Variants of $\alpha_1$-proteinase inhibitor in black and white South African patients with focal glomerulosclerosis and minimal change nephrotic syndrome

A C Halkas, M C Gaillard, P D Thomson, S L Green, H Ludewick, U Kala

Abstract

Objective—To determine the prevalence and biochemical characteristics of certain alleles of $\alpha_1$-proteinase inhibitor in black and white South African patients with two common types of pathology causing the nephrotic syndrome.

Design—A cross sectional study of black and white patients with focal glomerulosclerosis (FGS) or minimal change disease (MCNS) and black and white controls.

Setting—The patients were drawn from the Paediatric Nephrology Units at the Johannesburg and Baragwanath Hospitals and the controls were drawn from the South African Blood Transfusion Service and the Paediatric Nephrology Clinic in Johannesburg.

Results—There was a significant increase in the prevalence of the V allele in black patients with FGS (12%) as compared to black controls (1%) ($p=0.01$). None of the white patients with FGS had the V allele but two out the five coloured (mixed race) patients had the V allele (20%). An increase in the prevalence of the S allele of $\alpha_1$PI was found in white patients with FGS and MCNS (10%) as compared to white controls (2%). The plasma elastase inhibitory capacity (EIC) associated with the phenotypes (PI) $M1(Ala^{119})S$, $M1(Ala^{119})V$, and $M1(Ala^{119})M1(Ala^{119})$ was significantly decreased as compared to the EIC associated with PI $M1(Val^{119})M1(Val^{119})$ ($p=0.006$, $p=0.004$, and $p=0.025$, respectively). Twelve of 13 patients with FGS and infected with tuberculosis had either the $M1(Ala^{119})V$ or $F$ alleles and required transplantation owing to the severity of the disease. All of these patients were either black or coloured. However, eight of 12 patients with FGS who had the $M1(Ala^{119})V$ or $S$ alleles but were PPD negative did not require transplantation.

Conclusion—It is possible that the combination of functionally less efficient $\alpha_1$PI and an inflammatory challenge associated with an infection such as tuberculosis could predispose black and coloured nephrotic patients to more aggressive scarring in FGS.

Keywords: alpha-1-proteinase inhibitor; focal glomerulosclerosis; minimal change nephrotic syndrome; tuberculosis

Alpha-1-PI is the major inhibitor of serine proteinases in human plasma. It is a glycoprotein consisting of a polypeptide chain of 394 residues and a carbohydrate content of 12% and shows considerable genetic variation. More than 70 variants have been identified. The inheritance of the alleles follows an autosomal codominant pattern. The most common variant is type M, which consists of at least six subtypes: $M1(Val^{119})$, $M1(Ala^{119})$, $M2$, $M3$, $M4$, and $M5$. The $M2$ variant has recently been shown to be associated with asthma, and the prevalence of the $M1(Ala^{119})$ variant has been found to be significantly increased in black South Africans as compared to whites. This variant was also shown to be associated with a reduced plasma elastase inhibitory capacity and has been implicated as a contributory factor to the severity of atopic asthma in black patients. Atopy has been shown to be associated with steroid responsive nephrotic syndrome.

It is well known that the $PiZ$ in its homozygous form has been associated with the development of early onset emphysema in smokers, owing to the inefficient antiproteinase defense mechanism in the lung. Similarly, in the kidney, serine proteinases released by polymorphonuclear leucocytes (PMN) can modify the glomerular basement membrane (GBM) and have been shown to be directly involved in renal pathology, as shown by the loss of laminin of the GBM in kidney treated with elastase and catherspin G. Also proteinases can cleave IgG, thus liberating the Fc fragment which can augment the lymphocyte response. Furthermore, according to current concepts, glomerulosclerosis associated with nephrotic syndrome is primarily the result of extracellular matrix synthesis and degradation dysregulation.

The previously recognised association of atopy with the nephrotic syndrome, the possible role of $\alpha_1$PI in the regulation of elastase, and the suggested association of $\alpha_1$PI deficiency and membrane-proliferative glomerulonephritis prompted the present study.

