The p53Pro72Arg Polymorphism is Associated with Albuminuria among Aboriginal Australians

STEPHEN P. MCDONALD,*‡ WENDY E. HOY,* GRAEUME P. MAGUIRE,*§ NATALIA L. DUARTE,† DAVID E. L. WILCKEN,‡ and XING L. WANG,†¶

*Menzies School of Health Research, Darwin, Australia; †Cardiovascular Genetics Department, Prince of Wales Hospital, Sydney, Australia; ‡NT Clinical School, Flinders University of SA, Darwin, Australia; §Faculty of Medicine, University of Sydney, Sydney, Australia; ¶Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas.

Abstract. Albuminuria is a widely recognized marker of renal disease and cardiovascular risk. This is especially true in Aboriginal Australians living in remote communities who suffer high rates of end-stage renal disease and cardiovascular mortality. During a survey of risk factors for renal and cardiovascular disease in one such community, an association between a common polymorphism at codon 72 (Arg/Pro) of the p53 gene and markers of renal disease was sought. A cross-sectional community survey including 217 people was performed. Genotypes of the polymorphism were distributed in Hardy-Weinberg equilibrium, with p53Arg allele frequency of 0.45 (range, 0.41 to 0.50). Overall prevalence of albuminuria was high (31% microalbuminuria; 14% overt albuminuria). Urine albumin/creatinine ratio (ACR) was significantly associated with the number of p53Pro alleles (P = 0.01), and there was an interaction with tobacco smoking (P = 0.04). The p53 genotype was also associated with increasing HbA1c, but the relationship between p53 and ACR was independent of this. This is a previously unreported association. This study does not address the mechanism, but this finding, if confirmed, expands the described effects of p53 in cellular proliferation and apoptosis to include a role in the course of renal and possibly cardiovascular disease in this population.

Rates of renal failure among Aboriginal Australians in the Northern Territory of Australia (NT) are among the highest in the world (1,2). Rates of ischemic cardiac disease and death are also extremely high (3,4), as are rates of diabetes, dyslipidemia, and hypertension (5–8). Rates of tobacco smoking are also high (6,7,9,10). In this environment, early renal disease, manifest as albuminuria, is a strong predictor of total and cardiovascular mortality (11,12). The nature of the renal disease is relatively homogenous and is marked by steady progression through increasing degrees of albuminuria to end-stage renal disease (13). A number of factors have been suggested to contribute to this excess of renal disease, including low birth weight, poststreptococcal glomerulonephritis (PSGN), obesity, hypertension, and diabetes (14–17). Whether genetic factors play a particular role in either cardiovascular or renal disease in this environment is unknown.

p53 is a short-lived nuclear protein and is present at low levels in an inactive state in unstressed cells (18–20). It is a critical transcription factor that suppresses growth and triggers apoptosis (21). In response to cellular stress, p53 transcription and expression are enhanced, inducing the transcription of genes, including p21WAF1/CIP1, bax, GADD45, and hdm2 (22,23). These mediate the antiproliferative function of p53 by blocking cell cycle progression and provoking apoptosis. p53 may thus play a key role in any proliferation/apoptosis–related disorders such as cancer or atherogenesis. A number of studies have further suggested that some p53 mutations or polymorphisms are associated with increased risk for cancer, and an association with atherosclerosis has also been reported (24).

The pathologic mechanisms underlying the high prevalence of renal disease in the Aboriginal Australian population appears to involve glomerulomegaly (25,26), leading to subsequent sclerosis (27). Control of cellular proliferation in glomeruli may play a critical role in this process. Among many polymorphic markers in p53, a wild-type polymorphic variant at codon 72 (either p53Arg coded by CGC or p53Pro coded by CCC) appears to be common and functionally relevant. The two polymorphic variants of the wild-type 53 differ both biochemically and biologically (28). Many studies have further shown that the p53 polymorphisms are associated with increased risk of various forms of cancer, especially those related to virus infections (29). Associations have been shown with renal cancers (30), but no studies associating p53 polymorphisms with nonmalignant renal disease in humans have been published. Polymorphisms of p53 have, however, been associated with cardiovascular disease (including an interaction with smoking) in one study (24). In this study, we explored the role of the p53Arg/Pro variant in the development of albuminuria and renal impairment in a remote Aboriginal Australian community in the Northern Territory of Australia.
Materials and Methods

