients

Age of the 125 patients (53 males) was 37.6 ± 9.3, age at diabetes onset 15.1 ± 9.4, and diabetes duration 22.5 ± 10.0 years. HbA_{1c} at the time of biopsy was 8.5 ± 1.6%. Median AER was 8.9 (1.8-4630) µg/min. Eighty-eight patients were normoalbuminuric, 17 microalbuminuric, 19 proteinuric, and 1 could not be classified. GFR ranged from 33 to 166 ml/min/1.73m^2. Retinopathy was present in 83 (67.5%) patients, 38 of whom had proliferative changes. Hypertension was present in 56 (45%) of the patients; 31 were receiving antihypertensive drugs at the time of the studies, 21 angiotensin converting enzyme inhibitors (ACEI) or angiotensin II type 1 receptor blockers (AIIRB).

Demographic and Clinical Characteristics of Long-standing Type 1 Diabetic Patients

Demographic and clinical characteristics of the 124 type 1 diabetic patients classificable according to their AER categories are summarized in Table 1. Gender distribution and age were not different among normoalbuminuric, microalbuminuric, and proteinuric type 1 diabetic patients. Normoalbuminuric patients were older than microalbuminuric (p=0.024) or proteinuric (p=0.003) patients at diabetes onset. Normoalbuminuric patients had shorter diabetes duration (p=0.004) than proteinuric patients, but their duration was not different from microalbuminuric patients. HbA_{1c}

was lower in normoalbuminuric than in microalbuminuric ($p=0.01$) or proteinuric ($p=0.001$) patients, while microalbuminuric and proteinuric patients had similar HbA_{1c} values. Hypertension was more frequent in proteinuric than in normoalbuminuric ($p<0.0001$) or microalbuminuric patients ($p<0.01$), and also more frequent in microalbuminuric ($p<0.002$) than in normoalbuminuric patients. Despite that similar proportions of hypertensive proteinuric and normoalbuminuric ($p=0.128$) or microalbuminuric ($p=0.09$) patients were receiving antihypertensive therapy, proteinuric patients had higher SBP and DBP than normoalbuminuric ($p<0.001$ for both comparisons) and higher SBP than microalbuminuric ($p=0.006$) patients. Renin-angiotensin system (RAS) blockers (ACEI or AIIRB) were used at the same frequency by normoalbuminuric, microalbuminuric or proteinuric hypertensive diabetic patients. As expected, serum creatinine was higher and GFR lower in proteinuric *vs.* normoalbuminuric or microalbuminuric patients ($p<0.001$ for all comparisons). Retinopathy in normoalbuminuric was less frequent than in proteinuric patients ($p=0.003$), and tended to be less frequent than in microalbuminuric patients ($p=0.07$). There was a significant increase in the prevalence of proliferative retinopathy in proteinuric compared to normoalbuminuric ($p<0.0001$) or microalbuminuric ($p<0.0005$) patients.

Glomerular Structure of Long-standing Type 1 Diabetic Patients

Two to six (3.2 ± 0.7) glomeruli were evaluated per patient. There was a wide range of glomerular lesions among these long-standing type 1 diabetic patients, varying from measurements in the normal range to advanced disease (Table 2). GBM

width was increased in 74.4% of diabetic patients ($p<0.0001$ *vs.* controls). Vv(Mes/glom) varied from normal (0.14-0.26) to nearly 3 times normal, and was also greater than controls ($p<0.0001$). Mesangial components, Vv(MM/glom) ($p<0.0001$), Vv(MC/glom) ($p<0.0001$) and Vv[MM/(MM+MC)] ($p<0.001$), were increased in the glomeruli of these diabetic patients compared to controls, and Sv(PGBM/glom) was decreased when compared to controls ($p<0.0001$). Table 2 shows the glomerular structural values of according to AER categories. GBM width (Fig. 1), Vv(Mes/glom) (Fig. 2), Vv(MM/glom) and Vv[MM/MM+MC)] were increased, and Sv(PGBM/glom) was decreased in each of the AER categories of diabetic patients compared to the controls (Table 2). Vv(MC/glom) was greater in proteinuric patients *vs*. controls ($p<0.001$), while the comparison of control with microalbuminuric ($p=0.054$) or normoalbuminuric ($p=0.85$) patients did not reach statistical significance. GBM width increased progressively with increasing AER class from normoalbuminuria to microalbuminuria ($p<0.001$) or proteinuria ($p<0.001$) and from microalbuminuria to proteinuria ($p=0.036$). Vv(Mes/glom) also increased progressively from normoalbuminuric to microalbuminuric ($p=0.043$) or to proteinuric ($p<0.001$) patients, and was also greater in proteinuric *vs.* microalbuminuric ($p<0.001$) patients. The mesangial components, mesangial matrix and mesangial cell were also increased in proteinuric *vs*. normoalbuminuric ($p<0.001$ for all comparisons) and microalbuminuric ($p<0.001$ and $p=0.03$, respectively) patients. Mesangial matrix was greater ($p<0.05$) in microalbuminuric *vs*. normoalbuminuric patients. Vv[MM/(MM+MC)] was increased in proteinuric compared to normoalbuminuric

patients (p=0.012). Sv(PGBM/glom) decreased with increasing in AER levels ($p<0.001$ by ANOVA), but was not different between normoalbuminuric and microalbuminuric patients. MES increased progressively from normoalbuminuria to microalbuminuria (p=0.03) or proteinuria ($p<0.001$) and from microalbuminuria to proteinuria (p=0.002). Despite highly statistically significant differences, there was substantial overlap in glomerular structure among the normoalbuminuric, microalbuminuric, and proteinuric groups (Figs. 1 and 2).

Structural-Functional Relationships of Long-standing Type 1 Diabetic Patients

AER correlated inversely with age at diabetes onset (r=-0.32; $p<0.001$), and directly with diabetes duration (r=0.30; $p<0.001$), MBP (r=0.48; $p<0.001$), HbA_{1c} (r=0.35; $p<0.001$), and serum creatinine (r=0.56; $p<0.001$). AER also correlated inversely with GFR (r=-0.47; $p<0.001$). On the other hand, GFR correlated inversely with age (r=-0.18; $p<0.05$), diabetes duration (r=-0.29; $p<0.001$), MBP (r=-0.21; $p<0.02$), HbA_{1c} (r=-0.18; $p<0.05$) and serum creatinine (r=-0.60; $p<0.001$). GFR was directly correlated with age of diabetes onset (r=0.19; $p<0.04$). Clinical variables were also related to glomerular structure. Age was inversely related to GBM width (r=-0.27, p=0.003), while age at diabetes onset and diabetes duration were related to all structural variables. Age at onset was directly related to Sv(PGBM/glom) and inversely related to the other structural variables, and diabetes duration was inversely related to Sv(PGBM/glom) and directly related to the other structural variables. The relationships between MBP and structural parameters were similar to the ones described for diabetes duration. Vv(Mes/glom) (r=0.75; $p<0.001$) (Fig. 3) and GBM

width (r=0.63; p<0.001) (Fig. 4) correlated with AER across the entire range, from normoalbuminuria to proteinuria. Sv(PGBM/glom) had a negative correlation with AER (r=-0.62; p<0.001). The glomerular fractional volume of the mesangial components [mesangial matrix (r=0.71; p<0.001) and mesangial cell (r=0.50; p<0.001)] and MES (r=0.63; p<0.001) were also correlated with AER. All parameters of glomerular structure were related to GFR. The strongest relationships between GFR and glomerular structure were with Vv(MM/glom) (r=-0.53; p<0.001), Vv(Mes/glom) (r=-0.49; p<0.001), and Sv(PGBM/glom) (r=0.48; p<0.001) (Fig. 5). The relationships between glomerular structure and AER, and between glomerular structure and GFR, were similar when the 21 patients receiving ACEI or AIIRB were excluded from the analyses. The strong relationships between AER and glomerular structural parameters were present only when patients from all AER categories were included. When only patients within a given AER category were considered, these relationships were weaker in the normoalbuminuric and proteinuric groups and absent in the microalbuminuric group.