Materials and methods

Sixty patients (age range 3–18 years) at present being followed up at the Paediatric Nephrology...
Unit, Johannesburg and Baragwanath Hospitals with a diagnosis of nephrotic syndrome (defined as oedema, plasma albumin <25 g/l, and protein/creatinine ratio of >225 mg/mmol) caused by FGS or MCNS were included in the study. The groups consisted of 20 black patients with FGS (eight girls), eight black patients with MCNS (three girls), seven white patients with FGS (three girls), and 15 white patients with MCNS (five girls). There were also five coloured patients (mixed race) with FGS (one girl) and five coloured patients with MCNS (one girl). Nineteen of 32 of all FGS patients were on steroids. Nine were on high dose prednisone (2 mg/kg) and 10 were on lower doses (0.2 mg/kg). Among the patients with FGS, very few responded to steroid therapy. The majority of patients with MCNS (80%) were on lower doses (<0.2 mg/kg) of prednisone. Most of the patients with FGS and MCNS were in remission at the time of this study and 80% of those on steroids were on lower doses of prednisone. The diagnosis of FGS was histologically proven in all patients. Juxtamedullary glomeruli were evaluated in all cases. Children with secondary causes of proteinuria, such as immune complex mediated glomerulonephritis, congenital and hereditary forms of nephrotic syndrome, were excluded. Hepatitis B, C, and HIV were also excluded. None of the patients had membranoproliferative glomerulonephritis. None of the children whose kidneys had been biopsied had histological evidence of renal tuberculous infection.

One hundred and forty-two people were used as controls (age range 5–45 years). These consisted of 90 whites (38 women) and 52 blacks (18 women). Blood donors from the South African Blood Transfusion Service and patients at the Paediatric Nephrology Clinic who did not have any disease process associated with the immune system were used as controls, that is, patients with anatomical abnormalities. Each subject gave informed consent to participate in the study, which was passed by the Committee for Research on Human Subjects of the University of the Witwatersrand.

Plasma was separated on the day of collection and stored at −60°C until analysed. Phenotypes were identified by isoelectric focusing on polyacrylamide gels according to the method of Constans et al. modified as previously described.2 Control serum for the V variant was kindly supplied by Diane Cox (University of Toronto, Canada).

Genomic DNA was extracted from EDTA containing whole blood according to the method of Higushi.11 The polymerase chain reaction (PCR) was used to amplify exon III of the α,PI gene containing a BstEII restriction site, as previously described.1

Each blood sample was collected in a tube containing EDTA. Plasma was separated and assayed within 24 hours of venesection. Plasma concentrations of α,PI were determined by the antibody precipitation laser nephelometric method.12 The following reagents were obtained from Hoechst (Calbiochem Corp, San Diego, CA): the antibody to α,PI, the substrate for elastase (succinyltrialanyl-p-nitroanilide (SAPNA)), and N-protein standard plasma (that is, the standard used for α,PI determination). Porcine pancreatic elastase (PPE) was obtained from Boehringer Mannheim, Germany. All other reagents were of analytical grade (Merck, Darmstadt, Germany).

The elastase inhibitory activity was determined according to the subjects’ phenotype by a modification of our previously described method,11 modified as previously described.3 The assays were done randomly on the following groups: eight children (five white controls and one black control and two white patients with MCNS) with the M1(Val131)M1(Val131) phenotype; nine children (five black controls and four black patients with FGS) with the M1(Val131)M1(Ala131) phenotype; four black patients with FGS with the M1(Ala131)M1(Ala131) phenotype; five black patients with FGS with the M1(Ala131)V phenotype; and three white patients with MCNS with the M1(Ala131)S phenotype. Most of the patients were undergoing steroid therapy, but this did not have any effect on the EIC.

The purified protein derivative (PPD) obtained from the Statens Serum Institut (Copenhagen, Denmark) was used for evidence of exposure to tuberculosis.

The comparison of the distributions of the different variants in the various populations was done by means of the two tailed Fisher exact test. The biochemical characteristics of

### Table 1 Frequency of α,PI variants in black, white, and coloured patients with FGS

<table>
<thead>
<tr>
<th>Variant</th>
<th>Blacks Frequency</th>
<th>Whites Frequency</th>
<th>Coloureds Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1(Val131)</td>
<td>14 0.35</td>
<td>11 0.78</td>
<td>3 0.30</td>
</tr>
<tr>
<td>M1(Ala131)</td>
<td>20 0.50</td>
<td>2 0.14</td>
<td>5 0.90</td>
</tr>
<tr>
<td>V</td>
<td>5 0.12</td>
<td>2 0.20</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>Total 40</td>
<td>14 0.70</td>
<td>10</td>
</tr>
</tbody>
</table>

Comparison of the frequency of the V allele in black and coloured patients with FGS and black and coloured patients with MCNS by the two tailed Fisher’s exact test shows a significant difference (p=0.024).