Study Population

Subjects were from a cross-sectional survey that was conducted in a remote coastal Aboriginal Australian community in the East Arnhem region of NT, where prevalence rates of end-stage renal disease are in excess of 5000 per million. Total study population was 237 people (58% of community population). In conjunction with a broad spectrum of cardiovascular risk factors, serum urea and creatinine and spot urine albumin/creatinine ratios were measured. Blood samples were nonfasting, and urine samples were collected without regard to time of day. Whole blood (4 ml) for DNA analysis was collected into ethylenediaminetetraacetic acid tubes, which were stored at 4°C until DNA extraction. DNA specimens were available for 222 subjects. Five people of wholly European origin were excluded from further analysis, leaving specimens on 217 people, 203 of whom also had urine albumin/creatinine ratio (ACR) analyses on spot urine samples. Urinary cotinine concentrations were also measured on 171 samples after a period of storage at −70°C. A questionnaire was completed, and height and weight were recorded. BP was measured by a single observer using a mercury sphygmomanometer. The protocol was approved by the institutional ethics committee and written informed consent was obtained from all participants.

Laboratory Methods

The Arg/Pro variant at codon 72 was determined using PCR–restriction fragment-length polymorphism method because the CGC (p53Arg) can be digested by a specific BstUI restriction enzyme, whereas the CCC (p53Pro) region cannot be. The PCR thermal conditions were the same as described by Klaes et al. (31). The p53Pro homozygote (PP) has a single 182-bp band, the mutant AA homozygote has 133-bp and 49-bp bands, and the AP heterozygote has 182-bp, 133-bp, and 49-bp bands.

Electrolytes were analyzed at an accredited clinical pathology laboratory using a Hitachi 917 autoanalyzer (Hitachi, Japan). Urinary cotinine was measured by HPLC, and urinary cotinine/creatinine ratio was calculated. For urine cotinine concentration, 539 nmol/L (100 ng/ml) was used (32) as the discriminator between “smokers” and “nonsmokers,” and 610 (1 μg/mg) (33) for the cotinine/creatinine ratio (CCR). Cystatin C concentrations were measured by particle-enhanced turbidimetric assay (Dako, High Wycombe, UK).

Statistical Analyses

Polymorphism frequencies were compared with expected numbers on the basis of Hardy-Weinberg equilibrium using a χ² test. ACR was distributed with a right skew and was log-transformed before analysis. ACR was also analyzed in categories of “normal” (ACR <3.4 g/mol, <30 mg/g), “microalbuminuria” (3.4 to 34 g/mol; 30 to 300 mg/g), and “overt albuminuria” (≥34 g/mol, ≥300 mg/g). GFR was estimated by using the equation of Levey et al. (34). HbA1c was right skew in its overall distribution and was analyzed with nonparametric methods or as quartiles (when used as a covariate in multivariate analyses). However, when restricted to the nondiabetic group, HbA1c was normally distributed and analyzed. Cystatin C values were analyzed by using tobit regression to account for censoring of 27 values that were below the detection limit of the assay.

ACR was compared between genotypes by using ANOVA techniques. Statistical independence of potential confounders was assessed by adding other independent variables to a multiple regression with logACR as the dependent variable. First-order interactions were routinely sought with regard to tobacco smoking. HbA1c quartile was added as three indicator variables to avoid an assumption of linear effect. All results are presented after back-transformation; regression coefficients where ACR was used as a dependent variable are exponentiated and therefore refer to fractional or multiplicative change compared with baseline category. A P value of <0.05 was taken to indicate statistical significance.