In multiple linear regression analysis, Vv(Mes/glom) and GBM width were additive and explained 59% of the variability in AER (p<0.001). When clinical parameters were added to the model, 65% of AER variability was explained by Vv(Mes/glom), GBM width and MBP (p<0.001), and this was independent of RAS blockade. The other variables studied, HbA_{1c} and gender, were not significant and were excluded from the model. The same results were obtained when hypertension, instead of MBP, was used as an independent variable (r^2=0.64, p<0.001). Also, 33% of

the variability in GFR was predicted by Sv(PGBM/glom), AER, and gender ($p<0.0001$), while Vv(Mes/glom), GBM width, diabetes duration, HbA_{1c} and MBP were not independent GFR predictors.

Clinical variables also predicted glomerular structure. Thus, AER and GFR predicted Vv(Mes/glom) ($r^2=0.58$; $p<0.001$), while AER, diabetes duration, and retinopathy (present/absent) predicted MES ($r^2=0.47$; $p<0.001$).

Demographic, Clinical and Glomerular Structural Characteristics of "Fast-track" and "Slow-track" Type 1 Diabetic Patients

"Fast-track" patients were not different from "slow-track" patients regarding gender, age, age at diabetes onset and diabetes duration (Table 3). "Fast-track" patients had higher HbA_{1c}, DBP, MBP, serum creatinine and AER, and lower GFR, than "slow-track" patients. All but 2 "slow-track" patients were normoalbuminuric while 16 of the 25 "fast-track" patients were microalbuminuric (5 patients) or proteinuric (11 patients). Hypertension was present in 24% of "slow-track" and in 76% of "fast-track" patients. Proliferative retinopathy was present in only 2 of 25 "slow-track" *vs*. 13 of 25 "fast-track" patients ($p<0.001$) (Table 3). All glomerular structural parameters in Table 4 were different from normal in the "fast-track" patients (all $p<0.0001$). In contrast, while GBM width ($p<0.0001$) and Vv[MM/(MM+MC)] ($p<0.0001$) were increased in "slow-track" patients compared to controls, all other parameters including Vv(Mes/glom) (Fig. 6) and Sv(PGBM/glom) were not different from normal. As expected, "fast-track" patients had much more advanced glomerular

lesions than “slow-track” patients (Table 4; Fig. 6). MES was, by definition, greater in “fast-track” than in “slow-track” patients.

DISCUSSION

This is the largest single center study using uniform tissue processing and measurement methods to evaluate structural-functional relationships in long-standing type 1 diabetic patients with wide ranges of renal structure and function. Grouped according to their AER class, group ages were similar but proteinuric and microalbuminuric patients were younger at diabetes onset than normoalbuminuric patients, while duration was longer in the proteinuric group. These results could be interpreted as consonant with published evidence against the hypothesis that the prepuberal years of diabetes are protected from nephropathy (15). However, the rate of development of lesions may not be linear over time and longitudinal biopsy studies of children and adults will be necessary to answer this question.

This study confirmed the relationships of glycemia to the risk of diabetic complications while, at same time, the results suggest that other variables are of equal or greater importance in influencing nephropathy risk. Although blood pressure values were similar in normoalbuminuric and microalbuminuric patients, the prevalence of hypertension and antihypertensive treatment was greater in the microalbuminuric patients, and this may have masked blood pressure differences.

Blood pressures were highest, as expected (5) in proteinuric patients, this despite that almost all of these patients were receiving antihypertensive therapy.

Some debate still remains as to whether glomerular diabetic lesions are present in normoalbuminuric type 1 diabetic patients (16, 17). This study unequivocally confirmed that normoalbuminuric patients can have advanced glomerular lesions as well as clinical findings of renal disease, including low GFR and hypertension (2, 18). These results also support the observations that long-standing type 1 diabetic normoalbuminuric patients can progress to proteinuria (19-21). In fact, preliminary results of a 5-17 years follow-up study of normoalbuminuric patients with long-standing diabetes, showed that those progressing to microalbuminuria or proteinuria had worse glomerular lesions at baseline than those who remained normoalbuminuric (22). Also, as described here and elsewhere (2), there was a wide range of glomerular lesions in microalbuminuric patients and, despite that these patients have, as a group, more advanced lesions than normoalbuminuric patients, there was considerable overlap between these two groups for all glomerular parameters studied. Considering these findings, it is not surprising that only about 30-45% of long-standing microalbuminuric type 1 diabetic patients will progress to proteinuria over 6-10 years of follow-up (19-21, 23), while about 20% of long-standing normoalbuminuric patients will progress to microalbuminuria or proteinuria over this follow-up interval (19-21, 24, 25). On the other hand, microalbuminuric patients more frequently had hypertension and proliferative retinopathy compared to normoalbuminuric patients and, for many patients, microalbuminuria is a late indicator of diabetic nephropathy

risk. As expected, glomerular lesions were even more advanced in proteinuric patients and decreased GFR, hypertension and retinopathy were more frequently observed in these patients, paralleling the high risk of progression to ESRD and blindness in these patients.

There were strong correlations between structure and AER across the entire range from normoalbuminuria to proteinuria, and a large part of the AER variability was explained by two glomerular structural variables, Vv(Mes/glom) and GBM width. The strength of these relationships occurring despite the known day-to-day variability in AER, suggests that functional manifestations of diabetic nephropathy may appear earlier than heretofore appreciated and that progressive changes in AER, even within the normoalbuminuric range, could be significant (2, 3, 21, 22). Higher values of AER, still in the normoalbuminuric range have been associated with increased risk of progression to microalbuminuria and proteinuria (24, 26-28). We also observed that there was an increase in AER levels with worsening of glomerular structure within the normoalbuminuric range, albeit that the correlations of AER in this range and glomerular mesangial structure were weaker. However, some variability in AER remained unexplained by our studies, and other structural variables, such as percentage of glomerular sclerosis, arteriolar hyalinosis and interstitial fibrosis, epithelial cell structure and glomerular capillary wall biochemistry need to be studied in these patients. GFR also correlated with structural variables, however only about 35% of GFR variability was explained by structural and clinical parameters.

"Fast-track" and "slow-track" patients were classified based on their rate of mesangial expansion (MES). Mesangial fractional volume, as confirmed in the present study (5) is the glomerular structural parameter most closely related to the functional manifestations of diabetic nephropathy. This study confirms that Vv(Mes/glom) is not only highly correlated with AER but is also the strongest independent AER predictor in multiple linear regression models. Moreover, sequential biopsy studies (3) have shown that changes in AER over 5 years are associated with changes in Vv(Mes/glom) but not with other parameters. The overall design of these studies was to create two polarized groups with the ultimate goal of elucidate cellular/genetic factors associated with risk of or protection from diabetic nephropathy. Although the intent of the selection of patients in the upper and lower quintiles of MES distribution was to construct groups with different diabetic nephropathy risk, some "slow-track" patients were microalbuminuric and some "fast-track" patients were normoalbuminuric. This is not surprising, since there was a wide range of diabetes duration among patients (8-60 years) and some normoalbuminuric patients have advanced lesions while some microalbuminuric patients have mild lesions. As discussed here and elsewhere (21), normoalbuminuria is not a precise predictor of safety from diabetic nephropathy and microalbuminuria is not a precise predictor of diabetic nephropathy risk in long-standing type 1 diabetic patients. Since "fast-track" and "slow-track" patients were similar in diabetes duration, classification by MES did result in the creation of two groups that did not overlap in Vv(Mes/glom). As expected, "fast-track" patients had worse glycemic

control and had more frequently hypertension and proliferative retinopathy than "slow-track" patients. Despite no increase in Vv(Mes/glom) in "slow-track" patients compared to controls, this group had an increase in the proportion of mesangium made up by matrix {Vv[MM/(MM+MC)]}. Moreover, approximately 30% increase in GBM width was observed in "slow-track" patients when compared to control values. These suggest that certain structural glomerular changes occur at a very slow rate in all type 1 diabetic patients and probably have little prognostic significance. However, more severe GBM thickening and mesangial expansion associated with reduced filtration surface are strongly associated with the clinical manifestations of diabetic nephropathy and with ESRD risk.

