Genetic Variation at Selected SNPs in the Leptin Gene and Association of Alleles with Markers of Kidney Disease in a Xhosa Population of South Africa

Ikechi G. Okpechi1*, Brian L. Rayner1, Lize van der Merwe2, Bongani M. Mayosi1, Adebowale Adegbele3, Nicki Tiffin4, Rajkumar Ramesar4

1Department of Medicine, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa, 2Department of Statistics, University of the Western Cape, Cape Town, South Africa, 3Centre for Research on Genomics and Global Health, National Human Genome Research Institute, Bethesda, Maryland, United States of America, 4Division of Human Genetics, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Abstract

**Background:** Chronic kidney disease (CKD) is a significant public health problem that leads to end-stage renal disease (ESRD) with as many as 2 million people predicted to need therapy worldwide by 2010. Obesity is a risk factor for CKD and leptin, the obesity hormone, correlates with body fat mass and markers of renal function. A number of clinical and experimental studies have suggested a link between serum leptin and kidney disease. We hypothesised that variants in the leptin gene (LEP) may be associated with markers of CKD in indigenous black Africans.

**Methodology/Principal Findings:** Black South Africans of Xhosa (distinct cultural Bantu-speaking population) descent were recruited for the study and four common polymorphisms of the LEP (rs7799039, rs791620, rs2167270 and STS-U43653 [ENSSNP5824596]) were analysed for genotype and haplotype association with urine albumin-to-creatinine ratio (UACR), estimated glomerular filtration rate (eGFR), Serum creatinine (Scr) and serum leptin level. In one of the four single nucleotide polymorphisms (SNPs) we examined, an association with the renal phenotypes was observed. Hypertensive subjects with the T allele (CT genotype) of the ENSSNP5824596 SNP had a significantly higher eGFR (p = 0.0141), and significantly lower Scr (p = 0.0137). This was confirmed by haplotype analysis. Also, the haplotype GAAC had a modest effect on urine albumin-to-creatinine ratio in normotensive subjects (p = 0.0482).

**Conclusions/Significance:** These results suggest that genetic variations of the LEP may be associated with phenotypes that are markers of CKD in black Africans.
serum leptin concentration may be associated with markers of kidney disease such as urine albumin-to-creatinine ratio (UACR), Scr and eGFR. We therefore hypothesized that polymorphisms of the LEP may have significant effects on markers of renal function in black Africans.

**Methods**

The population sampling of this study, which is part of a larger study to determine the effects of obesity through the metabolic syndrome on kidney disease in an indigenous African population, was of a cross-sectional design and was carried out in Cape Town between May 2005 and July 2006. The study was approved by the joint Research Ethics Committee (REC) of the University of Cape Town and Groote Schuur Hospital. Written informed consent (approved by our REC) was obtained from each subject before they could enter the study. The method of recruitment has been previously described [14]. Briefly, two hundred and fifty three (253) ambulatory hypertensive subjects attending the Guguletu hypertension clinic and eighty-three (83) normotensive relatives in the community were recruited for the study. Although 336 subjects were recruited for the entire study, the sample sizes for the different single nucleotide polymorphisms (SNPs) that were examined differed and were fewer than that of the entire study population due to variation in the availability of high quality DNA, and incomplete successfully genotyping. We chose to study these non-coding polymorphisms because they capture the common haplotype variation across the LEP (figure 1) and also because they have been the commonly studied of the LEP SNPs in other populations, therefore providing a basis for comparison with our population.

The subjects were all of the same indigenous southern African tribal/cultural population group, namely of Xhosa origin, to ensure a homogenous population and to avoid confounding by population admixture which may lead to spurious results in gene association studies of unrelated individuals [17]. A questionnaire was administered to all participants to obtain relevant demographic information. Height, weight, waist and hip circumference were obtained. Body mass index was calculated from weight (kg) divided by height squared (m²). Blood pressure was measured in all the subjects using the same validated mercury sphygmomanometer. The average of 2 blood pressure measurements taken at least 2 minutes apart in the sitting position after about 5–10 minutes rest was recorded. Blood was drawn in the fasting state for routine chemistry including creatinine, lipids, glucose and for assay of leptin. Spot urine was also taken to measure the urine albumin-to-creatinine ratio (UACR). For all the tests, conventional assays were used in the chemical pathology laboratory on auto analysers with appropriate quality control. The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation [18]:

$$\text{eGFR} = 186 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female})$$

$$\times (1.210 \text{ if black}).$$

Serum leptin was measured using a commercially available human leptin radioimmunoassay kit (Linco Research, St. Charles, MO) with sensitivity 0.5 ng/ml, intra-assay precision 3.4–8.3%, and inter-assay precision 3.6–6.2%. All genetic analysis was carried out in the Division of Human Genetics at the University of Cape Town. Genomic DNA was isolated from peripheral blood lymphocytes using the Puregene DNA Isolation Kit (Gentra Systems, USA) according to the manufacturer’s protocol. Polymerase Chain Reaction (PCR) was carried out individually for the SNPs being tested. This was followed by restriction enzyme (RE) digest of the PCR products (table S1, S2, S3, S4). The primers (forward and reverse) used for the SNPs as well as the restriction enzymes used in the digest are shown in Table 1.

**Table 1. Primers and the restriction enzymes used for genotyping.**

| SNP          | Forward Primer      | Reverse Primer      | Restriction Enzyme |
|--------------|---------------------|---------------------|--------------------|
| rs7799039    | 5’TTCCTGTAAATTTCCTCTGAG 3’ | 5’AAAGCAAGACAGGCATAAAA 3’ | HhaI               |
| rs791620     | 5’CAACGGAGGGCCGCAGCGTGAT 3’ | 5’AGGTGCACCTGCGGGGCT 3’ | AscI               |
| rs2167270    | 5’GCCGGCCGGGTGGCCGTGACCTG 3’ | 5’GGCCGGCTGTCGCGCTCAAGG 3’ | MspAI              |
| ENSSNP5824596| 5’GGCACCTGGGAGACCTCGG 3’ | 5’GTCTGGATAAGGGGTGT 3’ | HpyCH4IV           |

Figure 1. Position of four polymorphisms typed across the leptin (LEP) gene on chromosome 7. Dark shading indicates coding sequence. doi:10.1371/journal.pone.0009086.g001
Results

The baseline features (demographic, clinical and biochemical) of all the participating subjects are summarised in table 2, stratified by diagnostic group. The median values of kidney disease phenotypes (Scr, eGFR and UACR) were not significantly different between the hypertensive and normotensive groups. The genotype frequencies of the different polymorphisms are summarised in table 3 and agree closely with the genotype frequencies of the different polymorphisms studied. In the hypertensive group, two phenotypes (Scr and eGFR), were associated with ENNSNP5624596, with the T allele significantly increasing eGFR (p = 0.0137) and decreasing Scr (p = 0.0186) (Table 4).

The haplotypes GCAC and GCGC occurred more frequently than other haplotypes in the hypertensive and normotensive groups, respectively (Table 5). In the hypertensive group, haplotype GCAT yielded significantly higher values of eGFR than GCGC (p = 0.0278). The 3-way haplotype, excluding the first SNP, showed a similar pattern with CAT being associated with significantly higher values of eGFR than both CGC (p = 0.0255). In the last two polymorphisms, AT was also associated with significantly higher eGFR than GC (p = 0.0233) (Table 6).

In the hypertensive group, haplotype GCAT yielded significantly lower values of Scr than GCGC (p = 0.0352). The 3-way haplotype, excluding the first SNP, showed a similar pattern with CAT being associated with significantly lower values of Scr than GCGC (p = 0.0310) and in the last two polymorphisms, AT was associated with significantly lower Scr than GC (p = 0.0293) (Table 6).

In the normotensive group, the only significant association was between UACR and the 4-way haplotype. Urine albumin-to-creatinine ratio was significantly higher in GAAC than in GCAC (p = 0.0482) (Table 7). Linkage disequilibrium (LD) plot of the 4 LEP SNPs and a comparison with LD of the same region in HapMap YRI population which contains additional markers across the same span, revealed low pairwise r2 values indicating that LD across the region is weak (figure S1).