Results

Population Characteristics

DNA results were analyzed for 217 participants. Current tobacco smokers numbered 148 (74 men); a further 15 (5 men) described themselves as “ex-smokers” and 51 (12 men) as “non-smokers” (with 3 nonresponses). The agreement between self-reported smoking status and categorization on the basis of cotinine concentration was good; levels of agreement for categories based on cotinine concentration (94% agreement; κ = 0.84 [0.69 to 0.98]) and CCR (agreement 94%; κ = 0.86 [0.72 to 1.0]) for CCR were similar (Table 1). Tobacco smoking was more common in men (odds ratio, 2.9 [1.5 to 5.8]; P = 0.001) but was not related significantly to age (mean age, 38.5 ± 1.1 yr in smokers versus 40.2 ± 1.7 in non-smokers; P = 0.4). Like many similar communities, there was a considerable shortage of housing, with a median (IQR) of 7 (5 to 10) people sharing each house of study participants and 3 (2 to 4) people sharing the bedroom of participants.

Spot urine samples were available for 203 of 217 participants. Overall rates of albuminuria were high, more so in women (Table 2). Prevalence rates also increased steadily with age (P < 0.0001), with no significant interaction with gender. ACR was significantly correlated with calculated GFR, but this was due to a close relationship in the lowest quartile of GFR (<72 ml/min per 1.73m²) (r = −0.53; P = 0.0001), with no significant association seen above the 25th GFR centile. Cys-tatin C was not significantly related to ACR (tobit analysis, P = 0.24). The prevalence of overt albuminuria (and to a lesser extent microalbuminuria) was higher in the group with GFR <72 ml/min per 1.73m² (odds ratio, 10.4 [3.6 to 30], P < 0.001 for overt albuminuria; odds ratio, 2.2 [1.0 to 4.9], P = 0.05 for microalbuminuria compared with “normal” ACR). Angiotensin-converting enzyme (ACE) inhibitors had been prescribed for 19% of the study sample (Table 3). Like many similar communities, there was a considerable shortage of housing, with a median (IQR) of 7 (5 to 10) people sharing each house of study participants and 3 (2 to 4) people sharing the bedroom of participants.

Frequency of the A allele was 0.45 (0.41 to 0.50), and genotype frequencies were in Hardy-Weinberg equilibrium (Table 3; χ²=1.8; P = 0.4).

Table 1. Agreement between self-reported smoking category and urine cotinine measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Never</th>
<th>Ex-Smoker</th>
<th>Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cotinine] &lt;520 nmol/L</td>
<td>36 (19)</td>
<td>8 (4)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>[Cotinine] ≥520 nmol/L</td>
<td>6 (3)</td>
<td>2 (1)</td>
<td>129 (70)</td>
</tr>
<tr>
<td>CCR &lt;610</td>
<td>39 (22)</td>
<td>7 (4)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>CCR ≥610</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>122 (7)</td>
</tr>
</tbody>
</table>

a CCR, cotinine/creatinine ratio.
There was significant variation in urine ACR between the different p53 genotypes (Figure 1; ANOVA $F = 3.2, P = 0.04$). This remained after including age and gender as covariates in the ANOVA (partial $F = 3.8, P = 0.02$). Furthermore, the results demonstrate a steady increase in ACR with increasing number of P alleles. When inserted as an ordinal predictor of ACR in a linear regression, each P allele was associated with an increase in ACR by a factor of 1.56 (1.10 to 2.22) ($R^2 = 0.03; P = 0.01$). When stratified by gender, the relationship was significant in men but not women (Table 4). An interaction term (p53 alleles · gender) was not significant ($P = 0.3$) when added to the main terms as a predictor of ACR. There was also no interaction of p53 genotype with age.