In conclusion, this paper describes clinical, renal functional, and renal structural characteristics of 125 long-standing type 1 diabetic patients that represent the cohort from which 25 "fast-track" and 25 "slow-track" patients were selected, based on rate of mesangial expansion, for studies of cellular markers of diabetic nephropathy risk (9). The overlap in renal structural and functional variables between the groups classified as normoalbuminuric, microalbuminuric and proteinuric is consistent with recent observations indicating that AER alone is a strong but imprecise indicator of diabetic nephropathy risk. The use of rate of mesangial expansion (MES) for classification into "fast-track" and "slow-track" groups has the advantage of allowing the inclusion of patients with varying diabetes duration. This strategy also helps to exclude normoalbuminuric patients with advanced diabetic nephropathy lesions from

the “slow-track” group. Our ongoing studies show that there are *in vitro* differences in the behavior of cells derived from these groups (9).

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Table 1. Demographic and Clinical Characteristics of Long-standing Type 1 Diabetic Patients.

	AER Categories			*p value*
	Normoalbuminuria	Microalbuminuria	Proteinuria	
Gender (male/female)	37/51	8/9	8/11	NS
Age (years)	38 ± 9	34 ± 8	39 ± 9	NS
Age at Diabetes Onset (years)	17 ± 10	11 ± 7	10 ± 5	0.001
Diabetes Duration (years)	21 ± 10	23 ± 11	29 ± 7	0.005
HbA_{1c} (%)	8.1 ± 1.4	9.3 ± 2.1	9.6 ± 1.4	<0.001
SBP (mm Hg)	122 ± 11	124 ± 13	138 ± 14	<0.001
DBP (mm Hg)	70 ± 8	72 ± 7	78 ± 8	0.001
MBP (mm Hg)	87 ± 8	89 ± 8	97 ± 9	<0.001
Hypertension (yes/no)	27/61	11/6	18/1	<0.0001
Antihypertensive (yes/no)	8/80	6/11	17/2	<0.001
RAS blocker (yes/no)	5/83	5/12	11/8	<0.001
Serum Creatinine (mg/dL)	0.9 ± 0.2	1.0 ± 0.3	1.3 ± 0.3	<0.001
GFR (ml/min/1.73m^2)	112 ± 23	102 ± 31	66 ± 22	<0.0001
AER* (µg/min)	6.2 (1.8-18.2)	30.9 (5.7-164.8)	839.0 (41.4-4630)	<0.001
Retinopathy (none/background/proliferative)	35/38/13	3/7/7	1/0/18	<0.001

Data are number of patients, mean ± SD or median (range).

AER: albumin excretion rate; HbA_{1c}: glycated hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; RAS: renin-angiotensin system; GFR: glomerular filtration rate. *AER values reflect the median of 3 measurements at time of kidney biopsy.

Table 2. Glomerular Structural Characteristics of Non-Diabetic Controls and Long-standing Type 1 Diabetic Patients.

	Controls	AER Categories			*p value*
		Normoalbuminuria	Microalbuminuria	Proteinuria	
GBM width (nm)	332±46	465±100	602±157	700±141	<0.001
Vv(Mes/glom)	0.20±0.03	0.28±0.07	0.34±0.09	0.50±0.12	<0.001
Vv(MM/glom)	0.09±0.02	0.16±0.05	0.19±0.08	0.31±0.09	<0.001
Vv(MC/glom)	0.08±0.02	0.09±0.02	0.10±0.03	0.14±0.06	<0.001
Vv[MM/MM+MC)]	0.51±0.08	0.63±0.07	0.64±0.11	0.70±0.10	<0.001
Sv(PGBM/glom) ($\mu m^2/\mu m^3$)	0.12±0.02	0.11±0.02	0.10±0.03	0.06±0.03	<0.001
MES	NA	0.39±0.32	0.66±0.48	1.12±0.61	<0.001

Data are mean ± SD.

AER: albumin excretion rate; GBM: glomerular basement membrane; Vv(Mes/glom): fractional volume of mesangium; Vv(MM/glom): fractional volume of mesangial matrix; Vv(MC/glom): fractional volume of mesangial cell; Vv[MM/(MM+MC)]: fractional volume of mesangial matrix per mesangium; Sv(PGBM/glom): surface density of peripheral glomerular basement membrane; MES: mesangium expansion score; NA: not applicable in non-diabetic controls since MES factors for diabetes duration.

Table 3. Demographic and Clinical Characteristics of "Fast-track" and "Slow-track" Type 1 Diabetic Patients.

	"Fast-track"	"Slow-track"	*p value*
Gender (male/female)	10/15	13/12	NS
Age (years)	35 ± 7	38 ± 10	NS
Age at Diabetes Onset (years)	14 ± 7	18 ± 11	NS
Diabetes Duration (years)	22 ± 8	20 ± 11	NS
HbA_{1c} (%)	9.2 ± 1.7	7.8 ± 1.2	0.002
SBP (mm Hg)	128 ± 15	122 ± 10	NS
DBP (mm Hg)	76 ± 9	71 ± 7	<0.05
MBP (mm Hg)	93 ± 10	88 ± 7	<0.05
Hypertension (yes/no)	19/6	6/19	<0.001
Antihypertensive (yes/no)	15/10	2/23	<0.001
RAS blocker (yes/no)	9/16	2/23	<0.02
Serum Creatinine (mg/dL)	1.0 ± 0.3	0.9 ± 0.1	<0.01
GFR (ml/min/1.73m^2)	90.5 ± 31.9	110.6 ± 14.2	<0.01
AER* (µg/min)	51.1 (4.9-4630)	5.7 (2.6-54.6)	<0.001
Retinopathy (none/background/proliferative)	4/8/13	12/11/2	0.002

Data are number of patients, mean ± SD or median (range).

HbA_{1c}: glycated hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; RAS: renin-angiotensin system; GFR: glomerular filtration rate; AER: albumin excretion rate. *AER values reflect the median of 3 measurements at time of kidney biopsy.

Table 4. Glomerular Structural Characteristics of "Fast-track" and "Slow-track" Type 1 Diabetic Patients.

	"Fast-track"	"Slow-track"	*p value*
GBM width (nm)	685 ± 168	440 ± 77	<0.001
Vv(Mes/glom)	0.47 ± 0.12	0.21 ± 0.31	<0.001
Vv(MM/glom)	0.28 ± 0.09	0.11 ± 0.02	<0.001
Vv(MC/glom)	0.13 ± 0.05	0.07 ± 0.02	<0.001
Vv[MM/MM+MC)]	0.68 ± 0.08	0.60 ± 0.07	0.001
Sv(PGBM/glom) ($\mu m^2/\mu m^3$)	0.07 ± 0.03	0.12 ± 0.02	<0.001
MES	1.27 ± 0.44	0.02 ± 0.17	NA

Data are mean ± SD.

GBM: glomerular basement membrane; Vv(Mes/glom): fractional volume of mesangium; Vv(MM/glom): fractional volume of mesangial matrix; Vv(MC/glom): fractional volume of mesangial cell; Vv[MM/(MM+MC)]: fractional volume of mesangial matrix per mesangium; Sv(PGBM/glom): surface density of peripheral glomerular basement membrane; MES: mesangium expansion score. NA: not applicable, different by design.