Discussion

We show from the results of this study that genetic variation in the LEP could have significant effects on renal disease phenotypes (markers of renal disease) in indigenous Africans. On the one hand, a marginal but significant effect was observed on microalbuminuria in normotensive subjects, while on the other hand a moderately significant and what is thought to be a “protective” effect was noticed with Scr and eGFR in hypertensive subjects. The reasons why this gene showed effects only on UACR

Table 2. Characteristics (median and interquartile range) of the study groups.

|                     | Hypertensive | Normotensive | p-value |
|---------------------|-------------|--------------|---------|
|                     | n  | Median  | LQ   | UQ   | n  | Median | LQ   | UQ   |         |
| Age (yrs)           | 253 | 57.0    | 49.0 | 63.0 | 83  | 32.0   | 24.0 | 42.0 | <0.0001  |
| BMI (Kg/m2)         | 252 | 33.7    | 28.2 | 39.9 | 82  | 28.2   | 22.1 | 33.3 | 0.0002   |
| SBP (mmHg)          | 252 | 151.5   | 137.0 | 165.3 | 83  | 122.0 | 114.0 | 138.0 | <0.0001  |
| DBP (mmHg)          | 252 | 94.0    | 87.0  | 102.0 | 83  | 82.0   | 76.0  | 89.0  | <0.0001  |
| FBG (mmol/L)        | 249 | 5.2     | 4.8   | 5.5  | 82  | 4.7    | 4.3   | 5.1   | 0.0008   |
| TG (mmol/L)         | 252 | 1.2     | 0.8   | 1.7  | 83  | 0.8    | 0.6   | 1.1   | 0.0292   |
| HDL-c (mmol/L)      | 252 | 1.4     | 1.1   | 1.6  | 83  | 1.5    | 1.3   | 1.9   | 0.0027   |
| Leptin (ng/ml)      | 247 | 26.9    | 11.0  | 45.5 | 83  | 20.0   | 8.6   | 35.8  | 0.0986   |
| Scr (µmol/L)        | 252 | 75.0    | 64.8  | 89.3 | 83  | 66.0   | 58.5  | 78.0  | 0.3067   |
| eGFR(ml/min/1.73 m2) | 252 | 99.0    | 85.8  | 114.0 | 78  | 125.0  | 109.3 | 143.8 | 0.1030   |
| UACR (mg/mmol)      | 252 | 0.8     | 0.3   | 2.6  | 83  | 0.6    | 0.2   | 1.4   | 0.0606   |

p-values are for test of difference in quantile normalised characteristic between diagnostic groups, adjusted for age and gender and relatedness. 

n = Number; Interquartile range is lower quartile (LQ) and upper quartile (UQ). BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; FBG = fasting blood glucose; TG = triglyceride; HDL-c = high density lipoprotein cholesterol Scr = serum creatinine; eGFR = estimated glomerular filtration rate; UACR = urinary albumin-to-creatinine ratio.

doi:10.1371/journal.pone.0009086.t002
in normotensives and then effects on Scr and eGFR alone in hypertensives are presently unclear. However, other factors associated with the hypertensive state may play a role. Although we consider that the effects of these polymorphisms on the phenotypes may be related to the renal actions of serum leptin, we have not shown that serum leptin has direct effects on the kidneys and can therefore not exclude autocrine and/or paracrine mediated actions of leptin in the kidney. The mechanism of action of serum leptin on the kidney has been previously described [15].

A number of studies have previously assessed the effects of the \textit{LEP} on phenotypes of cardiovascular disease (including obesity) and cancer [21,22,23,24,25]. However, the results from many such studies have been largely inconsistent and difficult to replicate in other populations. For instance, Shintani et al [23] reported a positive association between a polymorphic tetranucleotide repeat (TTTC)\textsubscript{m} polymorphism in the 3' flanking region of the \textit{LEP} and hypertension in a group of Japanese patients with essential hypertension. They found the frequency of the class I allele to be significantly higher in hypertensives compared with normotensive controls. In two other studies in South Americans and Italians in which the same polymorphism was examined, there was no association between the class I/II genotypes or alleles with hypertension or cardiovascular disease [24,25].