Regression of ACR against P allele number showed a significant interaction with smoking. In a multivariate equation, the interaction term for p53-current smoking was significant ($P = 0.04$), but the main term for current smoking category ($P = 0.09$) and the p53 allele number ($P = 0.9$) were not. Consistent with this were the results when the regression of ACR on P allele number was stratified in nonsmokers and smokers; the relationship in nonsmokers was not significant (Table 4). When the number of P alleles and tobacco smoking were inserted as independent variables, adding gender had little effect on overall model power, and gender was not a significant independent variable ($P = 0.13$).

Calculated GFR did not vary significantly across p53 categories.
Table 4. Relationship between presence and number of P alleles at p53 polymorphism and albuminuria by self-reported tobacco smoking

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fractional Change* in ACR per P Allele</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n = 87)</td>
<td>2.03 (1.23 to 3.33)</td>
<td>0.006</td>
</tr>
<tr>
<td>Women (n = 116)</td>
<td>1.33 (0.83 to 2.16)</td>
<td>0.2</td>
</tr>
<tr>
<td>Nonsmokers (n = 51)</td>
<td>0.8 (0.4 to 1.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Current smokers (n = 148)</td>
<td>2.0 (1.4 to 2.9)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Ex-smokers (n = 15)</td>
<td>2.5 (0.3 to 2)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*a Fractional change is the multiplicative factor outlining the difference (after back-transformation) in ACR per P allele for AP and PP compared with the AA genotype.

p53 Polymorphism and Other Variables

The distribution of key risk factors across p53 genotypes is summarized in Table 3. There was significant variation in HbA1c between the genotype groups (Kruskal-Wallis \( \chi^2_{2df} = 6.9; P = 0.02 \); Table 3). The trend in odds ratios for the upper quartile of HbA1c with increasing numbers of P alleles also significant.

No significant relationship was seen with a history of diabetes, although there was a trend toward higher rates in the PP genotype (Table 3). Diastolic and systolic BP did not vary significantly between p53 genotypes. HDL cholesterol did vary significantly between the three groups (Table 3), with the lowest mean in the heterozygote (AP) group. The variance significantly between the p53 genotypes was made using nonparametric tests, which showed that the differences were significant (Kruskal-Wallis, \( P = 0.03 \)). As the samples were nonfasting, triglycerides were not analyzed. There was no significant variation in the degree of overcrowding between p53 groups. C-reactive protein concentrations did not vary with p53 genotype, nor did fibrinogen concentration. The possibility of confounding was approached both by multivariate analysis and (in the case of glycemia) by restriction. Using the first approach, HbA1c quartile, age, systolic and diastolic BP, body mass index, alcohol use, tobacco smoking, and gender together with the number of p53 alleles were inserted in a multiple regression with ACR as dependent variable. The number of p53 alleles remained significant, with an increase by a factor of 1.4 (1.0 to 2.0) per P allele (\( P = 0.03 \)). Although the interaction term (smoking P allele) did not reach statistical significance (\( P = 0.11 \)), there was a positive association of p53 with ACR among smokers (increase, 1.7 [1.2 to 2.4] per allele; \( P = 0.006 \)) but not among nonsmokers (factor, 0.8 [0.4 to 1.6] per allele; \( P = 0.5 \)).

The relationship between p53 number and ACR was also analyzed in the subgroup (\( n = 151 \)) without a history of diabetes (in questionnaire response or chart review). Univariate analysis showed an increase in ACR by a factor of 1.7 (1.2 to 2.2) per P allele (\( P = 0.004 \)) in this group, and by a factor of 1.6 [1.2 to 2.3] (\( P = 0.002 \)) after adjustment for the factors above (including HbA1c as a continuous variable, as it is normally distributed in the nondiabetic group).

Discussion

We report here an association between the presence of the p53Pro polymorphism at codon 72 of the p53 gene and increasing ACR. We are not aware of previous published reports of an association of a polymorphism in this gene and renal failure (other than related to malignancy), albuminuria, or glycemia. Furthermore, the observations also suggest an interactive effect between this p53 polymorphism and cigarette smoking in their relationship with albuminuria.