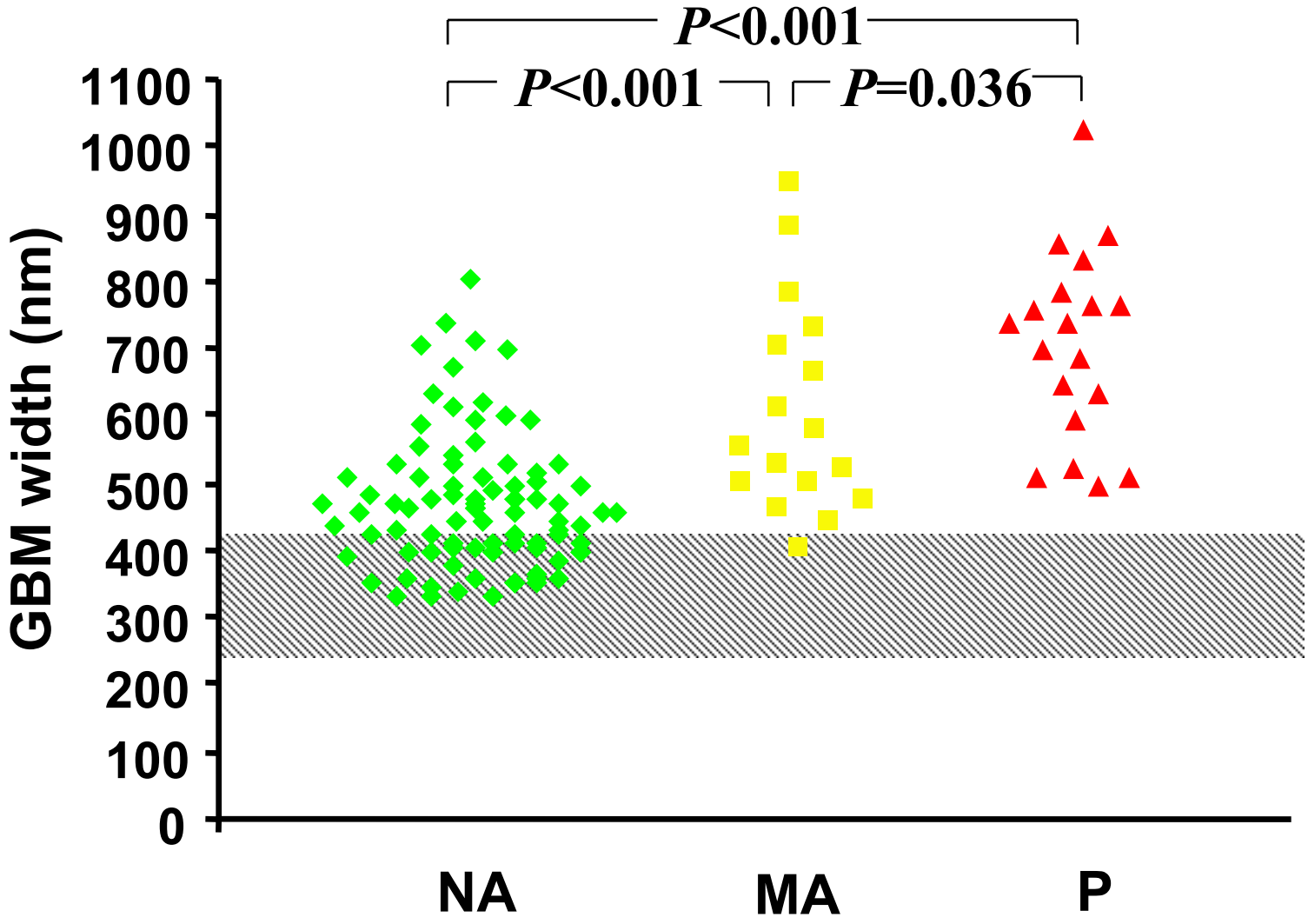

Figure 1. Glomerular basement membrane (GBM) width in 88 normoalbuminuric (NA), 17 microalbuminuric (MA) and 19 proteinuric (P) type 1 diabetic patients. The shaded area represents mean ± 2 SD in a group of 76 age-matched normal control subjects. All groups are different from controls.

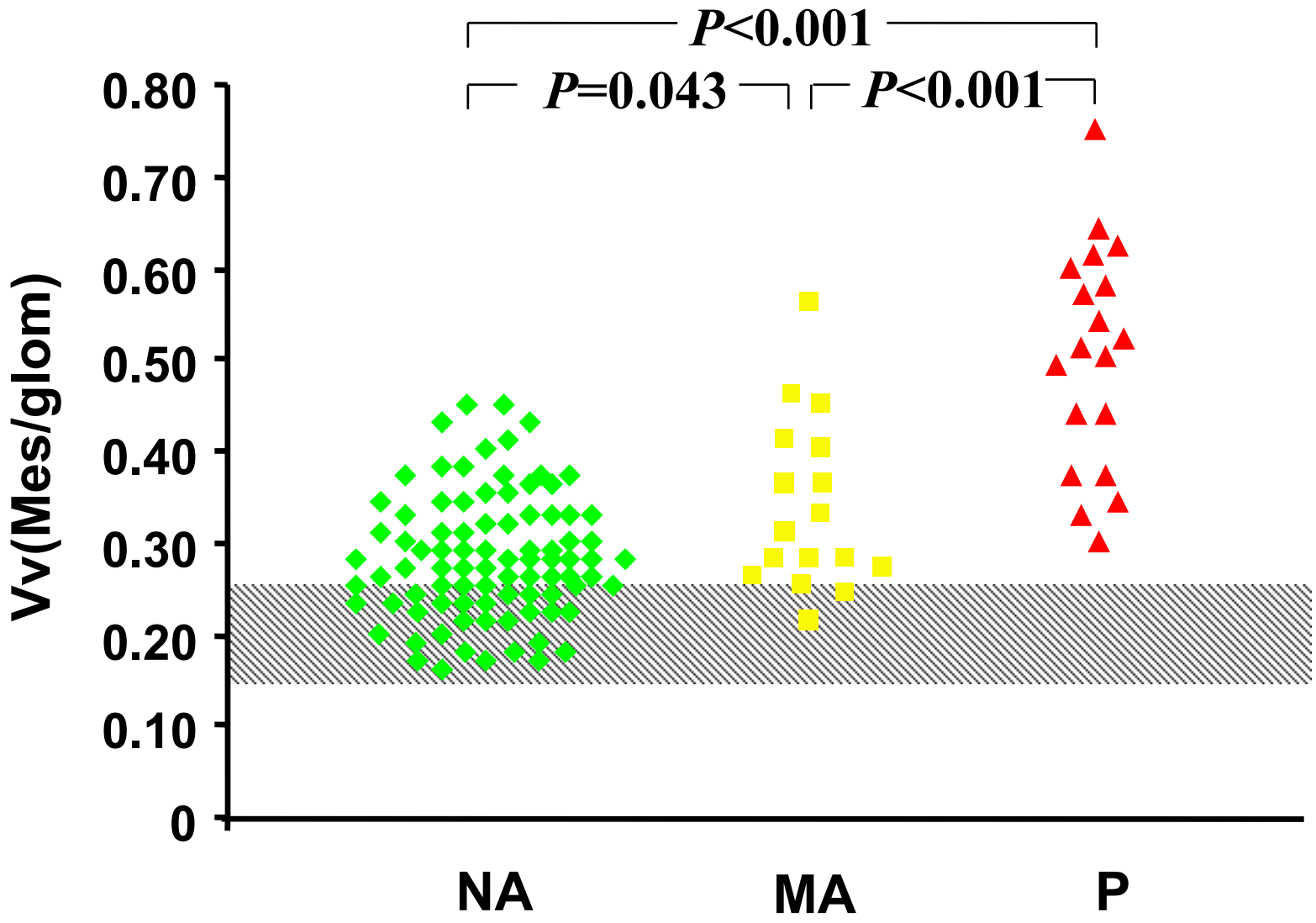

Figure 2. Mesangial fractional volume [Vv (Mes/glom)] in 88 normoalbuminuric (NA), 17 microalbuminuric (MA) and 19 proteinuric (P) type 1 diabetic patients. The shaded area represents mean ± 2 SD in a group of 76 age-matched normal control subjects. All groups are different from controls.