The result of our study may be confirmatory of previous studies that have reported association between the common polymorphisms of the \textit{LEP} and the various phenotypes they have assayed. One such confirmation relates to the “protective” effect of the T allele of the ENSSNP5824596 polymorphism from renal disease. To our knowledge, only one study [26] has reported on this “protective” effect from atherosclerosis in Caucasians. Gaukrodger et al demonstrated a significantly lower carotid intima medial thickness and pulse pressure in subjects with the T allele, compared to subjects without this allele \((p = 0.0076 \text{ and } p = 0.0001 \text{ respectively})\ [26]. Our study may have shown that this so-called “protective” association may exist in a different population using different phenotypes (Tables 4, 6 and 7).

This study was carried out on the assumption that common polymorphisms of the \textit{LEP} may be associated with kidney disease given that serum leptin has been clinically and pathogenetically linked with markers of kidney disease [15,16,27]. As we know it, ESRD is common and more severe in people of African origin,

| Table 3. Genotype counts, allele counts and frequency distributions. |
|---------------------------------------------------------------|
| **Hypertensives**  | **Normotensives**  | **p-value** |
|-------------------|-------------------|-------------|
| **rs7799039**     |                   |             |
| Typed             | 191               | 59          |
| G                 | 358               | 113         |
| G/A               | 24                | 5           |
| G/G               | 167               | 54          |
| **rs791620**      |                   |             |
| Typed             | 222               | 71          |
| C                 | 404               | 132         |
| A                 | 40                | 10          |
| C/C               | 187               | 61          |
| C/A               | 30                | 10          |
| A/A               | 5                 | 0           |
| **rs2167270**     |                   |             |
| Typed             | 219               | 74          |
| A                 | 238               | 84          |
| G                 | 200               | 64          |
| A/A               | 59                | 19          |
| A/G               | 120               | 46          |
| G/G               | 40                | 9           |
| **ENSSNP5824596** |                   |             |
| Typed             | 224               | 77          |
| C                 | 417               | 144         |
| T                 | 31                | 10          |
| C/C               | 193               | 68          |
| C/T               | 31                | 8           |
| T/T               | 0                 | 1           |

P-values are for test of difference in additive allelic and genotype distributions between diagnostic groups, adjusted for age and gender and relatedness. \(n = \text{count}; f = \text{frequency}. NC = \text{could not be calculated, because no minor allele homozygotes observed.}\)

doi:10.1371/journal.pone.0009086.t003
Table 4. Effect sizes ($\beta$) and p-values for genotype and allelic association with quantile normalised traits, adjusted for age and gender.

|                | Hypertensives | Normotensives |
|----------------|---------------|---------------|
|                | rs7799039     | rs791620      | rs2167270     | ENSSNP5824596 |
| Genotype       |               |               |               |               |
|                | Genotype      | Allelic       | Genotype      | Allelic       |
|                | G/A           | A             | C/A           | A/A           |
|                |                | $\beta$       | $\beta$       | $p$-value     |
| Leptin (ng/ml) | $-0.02$       | 0.9159        | 0.02          | 0.16          |
| Scr (µmol/L)   | 0.11          | 0.5206        | $-0.09$       | 0.24          |
| GFR (mL/min/1.73 m²) | $-0.14$ | 0.4428        | $0.02$        | $0.33$        |
| uACR (mg/mmol) | $-0.01$       | 0.9680        | $-0.09$       | 0.70          |
| Leptin (ng/ml) | $-0.28$       | 0.4670        | $-0.20$       | 0.4603        |
| Scr (µmol/L)   | 0.11          | 0.7760        | $-0.15$       | 0.5333        |
| GFR (mL/min/1.73 m²) | 0.25  | 0.5658        | $-0.09$       | 0.7813        |
| uACR (mg/mmol) | $-0.10$       | 0.8339        | $0.56$        | 0.0970        |

Empty column means genotype result is exactly the same as for allele, because no minor allele homozygotes were observed.