The association between p53 genotype and HbA1c raises the question of whether albuminuria is actually associated with p53 genotype or merely marks an association between p53 genotype and hyperglycemia. However, the association between p53 and ACR remains after adjustment for HbA1c quartile and in the analysis restricted to the nondiabetic group. Previous PSGN (17) and low birth weight (15) have both been associated with albuminuria in young adults in another similar community. Unfortunately, birth weight details were not available for most participants in this survey due to loss of records from the mission that previously provided medical care; records of who was affected in epidemics of PSGN were also not available. It is not known whether low birth weight or a vulnerability to PSGN are associated with p53 polymorphisms.

Whether these findings are true or represent \( \alpha \)-error must always be critically examined, particularly when findings such as these arise in the context of a larger observational study. The observations reported here were seen in a survey designed primarily to examine other issues, and thus the protocol did not include detailed collection of family pedigrees. Intrafamilial clustering of exposure to environmental or genetic factors could have caused the association seen here. The population of the community belongs to one cultural group, but we cannot directly examine the possibility of stratification related to admixture of genetic material from either European or Asian origin. There are no studies that have shown (or disproved) any relationship between other genetic markers and renal disease among Aboriginal Australian people, save for one poorly controlled study examining the ACE insertion/deletion polymorphism (35). Anecdotaly, we do not believe the environment differed between families. This is supported by the lack of association between p53 markers and either markers of crowding or acute inflammation.

The use of corrections of \( P \) values for multiple analyses (such as those of Bonferroni, Scheffe, or Sidak) has been suggested for studies where multiple comparisons are made and unexpected findings occur, particularly in post hoc analy-
sis of subgroups. Another effect of these corrections is to increase the likelihood of $\beta$ error, possibly dismissing a valid observation and nullifying the hypothesis-generating aspect of an observational study. These problems are compounded when addressing the issues of interactions that further reduce statistical power and illustrated by the different regression coefficients in men versus women in the absence of a significant interaction. The findings reported here clearly need to be confirmed in other communities, both indigenous and nonindigenous, with a protocol including detailed family pedigrees to facilitate appropriate statistical analysis.

These observations would be enhanced if they were reflected in other measures of renal function. Neither calculated GFR nor plasma cystatin C, however, vary across p53 genotype. Although GFR is correlated with ACR, albuminuria appears to be a more sensitive indicator of early renal damage than GFR. Longitudinal studies in a similar community have shown that elevated ACR is a strong risk marker for subsequent abnormal GFR (13) as well as cardiovascular and all-cause mortality (11). Thus the association of the p53 polymorphism with ACR but not GFR may be because ACR is a better epidemiologic marker of the milder degrees of renal disease seen on a community wide basis rather than calculated GFR, which marks a later stage of the process of decline (for which limited numbers are available here for analysis). Furthermore, the equations used in calculating GFR are sensitive to racial variation insofar as a correction factor is applied for African Americans (34). These equations have not been validated in Aboriginal Australians, but there is some evidence suggesting different body compositions (36). Cystatin C has been suggested as a more sensitive indicator of reduced GFR (37,38), but this has been challenged (39). Again, validity in this racial group has not been established.

Although the sample size here is large relative to other studies based in Aboriginal Australian communities, it is small compared with studies elsewhere. The nature of the interaction with smoking in the relationships further compromises statistical power and leads to the situation where substantially different relationships are demonstrated in some subgroups but interaction terms are not significant at usual significance levels. The need to examine interactions should be incorporated in the design and power estimations of subsequent studies.