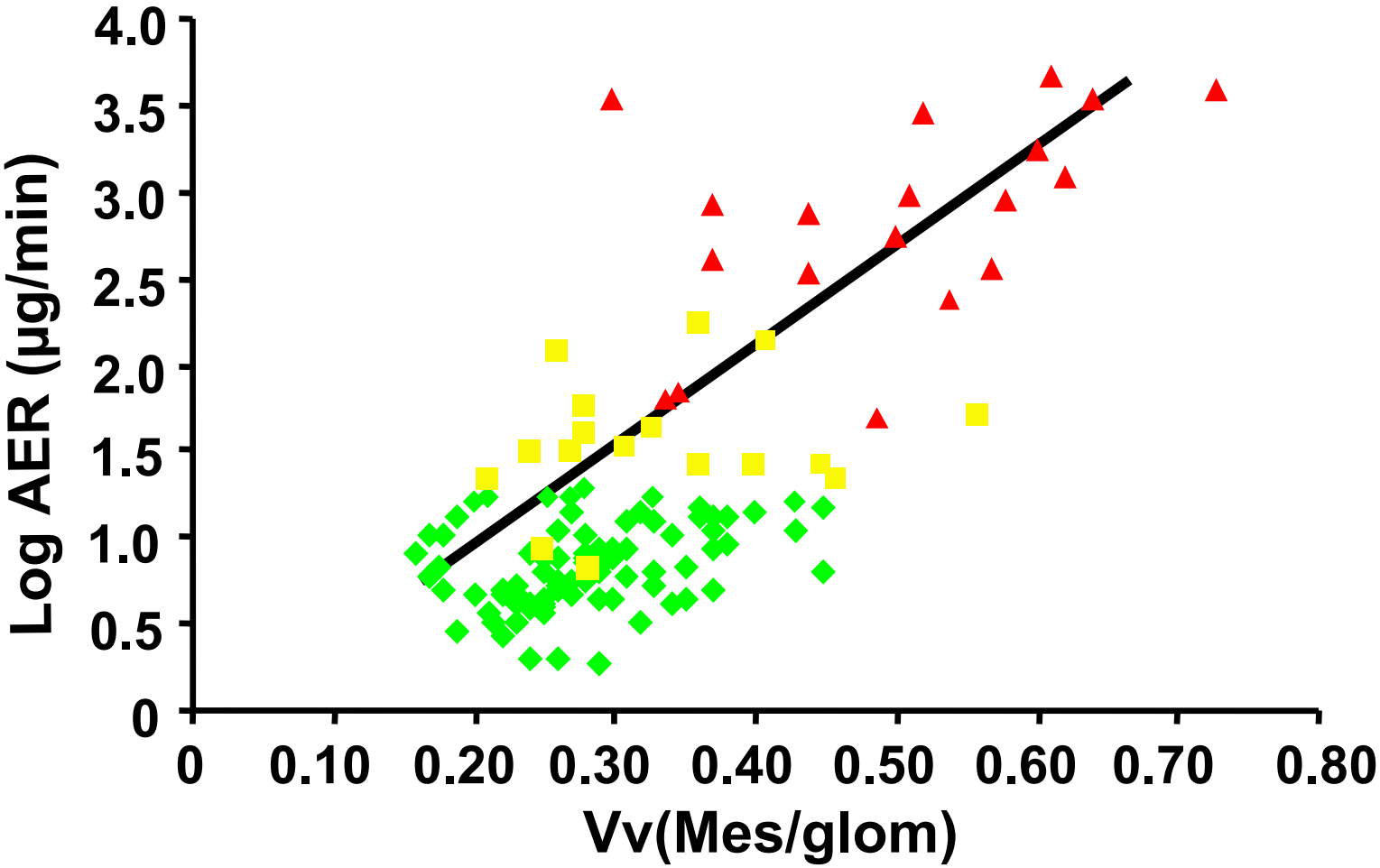

Figure 3. Correlation between mesangial fractional volume [Vv (Mes/glom)] and albumin excretion rate (AER) in ◆ normoalbuminuric; ■ microalbuminuric and ▲ proteinuric type 1 diabetic patients. r=0,75; p<0,001.