### Table 2 Frequency of α,PI variants in black, white, and coloured patients with MCNS

<table>
<thead>
<tr>
<th>Variant</th>
<th>Blacks Frequency</th>
<th>Whites Frequency</th>
<th>Coloureds Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1(Val131)</td>
<td>9 0.56</td>
<td>17 0.57</td>
<td>7 0.70</td>
</tr>
<tr>
<td>M1(Ala131)</td>
<td>6 0.37</td>
<td>8 0.27</td>
<td>2 0.20</td>
</tr>
<tr>
<td>M2</td>
<td>1 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>1 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>Total 16</td>
<td>3 0.10</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 3 Frequency of α,PI variants in black and white controls

<table>
<thead>
<tr>
<th>Variant</th>
<th>Blacks Frequency</th>
<th>Whites Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1(Val131)</td>
<td>46 0.44</td>
<td>128 0.70*</td>
</tr>
<tr>
<td>M1(Ala131)</td>
<td>55 0.52</td>
<td>34 0.20†</td>
</tr>
<tr>
<td>M2</td>
<td>2 0.01</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1 0.01</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>4 0.02</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>1 0.01</td>
<td></td>
</tr>
<tr>
<td>Total 104</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

*Comparison of M1(Val131) in black controls v white controls, p<0.0001.
†Comparison of M1(Ala131) in black controls v white controls, p<0.0001.
the variants were compared by means of the Student t test.

**Results**

The distributions of the different PIs of α-PI in all nephrotic patients and controls are shown in tables 1, 2, and 3 respectively. A significant increase in the prevalence of the V variant was found in the black patients with FGS (12%) as compared to the black controls (1%) (p=0.01). Similarly, in the five coloured patients with FGS, 20% had the V variant. The V variant was not present in the five coloured patients with MCNS. An increase in the S variant was found in the white patients with FGS and MCNS (10%) as compared to the white controls (2%), but did not reach significance.

Table 4 shows the biochemical characteristics of the five different phenotype groups of α-PI. There was no difference in plasma concentrations between the groups. However, the specific elastase inhibitory capacity was found to be significantly decreased in plasma of patients and controls containing PIs M1(Val\(^{213}\))M1(Ala\(^{213}\))M1(Ala\(^{213}\)), M1(Ala\(^{213}\))M1(Ala\(^{213}\)), M1(Ala\(^{213}\))V, and M1(Ala\(^{213}\))S as compared to plasma containing the PI M1(Val\(^{213}\))M1(Val\(^{213}\)) (p=0.048, p=0.025, p=0.004, and p=0.006, respectively). Furthermore, as shown in table 5, patients with FGS were found to have a significantly reduced EIC as compared to patients with MCNS or controls (p=0.02 and p=0.005, respectively).

In all the FGS patients, 12 out of the 13 who required transplantation had previous exposure to tuberculosis (evidenced by PPD >15 mm on presentation of the nephrotic syndrome) and all these were black or coloured patients. The phenotypes of these 12 patients consisted of six M1(Ala\(^{213}\))M1(Val\(^{213}\))F, one M1(Ala\(^{213}\))V, and one M1(Val\(^{213}\))F(Val\(^{213}\)). The eight of 12 black and coloured or white patients with FGS who had the M1(Ala213)\(^{V}\) or S alleles but were PPD negative did not require transplantation. The patients who required transplantation and those who did not had been followed over a similar period and the PPD positivity was not related to the duration of the nephrotic syndrome, being present at the onset of the disease.

**Discussion**

FGS is found in 25-35% of black South African children with idiopathic nephrotic syndrome.\(^{14,15}\) Furthermore FGS is the most common cause of end stage renal disease (ESRD) in our population of patients. The association between FGS in black South African children and tuberculosis has previously been reported.\(^{15}\)

Tuberculosis was found in 37.5% of children with FGS as compared to 6% in a group of children with MCNS,\(^{15}\) the latter being similar to the control population. In our previous study, nine of the 10 patients with FGS who required transplantation had evidence of infection by Mycobacterium tuberculosis.\(^{15}\) It has also been noted that black and Hispanic children experience a disproportionate frequency of FGS with progression to ESRD, suggesting that both genetic and environmental factors play a role.\(^{16}\)

Alpha-1-PI is the major inhibitor of serine proteinases and plays a role in modulating interleukin-1 (IL-1) mediated inflammatory responses by inducing the synthesis of IL-1 receptor antagonist.\(^{17}\) It has been shown that α-PI can bind to extracellular matrix (ECM) in vitro, and once bound retains 50% of its ability to inhibit elastase mediated ECM proteolysis.\(^{18}\)

The serine proteinases elastase and plasminogen activators play an important role in the remodelling of the kidneys' ECM,\(^{17}\) and may also play a role in the inflammatory response by augmenting the lymphocyte response.\(^{20}\)