Scr = Serum creatinine, eGFR = Estimated Glomerular filtration rate, UACR = Urine Albumin-to-creatinine ratio.

$\beta$ (genotype) = effect size (regression coefficient), estimated difference in transformed (quantile normalised) phenotype between individuals with a given genotype and individuals with the major allele homozygote genotype.

$\beta$ (allelic) = effect size (regression coefficient), estimated difference in transformed (quantile normalised) phenotype for each additional allele.

doi:10.1371/journal.pone.0009086.t004
although the exact reasons for this remain elusive. Differences in socio-economic status, higher prevalence of hypertension and an increased inherited susceptibility of indigenous Africans to kidney disease are all possible explanations [28,29]. Additionally, as the prevalence of obesity continue to increase, its contribution to kidney disease globally and especially amongst the indigenous Africans cannot be ignored [30]. The overall median BMI of our study population was 32.5 kg/m² (33.7 kg/m² in the hypertensives and 28.2 kg/m² in the normotensive group) (table 2).

This study is important in two ways: firstly, its focus on the relationship between the LEP and renal disease phenotypes. However, the value of this is diminished as the only significant effect we observed after multiple testing was the association of the T allele at ENSSNP5824596 among the hypertensives. This may have been due to the smaller sample size in the normotensive group

| Table 5. Inferred haplotype frequencies in the study population. |
|---------------------------------------------------------------|
| **Frequency**                                                 |
| **Haplotype** *                                              | **Hypertensive** | **Normotensive** |
| GCAC                                                        | 0.37            | 0.46            |
| GGGC                                                        | 0.42            | 0.36            |
| GAAC                                                        | 0.08            | 0.07            |
| AGGC                                                        | 0.04            | 0.05            |
| GCAT                                                        | 0.06            | 0.03            |
| ACAC                                                        | 0.03            | 0.00            |

* Haplotypes are in their order on chromosome 7 (see Figure 1). Frequencies in bold characters denote the base (common) haplotypes.

| Table 6. Results of test of association of haplotypes with markers of renal disease, adjusted for age and gender, in the hypertensive subjects. |
|---------------------------------------------------------------|
| **Scr** | **eGFR** | **UACR** |
| **β** | **p** | **β** | **p** | **β** | **p** |
| A C A C | 0.29 | 0.3750 | -0.41 | 0.1760 | -0.03 | 0.9210 |
| A C G C | -0.12 | 0.7160 | 0.21 | 0.5210 | -0.16 | 0.6490 |
| G A A C | 0.00 | 0.9670 | -0.06 | 0.7420 | -0.29 | 0.1480 |
| G C A C | -0.04 | 0.7230 | 0.07 | 0.5340 | -0.08 | 0.4970 |
| G C A T | -0.38 | 0.0352 | 0.42 | 0.0278 | -0.12 | 0.5960 |
| - C A T | -0.38 | 0.0318 | 0.42 | 0.0255 |       |       |
| - - A T | -0.36 | 0.0293 | 0.39 | 0.0233 |       |       |
| G C G C | Base haplotype |

Tests are adjusted for age and sex.

**β** = estimated difference in transformed phenotype between individuals with a given haplotype and individuals with the base haplotype.

**Scr** = serum creatinine, **eGFR** = Estimated Glomerular filtration rate, **UACR** = Urine Albumin-to-creatinine ratio.

**Table 7. Results of tests of association of haplotypes with markers of renal disease, adjusted for age and gender, in the normotensive subjects.**

| **Scr** | **eGFR** | **UACR** |
|---------|----------|----------|
| **β**   | **p**    | **β**    | **p**    | **β**    | **p**    |
| A C G C | -0.11 | 0.7650 | 0.29 | 0.4920 | 0.18 | 0.6835 |
| G A A C | -0.21 | 0.4650 | 0.22 | 0.4930 | 0.71 | 0.0482 |
| G C A T | -0.28 | 0.4790 | 0.45 | 0.4820 | 1.35 | 0.0743 |
| G C G C | -0.01 | 0.9480 | 0.12 | 0.5380 | 0.19 | 0.3194 |
| G C A T | -0.34 | 0.4540 | 0.47 | 0.3000 | -0.18 | 0.6845 |
| G C A C | Base haplotype |

β = estimated difference in transformed phenotype between individuals with a given haplotype and individuals with the base haplotype.