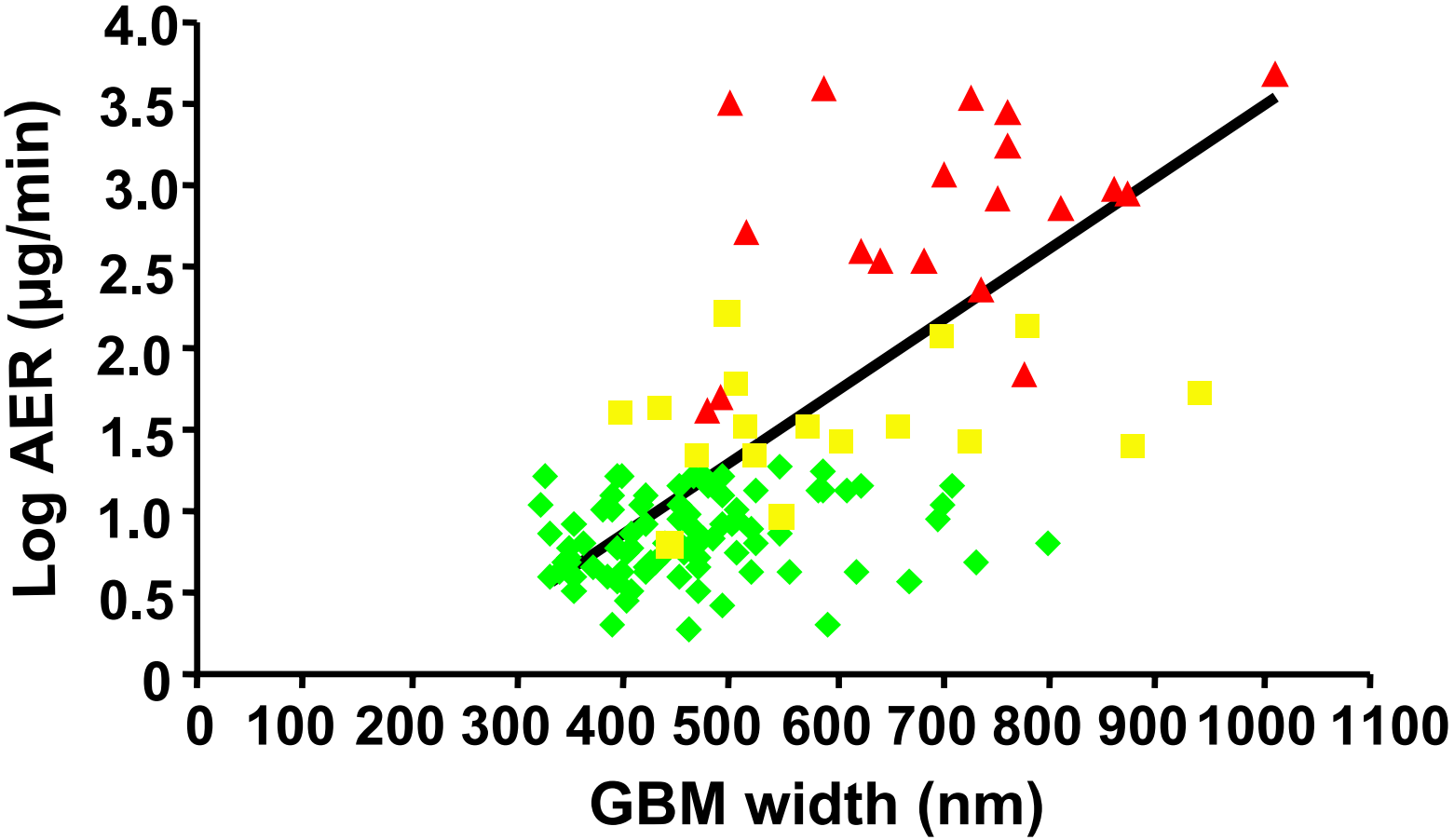

Figure 4. Correlation between glomerular basement membrane (GBM) width and albumin excretion rate (AER) in ◆ normoalbuminuric; ■ microalbuminuric and ▲ proteinuric type 1 diabetic patients. r=0,63; p<0,001.

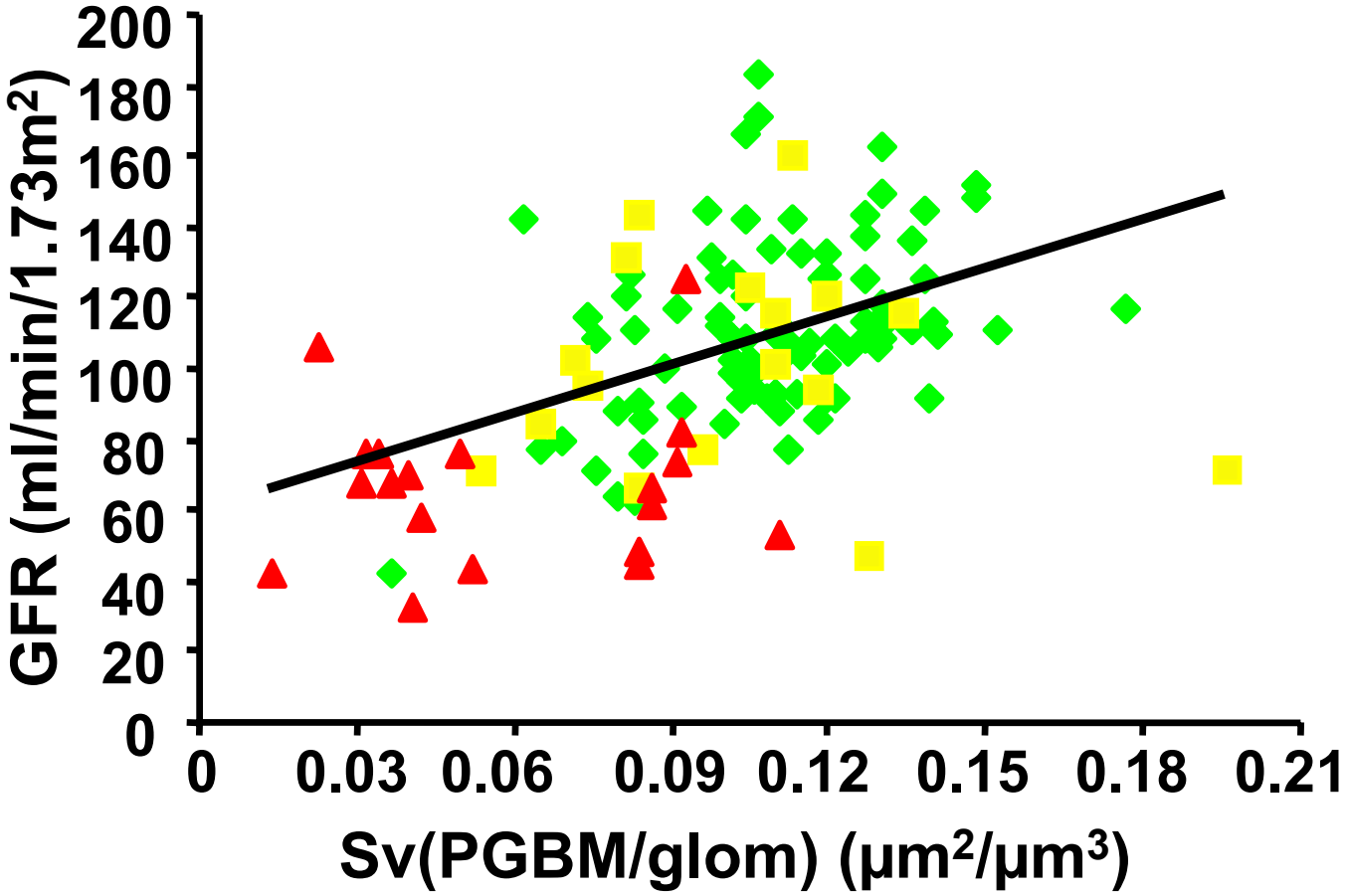

Figure 5. Correlation between surface density of peripheral basement membrane [Sv(PGBM/glom)] and glomerular filtration rate (GFR) in ◆ normoalbuminuric; ■ microalbuminuric and ▲ proteinuric type 1 diabetic patients. r=0,48; p<0,001.

