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GENDER-SPECIFIC ASSOCIATION OF THE K121Q ECTONUCLEOTIDE PYROPHOSPHATASE PHOSPHODIESTERASE (ENPP) 1 POLYMORPHISM WITH SUBCUTANEOUS ADIPOSITY IN A SOUTH AFRICAN BLACK POPULATION

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ABSTRACT

Purpose: **This study aimed to determine whether the ENPP-1 K121Q (rs1044498) polymorphism was associated with adiposity and cardiometabolic disease markers within a South African Black population.**

Methods: **Black participants from the greater Johannesburg–Soweto area in South Africa, for whom metabolic syndrome status, cardiometabolic disease markers, adipokine levels and body fat distribution had already been measured, were genotyped by PCR-RFLP for the presence of the K121Q polymorphism.**

Results: **Within a cohort of 345 Black South Africans, the frequency of the ENPP-1 K121Q C allele was 0.89. In the total cohort, the K121Q polymorphism was not significantly associated with any cardiometabolic disease markers or adipokine levels. However, in the female population the CC genotype was shown to be associated with increased abdominal subcutaneous fat levels (p=0.007).**

Conclusion: **This cohort of Black South Africans had a higher frequency of the C allele when compared to reported data for Asian and Caucasian populations, however this frequency was comparable to other African data. The presence of the rs1044498 polymorphism was not associated with markers of cardiometabolic disease, suggesting that these associations may be population dependent. The gender-specific genotypic association** with **subcutaneous fat levels is a novel finding requiring further investigation**.

INTRODUCTION

The incidence of overweight and obesity is rising rapidly, with the number of cases worldwide doubling between 1980 and 2008 (1). The rise in the number of overweight and obese individuals is associated with increased co-morbidities such as cardiac disease and diabetes (2). In the South African context, the prevalence of obesity and overweight is exceptionally high, particularly in women. Recently the South African National Health and Nutrition Examination Survey, showed obesity within >15 year olds to be 40.1% in females and 11.6% in males (3).

Several genetic factors involved in the development of obesity have been identified, however the majority of these associations have not been fully investigated within African populations. One such factor is ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP-1), a transmembrane glycoprotein that plays an important role in bone mineralisation where it catalyses the hydrolysis of ATP to AMP and pyrophosphate (4). In addition, ENPP-1 binds to the insulin receptor, inhibiting its tyrosine kinase signalling activity (5). This enzyme is overexpressed in insulin-responsive tissues of individuals with type 2 diabetes and this increase is seen prior to the development of diabetes (6).

Intracellular lipid accumulation within adipocytes is mediated through the interaction of insulin with its receptor. Binding of insulin activates tyrosine kinase activity of the receptor leading to phosphorylation of insulin receptor substrate (IRS) 1 which in turn initiates a signalling cascade (7) that enhances numerous sub-cellular processes leading to increased adipocyte triglyceride deposition. An ENPP-1 non-synonymous A>C polymorphism (rs1044498) located in the protein binding region, imparts an amino acid change of lysine (K) to glutamine (Q) at codon 121. This amino acid change enhances ENPP-1 binding to the insulin receptor, which in turn decreases the tyrosine kinase activity of the receptor (8). The presence of this polymorphism has been linked to numerous pathologies including obesity (9), insulin resistance (10), type 2 diabetes (11) and coronary artery disease (12). However, these associations are not consistently observed (13, 14).

Whilst the ENPP-1 polymorphism has been investigated within the South African mixed ancestry population (15, 16), the prevalence of the ENPP-1 K121Q polymorphism within the South African Black population has yet to be determined. We therefore aimed to determine the prevalence of this polymorphism within a cohort of Black South African subjects, and whether it is associated with obesity. We further aimed to determine whether the K121Q polymorphism is associated with body fat distribution i.e. visceral and subcutaneous fat thickness and waist-to-hip ratio. Studies have shown that the K121Q polymorphism is also associated with insulin resistance (6, 10), and therefore we also determined if a similar association was present in our study population.