Mechanisms for the association between p53Pro and albuminuria are not clear. Active interactions with environmental factors, including infections and metabolic influences and possible cell type-specific functions of the p53 gene, further complicate the speculation about the mechanisms. The environment in which this association has been observed (especially the high prevalence of albuminuria) is typical of that reported in other remote Australian Aboriginal communities, as is the high prevalence of related conditions including diabetes and hypertension (14,40–42) as well as the degree of crowding (43). Moreover, the presence of albuminuria strongly marks risk of both cardiovascular and all-cause mortality (11,12). Cardiovascular mortality accounts for a large part of the excess mortality of this group, especially in the <65 yr age group (44,45).

The clinical and histopathologic nature of the renal disease seen in Aboriginal Australian communities is fairly homogeneous (1,46). Glomerular enlargement and subsequent glomerulosclerosis are the hallmarks of Aboriginal Australian renal disease (26,27). We speculate that cells carrying p53Pro may have more potential to hypertrophy and develop these changes in response to the various stressors placed on the kidney in this environment. Alternatively there may be an effect on renal mass, predisposing to reduced renal functional reserve to compensate for subsequent renal insults. In one report, alterations in expression of murine p53 have been associated with defective development of the mesenchymal ureteric bud and reduced glomerular number (47). If a similar (but more subtle) situation applied to the Arg/Pro polymorphism, then AP or PP genotype kidneys might then be more likely to develop albuminuria. Possible approaches to examining this hypothesis might include assessing renal volume in the different groups by ultrasound, or assessing material obtained at autopsy for unrelated causes.

Can these results be generalized to other environments? The frequency distribution of the p53Pro in Aboriginal Australians (0.55) is higher than that in people of European origin (0.29) but lower than African black people (0.63) (48). This fits the hypothesis by Beckman et al. (48) that the p53Pro allele increases with increased latitude. Other transitional populations also suffer high rates of renal disease, diabetes, hypertension, and associated conditions. The role of the p53 polymorphism is unlikely to differ substantially between different populations. If, as appears likely from the smoking interaction, the p53 polymorphism governs response to other renal insults then these results might be more applicable to environments and populations similar to this one with a high prevalence of these risk factors. Given the relationships between albuminuria and atherosclerosis, it also appears consistent with the previously observed relationship between p53 polymorphisms and coronary artery disease (24) in an urban European population with much lower rates of renal disease and in which generalized vascular disease is more manifest as cardiac disease.

In conclusion, we have observed an association between a p53 polymorphism and urine ACR in an environment marked by high prevalence of renal disease. There are also suggestions that there is a relationship with glycemia as measured by HbA1c, but this does not appear to explain the relationship with albuminuria. Further work is required, which should include clinical studies of large enough scale in different populations to confirm this finding and also explore interaction effects. End points for these studies should include both cardiovascular and renal outcomes, possibly including markers of endothelial function, as this might explain the links between coronary artery disease, albuminuria, and smoking. If this association is confirmed that there is also clearly a role for laboratory studies to better elucidate the mechanisms.

Acknowledgments

We are grateful for the advice and support from the Angurugu Community Government Council and Health Clinic and the assistance
of Loyla Lesley, Norma Benger, Paul Lalara, and Mary Amagula in conducting the study. This material was presented in abstract form at the World Congress in Nephrology, San Francisco, October 2001. Financial or material support was received from the Australian Pharmaceutical Manufacturers Association, the National Health and Medical Research Council of Australia, the Australian Kidney Foundation, the Groote Eylandt Mining Company, and Territory Health Services (East Arnhem).

References

8. Sladden T: Cardiovascular Disease Risk Factors in Adults of an Aboriginal Community [MSc thesis]. Darwin, Menzies School of Health Research, University of Sydney: p. 135, 1990
33. Ismail AA, Gill GV, Lawton K, Houghton GM, MacFarlane IA: Comparison of questionnaire, breath carbon monoxide and urine


40. Eiser D: *Microalbuminuria and Cardiovascular Risk Factors in Central Australian Aboriginal Communities* [BMedSc thesis], Department of Medicine, St., Vincent’s Hospital. Melbourne, University of Melbourne, 1995: p 73.