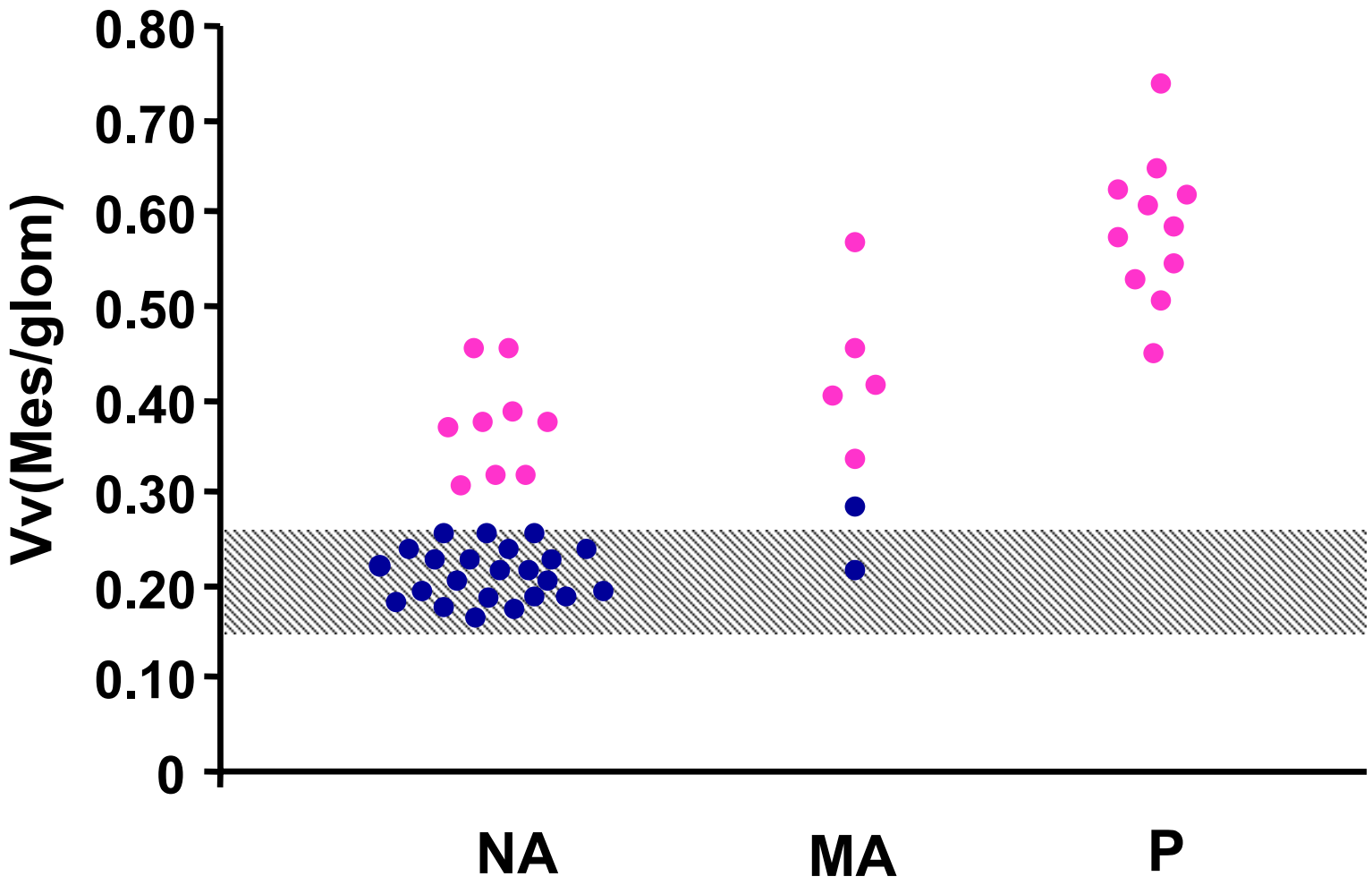

Figure 6. Mesangial fractional volume [Vv (Mes/glom)] in "fast-track" (●) and "slow-track" (●) normoalbuminuric (NA), microalbuminuric (MA) and proteinuric (P) type 1 diabetic patients. The shaded area represents mean ± 2 SD in a group of 76 age-matched normal control subjects.

REFERENCES

1. Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Cell and matrix components of the glomerular mesangium in type I diabetes. *Diabetes* 41:679-684, 1992.

2. Fioretto P, Steffes MW, Mauer M: Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. *Diabetes* 43:1358-1364, 1994.

3. Fioretto P, Steffes MW, Sutherland DE, Mauer M: Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. *Kidney Int* 48:1929-1935, 1995.

4. Berg UB, Torbjornsdotter TB, Jaremko G, Thalme B: Kidney morphological changes in relation to long-term renal function and metabolic control in adolescents with IDDM. *Diabetologia* 41:1047-1056, 1998.

5. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143-1155, 1984.

6. Jacobsen P, Rossing K, Rossing P, Tarnow L, Mallet C, Poirier O, Cambien F, Parving HH: Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int* 53:1002-1006, 1998.

7. Parving HH, Jacobsen P, Tarnow L, Rossing P, Lecerf L, Poirier O, Cambien F: Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin converting enzyme: observational follow up study. *Br Med J* 313:591-594, 1996.

8. Brenner BM: Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int* 23:647-655, 1983.

9. Huang C, Caramori ML, Kim Y, Rich S, Miller ME, Fish AJ, Mauer M: Alterations of Na^+/H^+ exchanger (NHE-1) mRNA expression in type 1 diabetic patients with rapid *versus* slow development of diabetic nephropathy lesions. *Diabetes*, in press.

10. The Sixth Report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med* 157:2413-2446, 1997.

11. Chavers BM, Simonson J, Michael AF: A solid phase fluorescent immunoassay for the measurement of human urinary albumin. *Kidney Int* 25:576-578, 1984.

12. Rocco MV, Buckalew VM Jr., Moore LC, Shihabi ZK: Measurement of glomerular filtration rate using nonradioactive Iohexol: comparison of two one-compartment models. *Am J Nephrol* 16:138-143, 1996.

13. Ellis EN, Steffes MW, Goetz FC, Sutherland DE, Mauer SM: Glomerular filtration surface in type I diabetes mellitus. *Kidney Int* 29:889-894, 1986.

14. Jensen EB, Gundersen HJ, Østerby R: Determination of membrane thickness distribution from orthogonal intercepts. *J Microsc* 115:19-33, 1979.

15. Ellis EN, Warady BA, Wood EG, Hassanein R, Richardson WP, Lane PH, Howard C, Kemp SF, Aceto T, Garibaldi L, Wiegmann TB, Savin VJ: Renal structural-functional relationships in early diabetes mellitus. *Pediatr Nephrol* 11:584-591, 1997.

16. Mogensen CE: Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. *Diabetologia* 42:263-285, 1999.

17. Østerby R: Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia* 35:803-812, 1992.

18. Lane PH, Steffes MW, Mauer SM: Glomerular structure in IDDM women with low glomerular filtration rate and normal urinary albumin excretion. *Diabetes* 41:581-586, 1992.

19. Forsblom CM, Groop PH, Ekstrand A, Groop LC: Predictive value of microalbuminuria in patients with insulin-dependent diabetes of long duration. *Br Med J* 305:1051-1053, 1992.

20. Rudberg S, Persson B, Dahlquist G: Increased glomerular filtration rate as a predictor of diabetic nephropathy--an 8-year prospective study. *Kidney Int* 41:822-828, 1992.

21. Caramori ML, Fioretto P, Mauer M: The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? *Diabetes* 49:1399-1408, 2000.

22. Caramori ML, Fioretto P, Mauer M: Long-term follow-up of normoalbuminuric longstanding type 1 diabetic patients: Progression is associated with worse baseline glomerular lesions and lower glomerular filtration rate (Abstract). *J Am Soc Nephrol* 10:126A, 1999.

23. Rossing P, Hougaard P, Borch-Johnsen K, Parving H-H: Progression of microalbuminuria in type 1 diabetes: 10 years observation and follow-up (Abstract). *Diabetologia* 43 (suppl 1):A254, 2000.

24. Mathiesen ER, Ronn B, Storm B, Foght H, Deckert T: The natural course of microalbuminuria in insulin-dependent diabetes: a 10-year prospective study. *Diabet Med* 12:482-487, 1995.

25. Chiarelli F, Catino M, Tumini S, de Martino M, Mezzetti A, Verrotti A, Vanelli M: Increased Na+/Li+ countertransport activity may help to identify type 1 diabetic adolescents and young adults at risk for developing persistent microalbuminuria. *Diabetes Care* 22:1158-1164, 1999.

26. Risk factors for development of microalbuminuria in insulin dependent diabetic patients: a cohort study. Microalbuminuria Collaborative Study Group, United Kingdom. *Br Med J* 306:1235-1239, 1993.

27. Mogensen CE ,Poulsen PL: Epidemiology of microalbuminuria in diabetes and in the background population. *Curr Opin Nephrol Hypertens* 3:248-256, 1994.

28. Royal College of Physicians of Edinburgh Diabetes Register Group: Near-normal urinary albumin concentrations predict progression to diabetic nephropathy in Type 1 diabetes mellitus. *Diabet Med* 17:782-791, 2000.

RESUMO DOS RESULTADOS

CAPÍTULO I

1. Aproximadamente 10% dos pacientes normoalbuminúricos e 30-45% dos pacientes microalbuminúricos com DM tipo 1 de longa duração irão progredir para proteinúria após 10 anos de acompanhamento.

2. Aproximadamente 30% dos pacientes microalbuminúricos com duração de DM tipo 1 entre 7-40 anos irão, espontaneamente ou em vigência de tratamento antihipertensivo e controle glicêmico intensificado, reverter para normoalbuminúria.

3. Considerando que a maioria dos pacientes com DM tipo 1 de longa duração de doença apresenta normoalbuminúria, pode-se estimar que o número absoluto de pacientes que irá progredir para proteinúria nos próximos 10 anos neste grupo de pacientes é similar ao observado no grupo de pacientes com microalbuminúria.