MATERIAL AND METHODS

Study subjects: A cohort of 345 South African Black participants were selected from a larger study as described by George *et al* (17). Briefly, the participants were recruited from the greater Johannesburg-Soweto metropolitan area in South Africa and consisted of family members/acquaintances of participants recruited to the Birth to Twenty study (a longitudinal analysis of more than 3200 children and their caregivers) (18). African ethnicity was self-reported (17).

Detailed anthropometric data were available for all participants including ultrasound-

measured visceral and abdominal subcutaneous fat thickness as well as weight, height, waist and hip circumference. Cardiometabolic variables including fasting levels of glucose, HbA1c, insulin and insulin resistance (from HOMA method; (19)) were also available, together with adiponectin and leptin serum levels. The smoking habits of all subjects were also analysed, and each individual was defined as either a current smoker or non-smoker. The methods of measurement for all these variables have been described elsewhere (17). Participants were categorised as having metabolic syndrome using the harmonized definition (20).

Ethics clearance was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (ethics clearance number M10411).

Genotyping: DNA was extracted from stored buffy coats using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

All samples were genotyped for the K121Q polymorphism (rs1044498) using the primers described by Santoro et al (5). Briefly, a 208bp region of the *ENPP-1* gene flanking the polymorphism was amplified using the Q5 high fidelity DNA polymerase (New England Biolabs, Ipswich, Massachusetts, United States). Following an initial denaturation step at 98°C for one minute, the PCR reaction was allowed to proceed for 35 cycles (98°C for 1 second; 57°C for 5 seconds; 72°C for 7 seconds). The PCR amplicons were sequenced (Inqaba Biotechnology, Pretoria, South Africa) to confirm the correct region of the gene was amplified. The amplified fragments were digested with AvaII (New England Biolabs, Massachusetts, USA), which recognises the sequence encoding the glutamine (Q) variant generating two fragments of 154 and 54bp. The digested PCR products were run on a 2% agarose gel and genotyped according to the number of fragments visible.

Statistical analysis: A χ^2 test was performed to confirm the genotype frequencies were in Hardy Weinberg equilibrium. The CA and AA genotype were combined for analysis as the AA genotype alone was found at a frequency which was too low to analyse separately. Student's non-paired t-test was used to compare the mean variable levels between the two genotypic groups (CC vs CA/AA). Multivariable linear regression analysis was performed using abdominal subcutaneous fat as the dependent variable to determine if the effect of the K121Q genotype was retained after adjusting for possible confounders i.e. age, gender and smoking. B values are represented as standardized values. All statistical analyses were performed using Statistica version 13 (StatSoft, Tulsa, OK, USA).

RESULTS

Clinical characteristics of study participants: The clinical characteristics of the cohort (N = 345) are summarised in Table 1. The study population consisted of approximately equal numbers of males and females (47.8 vs 52.2 %, p=0.42). The body mass index (BMI) ranged from 15.5 to 52.5kg/m^2. The prevalence of obesity was 32.9 %, and the prevalence of metabolic syndrome was 29.2 %. Males had statistically lower BMI, leptin, adiponectin, insulin, insulin resistance, waist-to-hip ratio and subcutaneous fat mass when compared to females. In addition, there were significantly less males than females with metabolic syndrome (18.2 vs 39.1 %, respectively).

Table 1

General characteristics of the study population

Variables	**Total (n=345)**	Male (n=165)	Female (n=180)	p value
Age (years)	42.0 [29.0; 51.5]	41.0 [29.0; 51.0]	42.0 [28.0; 52.0]	0.792
Gender (% male)	47.8	-	-	-
BMI (kg/m^2)	26.0 [21.3; 32.4]	22.8 [20.2; 26.9]	30.4 [24.3; 35.3]	<0.0001
Obesity (%)	32.9	13.3	50.5	<0.0001
Leptin (ng/mL)	9.23 [2.55; 26.0]	2.80 [0.82; 6.80]	24.6 [11.3; 43.7]	<0.0001
Adiponectin (ng/mL)	7.30 [4.88; 10.9]	6.34 [4.24; 10.0]	7.87 [5.49; 11.6]	<0.0001
Metabolic syndrome (%)	29.1	18.2	39.2	<0.0001
Glucose (mmol/L)	4.90 [4.60; 5.40]	4.90 [4.60; 5.40]	4.90 [4.60; 5.30]	0.310
HbA1c (%)	5.40 [5.09; 5.70]	5.37 [5.07; 5.68]	5.42 [5.11; 5.72]	0.565
HOMA-IR	1.77 [1.08; 2.89]	1.41 [0.77; 2.76]	2.04 [1.26; 2.93]	<0.0001
Insulin (mU/L)	7.71 [4.88; 12.2]	6.30 [3.61; 10.3]	9.38 [5.95; 12.7]	<0.0001
Waist-to-hip ratio	0.86 ± 0.09	0.85 ± 0.09	0.88 ± 0.08	<0.0001
Visceral fat (cm)	5.05 ± 1.84	5.14 ± 1.79	4.95 ± 1.90	0.339
Subcutaneous fat (cm)	2.34 [1.58; 3.47]	1.65 [1.27; 2.34]	3.20 [2.30; 4.24]	<0.0001
Smoking (%)	34.6	56.1	15.0	<0.0001