**Scr** = Serum creatinine, **eGFR** = Estimated Glomerular filtration rate, **UACR** = Urine Albumin-to-creatinine ratio.

Tests are adjusted for age and gender.

Supporting Information

| Table S1 | PCR assay of rs7799039 |
|----------|------------------------|
| Found at: doi:10.1371/journal.pone.0009086.s001 (0.04 MB DOC) |

| Table S2 | PCR assay of rs791620 |
|----------|------------------------|
| Found at: doi:10.1371/journal.pone.0009086.s002 (0.03 MB DOC) |

| Table S3 | PCR assay of rs2167270 |
|----------|------------------------|
| Found at: doi:10.1371/journal.pone.0009086.s003 (0.03 MB DOC) |

| Table S4 | PCR assay of ENSSNP5824596 |
|----------|---------------------------|
| Found at: doi:10.1371/journal.pone.0009086.s004 (0.03 MB DOC) |

| Figure S1 | Linkage Disequilibrium (LD) plot visualized as a GOLD heat map |
|-----------|---------------------------------------------------------------|
| Found at: doi:10.1371/journal.pone.0009086.s005 (1.49 MB TIF) |
Acknowledgments

We wish to thank Ms Donette Baines, Ms Nicola Baines and Mr. Deane Burton who assisted with collection of the data in Guguletu, Cape Town. We also wish to thank Ms Gabi Solomon, Ms Zemio Latief and Ms Alvera Vorster of the Human Molecular Genetics laboratory of the University of Cape Town who managed and assisted with the analysis of the DNA samples. Finally we wish to thank Drs. Judy King and Helen Vreede of the National Health Laboratory Services (NHLS) at the Groote Schuur Hospital who provided support with chemical and hormone assays.