4. Aproximadamente 12% dos pacientes normoalbuminúricos com DM tipo 2 irão progredir para proteinúria após 10-15 anos de acompanhamento.

5. Aproximadamente 40% dos pacientes microalbuminúricos com DM tipo 2 irão progredir para proteinúria após 5-6 anos de acompanhamento.

6. A proporção de pacientes microalbuminúricos com DM tipo 2 que irá retornar à normoalbuminúria não está definida.

7. Considerando que cerca de 70% dos pacientes com DM tipo 2 é normoalbuminúrico na avaliação inicial, pode-se estimar que, dos dos pacientes que irão progredir para proteinúria nos próximos 10-15 anos, 40% se originará do grupo inicialmente normoalbuminúrico e 60% do grupo inicialmente microalbuminúrico.

CAPÍTULO II

1. Pacientes normoalbuminúricos com DM tipo 1 de longa duração apresentam lesões glomerulares renais, incluindo aumento no volume fracional do mesângio, quando comparados a controles não-diabéticos.

2. Pacientes microalbuminúricos com DM tipo 1 apresentam, em média, lesões glomerulares mais avançadas do que pacientes normoalbuminúricos.

3. Pacientes proteinúricos com DM tipo 1 apresentam, em média, lesões glomerulares mais avançadas do que pacientes microalbuminúricos ou normoalbuminúricos.

4. Existe uma sobreposição importante na gravidade das lesões glomerulares renais em pacientes com DM tipo 1 de longa duração nas diferentes classes de EUA.

5. Em pacientes com DM tipo 1 de longa duração a estrutura glomerular está correlacionada com a EUA. Entretanto, existe uma importante variação individual na gravidade das lesões renais associadas a aumento da EUA nestes pacientes.

COMENTÁRIOS FINAIS E PERSPECTIVAS

Apesar de medidas da EUA serem o método não-invasivo que melhor identifica pacientes com risco aumentado para o desenvolvimento de nefropatia diabética, a presença isolada de microalbuminúria não é suficiente para este fim e estudos buscando identificar marcadores de risco mais precisos e precoces se fazem necessários.

Nossos estudos sugerem que lesões glomerulares graves podem se desenvolver antes do que as manifestações clínicas da nefropatia diabética ocorram. Além disto, a presença de lesões glomerulares avançadas em alguns pacientes normoalbuminúricos e de lesões iniciais em alguns pacientes com microalbuminúria é compatível com as taxas de progressão para proteinúria e de reversão para normoalbuminúria observadas em estudos mais recentes e aqui descritas em detalhe. É improvável que lesões glomerulares avançadas sejam de pouco valor prognóstico em pacientes com normoalbuminúria. Neste sentido, os resultados iniciais de nossos estudos longitudinais em pacientes com DM tipo 1 de longa duração (1) demonstraram que os pacientes normoalbuminúricos que progrediram para nefropatia diabética após 5-17 anos de seguimento apresentavam lesões glomerulares renais mais avançadas no início do estudo do que os pacientes que permaneceram normoalbuminúricos. Estudos envolvendo um pequeno número de pacientes microalbuminúricos com DM tipo 1 (2)

descreveram resultados semelhantes. Neste momento, nosso estudo longitudinal está sendo ampliado, e uma coorte de pacientes microalbuminúricos que tiveram biópsias renais realizadas para fins de pesquisa na Universidade de Minnesota também está sendo avaliada. É possível que o estudo detalhado da estrutura glomerular em pacientes não-proteinúricos com DM tipo 1 de longa duração possa identificar pacientes com risco aumentado para nefropatia clínica. Contudo, não seria prático sugerir que todos os pacientes com DM de longa duração, na ausência de proteinúria, sejam submetidos à biópsia renal. Entretanto, é importante notar que, apesar da biópsia renal ser um procedimento invasivo, os riscos inerentes ao procedimento são baixos se a biópsia é realizada por profissionais experientes e em condições clínicas adequadas. Além disto, a maioria das biópsias renais realizadas na prática clínica são para fins de diagnóstico e de prognóstico, e em apenas 20-30% dos casos as decisões terapêuticas são influenciadas pelos achados da biópsia *per se*. Biópsias renais são utilizadas rotineiramente, por exemplo, para a avaliação de hematúria microscópica persistente em crianças, uma condição geralmente benigna e que apresenta uma taxa de progressão para doença renal terminal de cerca de 10%, muito menor do que os 25-35% observados em pacientes com DM tipo 1.

O número alarmante de pacientes desenvolvendo nefropatia diabética indica que a busca de outros marcadores de risco, que possam ser utilizados isoladamente ou em associação com medidas da EUA e da filtração glomerular, precisa ser intensificada. Medidas, no sangue ou na urina, de moléculas como a pró-renina, os produtos avançados da glicação não-enzimática e os fatores de crescimento, entre outros, seriam

possíveis alternativas. Além disto, a identificação dos genes associados à proteção ou ao risco para nefropatia diabética, assim como medidas de função celular em fibroblastos, poderiam ser utilizadas não só na identificação de pacientes com risco aumentado mas também para elucidar os mecanismos envolvidos na gênese e na progressão da nefropatia. Nossos estudos quantificando o RNA mensageiro para moléculas possivelmente associadas à patogênese da nefropatia diabética nos fibroblastos cultivados a partir de biópsias de pele realizadas nos pacientes com DM tipo 1 de longa duração descritos no capítulo II, e portanto classificados de acordo com a taxa de progressão de lesões glomerulares como pacientes com lento ("slow-track") ou rápido ("fast-track") desenvolvimento de nefropatia diabética, têm gerado resultados promissores. Quando a expressão do RNA mensageiro para a cadeia alfa 1 do colágeno tipo I foi avaliada, através de PCR quantitativo, pacientes "fast-track" apresentaram níveis reduzidos de RNA mensageiro para esta molécula quando comparados a controles não-diabéticos, entretanto não foram observadas diferenças entre pacientes "fast-track" e "slow-track" (dados não-publicados). Nossos planos para o futuro envolvem a avaliação do RNA mensageiro para outras moléculas possivelmente relacionadas à nefropatia diabética. As mensagens a serem avaliadas estão sendo selecionadas baseado no conhecimento vigente dos fatores envolvidos na gênese e na progressão da nefropatia diabética. O estudo de fibroblastos, que são células de fácil obtenção e cultivo, e cujo comportamento parece refletir a presença de risco ou não para nefropatia diabética, poderá indicar fatores de risco ou de proteção a serem pesquisados precocemente no curso do DM tipo 1.

Em resumo, diversos mecanismos têm sido propostos para explicar as complicações renais causadas pelo DM e é provável que múltiplas rotas metabólicas estejam envolvidas e que interajam em um processo que é, provavelmente, regulado geneticamente. Os avanços recentes na área de biologia molecular e em genética tornam real a possibilidade de elucidar os mecansimos e os genes envolvidos no desenvolvimento da nefropatia diabética. Estes avanços irão permitir a identificação precoce de pacientes em risco e, possivelmente, levar à criação de estratégias de prevenção baseadas no conhecimento da patogênese da nefropatia diabética.

BIBLIOGRAFIA

1. Caramori ML, Fioretto P, Mauer M: Long-term follow-up of normoalbuminuric longstanding type 1 diabetic patients: Progression is associated with worse baseline glomerular lesions and lower glomerular filtration rate (Abstract). *J Am Soc Nephrol* 10:126A, 1999.

2. Bangstad HJ, Østerby R, Hartmann A, Berg TJ, Hanssen KF: Severity of glomerulopathy predicts long-term urinary albumin excretion rate in patients with type 1 diabetes and microalbuminuria. *Diabetes Care* 22:314-319, 1999.