Data is shown as mean ± SD or median [interquartile range] or percentage.

The C allele is the predominant allele in the South African Black population: Within this cohort, the C (glutamine; Q) allele was found to be the predominant allele with a frequency of 0.89 (Table 2). The CC genotype was present in 269 individuals (77.9 %), 74 individuals were heterozygous (21.4 %) and 2 individuals had the AA genotype (0.6 %) (Table 2). There were no gender differences in allelic frequency (female C allele = 0.91 vs male C allele = 0.87; p=0.227). The allele frequencies of this cohort were in Hardy Weinberg equilibrium (χ^2 = 1.631; p=0.196).

Table 2

Genotypic and allelic frequencies of rs1044498 in 345 black South African study participants

Genotype frequency			**Allele frequency**	
CC (n = 269)	CA (n = 74)	AA (n = 2)	C (n = 612)	A (n = 78)
0.78	0.21	0.01	0.89	0.11

The association of the K121Q polymorphism with markers of obesity: Within our cohort, K121Q was not significantly associated with BMI, nor any other markers of metabolic disease (Table 3). Interestingly, whilst BMI was not associated with the K121Q polymorphism, individuals carrying the 121Q C allele were found to have significantly higher levels of abdominal subcutaneous fat (2.45 [1.62; 3.55] vs 1.98 [1.49; 3.00] cm; p=0.049).

Table 3

Clinical characteristics of study participants and their association with ENPP-1 genotype

Variable	CC (n= 269)	CA and AA (n=76)	p value
Age (years)	42.0 [28.0; 51.0]	41.5 [31.5; 53.0]	0.740
BMI (kg/m²)	26.3 [21.4; 32.0]	25.1 [20.8; 32.8]	0.745
Obesity (%)	32.2	31.6	0.897
Visceral fat (cm)	5.10 ± 1.80	4.86 ± 2.01	0.317
Subcutaneous fat (cm)	2.45 [1.62; 3.55]	1.98 [1.49; 3.00]	0.049
HBA1c (%)	5.42 [5.10; 5.71]	5.34 [5.07; 5.70]	0.845
Male gender (%)	46.0	52.6	0.344
Insulin (mU/L)	7.64 [4.89; 12.2]	7.81 [4.87; 11.0]	0.915
Insulin resistance	1.73 [1.08; 2.88]	1.82 [1.13; 2.90]	0.954
Leptin (ng/mL)	9.60 [3.30; 28.5]	8.80 [2.35; 21.1]	0.231
Adiponectin (ng/mL)	7.53 [4.93; 11.1]	6.38 [4.86; 9.83]	0.575
Glucose (mmol/L)	4.90 [4.60; 5.30]	4.95 [4.60; 5.40]	0.633
Waist-to-hip ratio	0.85 ± 0.09	0.84 ± 0.08	0.655
Metabolic syndrome (%)	29.0	28.9	0.964

Data is shown as mean ± SD or median [interquartile range] or percentage.

As many of the variables examined in this study showed gender differences, we analysed each gender individually to determine whether there were any gender-specific associations related to the presence of the polymorphism (Tables 4 and 5). Within males, none of the variables were associated with the rs1044498 genotype. In the female group the association with subcutaneous adiposity remained, but no other associations were seen.