References

1. Lysaght MJ (2002) Maintenance dialysis population dynamics: Current trends and long-term implications. J Am Soc Nephrol 13: 37–40.
2. Xu J, Ma JF, Louis TA, Collins AJ (2001) Forecast of the number of patients with end-stage renal disease in United States to the year 2010. J Am Soc Nephrol 12: 2753–2758.
3. Wackers K, Ihem H, Olsen MH, Berch-Johnsen K, Lindholm LH, et al. (2003) Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med 139: 901–906.
4. Hilleges HL, Fedler V, Diercks GF, van Gilst WH, de Zeeuw D, et al. (2002) Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation 106: 1777–1782.
5. Santopinto JJ, Fox KA, Goldberg RJ, Budaj A, Pitera G, et al. (2003) on behalf of the GRACE Investigators. Creatinine clearance and adverse hospital outcomes in patients with acute coronary syndromes: findings from the global registry of acute coronary events (GRACE). Heart 89: 1003–1008.
6. Goreesh J, Wei GL, McGuunian G, Brancati FL, Levy AS, et al. (2001) Prevalence of high blood pressure and elevated serum creatinine level in the United States: findings from the third National Health and Nutrition Examination Survey (1988–1994). Arch Intern Med 161: 1207–1216.
7. Jones CA, Francis ME, Eberhardt MS, Chavers B, Goreesh J, et al. (2002) Microalbuminuria in the US population: third National Health and Nutrition Examination Survey. Am J Kidney Dis 39: 445–459.
8. Stengel B, Tarver-Carr ME, Powe NR, Eberhardt MS, Brancati FL (2003) Lifestyle factors, obesity and the risk of chronic kidney disease. Epidemiology 14: 479–487.
9. Fox CS, Larson MG, Leip EP, Cullerton B, Wilson PW, Levy D (2004) Predictors of new-onset kidney disease in a community-based population. JAMA 291: 144–1450.
10. Zhang Y, Pronca R, Maffir R, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432.
11. Conidino RV, Sinha MK, Heimann ML, Kriaucienas A, Stephens TW, et al. (1996) Serum immunoactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334: 292–295.
12. Strobel A, Isaad T, Camoin L, Ozata M, Sirosgard AD (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet 18: 212–215.
13. Rudberg S, Persson B (1998) Serum leptin levels in young females with insulin-dependent diabetes and the relationship to hyperandrogenicity and microalbuminuria. Horm Res 50: 297–302.
14. Okpechi K, Pascoe MD, Swaapenpil CR, Rayner BL (2007) Microalbuminuria and the metabolic syndrome in non-diabetic black Africans. Diab Vasc Dis Res 4: 363–367.
15. Wolf G, Hamann A, Han DC, Helmchen U, Thais F, et al. (1999) Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis. Kidney Int 56: 860–872.
16. Balleman BJ (1999) A role for leptin in glomerulosclerosis? Kidney Int 56: 1154–1155.
17. Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 256: 2037–2048.
18. Leve A, Greene T, Kusek J, Beck G. MDRD Study Group (2001) A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. J Am Soc Nephrol 11: 155.
19. Sinnwell JP, Schaid DJ (2008) Haplo Stats (version 1.4.0): Statistical Methods for Haplotype Wherm Linkage Phase is Ambiguous. Mayo website (2010) http://mayoresearch.mayo.edu/mayo/research/schaid_lab/upload/manualHaploStats.pdf.
20. The International HapMap Project. HapMap website (2010) http://www.hapmap.org.
21. Luc anto R, Ponti E, Berselli ME, Saxia G, Minacci A, et al. (2000) The A19G polymorphism in the 5’ untranslated region of the human obese gene does not affect leptin levels in severely obese patients. J Clin Endocrinol Metab 85: 3569–3591.
22. Shibola CF, Holly EA, Forrest MS, Hubbard B, Bracchi PM, et al. (2004) Body Mass Index, Leptin and Leptin Receptor Polymorphisms, and Non-Hodgkin Lymphoma. Cancer Epidemiol Biomarkers Prev 13: 779–786.
23. Shintani M, Ikegami H, Kasaizumi Y, Ohishi M, et al. (2002) Leptin gene polymorphism is associated with hypertension independent of obesity. J Clin Endocrinol Metab 87: 2909–2912.
24. Hiruy HM, Hirata MH, Sampiao MF, Armaganiin D, et al. (2006) LEP 3′ HVR is associated with obesity and leptin levels in Brazilian individuals. Molecular Genetics and Metabolism 89: 374–380.
25. Forrce E, Di Febbio C, Pintor S, Baccante G, Gatta V, et al. (2006) Microsatellite polymorphism of the human leptin gene (LEP) and risk of cardiovascular disease. Int J Obes (Lond) 30: 209–213.
26. Gaukrodger N, Mayosi BM, Incir H, Avery P, Baker M, et al. (2005) A rare variant of the leptin gene has large effects on blood pressure and carotid intima-media thickness: a study of 1428 individuals in 246 families. J Med Genet 42: 474–476.
27. Briley LP, Szczez LA (2006) Leptin and Renal Disease. Seminars in Dialysis 19: 54–59.
28. Seedat YK (1999) Improvement in treatment of hypertension has not reduced incidence of end-stage renal disease. J Hum Hypertens 13: 747–751.
29. Krop JS, Goreesh J, Chambless LE, Shahar E, Watson RL, et al. (1999) A community-based study of explanatory factors for the excess risk for early renal function decline in Blacks vs Whites with diabetes: The Atherosclerosis Risk in Communities Study. Arch Intern Med 159: 1777–1783.
30. Tarver-Carr ME, Powe NR, Eberhardt MS, Laveist TA, Kington RS, et al. (2002) Excess Risk of Chronic Kidney Disease among African-American versus White Subjects in the United States: A Population-Based Study of Potential Explanatory Factors. J Am Soc Nephrol 13: 2363–2370.

Author Contributions

Conceived and designed the experiments: IGO BLR BM RR. Performed the experiments: IGO NT. Analyzed the data: IGO BLR LvdM BM AA NT RR. Contributed reagents/materials/analysis tools: IGO BLR LvdM BM AA NT RR. Wrote the paper: IGO BLR LvdM BM AA NT RR.