In this study, patients with the M1(Ala\(^{213}\))V and S alleles of α-PI were found to have reduced EIC. Recently Vaziri et al\(^{22}\) reported a decreased α-PI protease inhibitory activity in a group of nephrotic patients, who also had markedly raised tissue type plasminogen activator levels. In our study we found a significant increase in the prevalence of the V allele of α-PI in black and coloured patients with FGS and 40% of black patients who required transplantation were PI M1(Ala\(^{213}\))V. The V allele was not found in any of the white groups, but was found in two of the five coloured patients with FGS. Similarly, an increase of the S allele was found in white patients with FGS and MCNS and this allele was not found in any of the other population groups.

The PI MS has also been associated with asthma\(^{20}\) and a number of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and anterior uveitis.\(^{21}\) These associations suggest that people with these variants are subject to an enhanced immune response. An increased IL-2R expression in T lymphocytes in patients with MCNS has recently been observed, suggesting an increased activation of the T cells in these patients.\(^{21}\) Furthermore, in vitro studies have shown an α-PI dose dependent inhibition of lymphocyte responsiveness to mitogens.\(^{22}\)

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### Table 4 Biochemical characteristics of α-PI in the different phenotype groups

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>No</th>
<th>Plasma level (g/l)</th>
<th>EIC* (KU/g)</th>
<th>Specific activity (KU/g)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1(Val(^{213}))M1(Val(^{213})) 8</td>
<td>1.44 (0.015)</td>
<td>47.99 (4.05)</td>
<td>33.76 (2.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1(Val(^{213}))M1(Ala(^{213})) 9</td>
<td>1.59 (0.68)</td>
<td>41.76 (3.34)</td>
<td>26.32 (1.94)</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>M1(Ala(^{213}))M1(Ala(^{213})) 4</td>
<td>1.41 (0.197)</td>
<td>30.77 (7.73)</td>
<td>21.19 (3.21)</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>M1(Ala(^{213}))V 5</td>
<td>1.74 (0.22)</td>
<td>36.89 (4.62)</td>
<td>20.78 (1.66)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>M1(Ala(^{213}))S 3</td>
<td>1.30 (0.14)</td>
<td>34.82 (5.95)</td>
<td>20.63 (2.07)</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

Mean values are mean (SEM).

*EIC=elastase inhibitory capacity.

†a versus b, c, d, and e.

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### Table 5 Comparisons of plasma elastase inhibitory capacity of α-PI in patients with FGS or MCNS and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Plasma elastase inhibitory capacity (KU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>33.44 (8.39)</td>
</tr>
<tr>
<td>Patients with FGS</td>
<td>13</td>
<td>22.77 (5.05)</td>
</tr>
<tr>
<td>Patients with MCNS</td>
<td>5</td>
<td>32.51 (7.75)</td>
</tr>
</tbody>
</table>

Patients with FGS = controls, p=0.005.

Patients with FGS = patients with MCNS, p=0.02.
Variants of α₁-protease inhibitor in South African patients

could also play a role in our patients as shown by decreased EIC, which on the same basis should lead to increased lymphocyte responsiveness. The M1(Ala¹⁷⁵)V phenotype was more prevalent in the black patients with FGS. We have previously shown that the M1(Ala¹⁷⁵) allele is significantly increased in the black South African population, and may contribute to the severity of asthma owing to a reduced EIC. The black patients with FGS may therefore be predisposed to a more severe course of the disease because of a decrease in the capacity to inhibit proteinases. In our local experience, FGS leading to end stage renal failure is far more prevalent in black patients, often with previous exposure to tuberculosis (37%), than in white patients (6%). Besides degrading ECM components, extracellular proteinases may also contribute to mobilising growth factors and cytokines, which further enhance the immune response. Recent evidence has shown an increased in vivo elastase activity in subjects with α₁PI deficiency, homozygous PIZ and heterozygous PIMS.

In conclusion, we have shown that black and coloured nephrotic patients with FGS had an increased prevalence of the M1(Ala¹⁷⁵)V phenotype of α₁PI and a corresponding decrease in EIC. In white patients with FGS and MCNS, an increased prevalence of the S variant was also associated with a decrease in EIC. It is possible that, in the face of an inflammatory challenge such as infection with tuberculosis, there may be diminished downregulation of proteinases and cytokines, leading to dysregulation of the extracellular matrix with resulting increase in scarring.

11 Higushi R. Rapid efficient DNA extraction for PCR from cells or blood. Amplifications 1989;2:1-12.
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doi: 10.1136/jmg.35.1.6

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