Table 4

Clinical characteristics of male participants and their association with ENPP-1 genotype

Variable	CC (n=125)	CA and AA (n=40)	p value
Age (years)	41.0 [29.0; 53.0]	39.5 [30.5; 50.5]	0.820
BMI (kg/m²)	22.5 [20.3; 27.3]	24.0 [20.1; 26.8]	0.394
Obesity (%)	12.8	15.0	0.929
Visceral fat (cm)	4.98 ± 1.88	4.86 ± 2.01	0.734
Subcutaneous fat (cm)	1.66 [1.23; 2.34]	1.55 [1.33; 2.13]	0.941
HBA1c (%)	5.40 [5.06; 5.70]	5.35 [5.09; 5.64]	0.431
Insulin (mU/L)	5.44 [3.58; 10.0]	7.81 [4.83; 11.2]	0.135
Insulin resistance	1.27 [0.75; 2.55]	1.84 [1.21; 2.94]	0.150
Leptin (ng/mL)	3.20 [0.75; 6.80]	2.55 [1.51; 6.95]	0.384
Adiponectin (ng/mL)	6.77 [4.16; 10.51]	5.75 [4.37; 7.68]	0.448
Glucose (mmol/L)	4.90 [4.60; 5.30]	5.00 [4.60; 5.40]	0.601
Waist-to-hip ratio	0.88 ± 0.07	0.88 ± 0.09	0.855
Metabolic syndrome (%)	16.8	22.5	0.563

Data is shown as mean ± SD or median [interquartile range] or percentage.

Table 5

Clinical characteristics of female participants and their association with ENPP-1 genotype

Variable	CC (n= 144)	CA and AA (n=36)	p value
Age (years)	42.0 [28.0; 51.0]	42.0 [34.0; 55.0]	0.394
BMI (kg/m²)	30.4 [24.6; 35.4]	30.2 [22.8; 34.9]	0.597
Obesity (%)	50.7	50.0	0.941
Visceral fat (cm)	5.21 ± 1.72	4.86 ± 2.04	0.301
Subcutaneous fat (cm)	3.29 [2.50; 4.24]	2.39 [1.92; 4.26]	0.019
HBA1c (%)	5.44 [5.15; 5.71]	5.29 [5.05; 5.89]	0.576
Insulin (mU/L)	9.88 [6.21; 13.5]	7.83 [5.16; 11.0]	0.074
Insulin resistance	2.23 [1.32; 3.06]	1.82 [1.10; 2.73]	0.116
Leptin (ng/mL)	26.0 [11.9; 43.7]	20.6 [11.0; 39.4]	0.219
Adiponectin (ng/mL)	7.83 [5.55; 11.6]	8.10 [5.42; 13.2]	0.782
Glucose (mmol/L)	4.90 [4.60; 5.30]	4.90 [4.55; 5.35]	0.910
Waist-to-hip ratio	0.85 ± 0.09	0.84 ± 0.08	0.681
Metabolic syndrome (%)	40	36	0.566

Data is shown as mean ± SD or median [interquartile range] or percentage.

To further investigate the association found between subcutaneous adiposity and the K121Q polymorphism in the total population and in females, multivariable linear regression analyses were performed with adjustment for possible confounding variables i.e. age, gender (included only in the total population analysis) and smoking. Table 6 shows the multivariable regression model for the total population whilst Table 7 shows the model for females. When doing a multivariable linear regression for subcutaneous adiposity in the male population, genotype did not reach significance (p=0.950), however smoking (p=0.024) and age (p=0.008) were both significantly associated.

Table 6

Multivariable linear regression model for subcutaneous fat in the whole population

Dependent Variable	Independent Variable	b value	p value
Log Subcutaneous Fat (n = 342)* Unadjusted $r^2 = 0.38$ p <0.0001	Gender[a]	-0.50	< 0.0001
	Age	0.21	< 0.0001
	Smoking[b]	-0.12	0.010
	Genotype coding[c]	0.08	0.057

*[a]Gender code, 1=male and 0=female; [b]smoking code, 1=smoker and 0=non-smoker; [c]genotype coding, 1=CC and 0=CA/AA, *smoking data unavailable for three participants*

Table 7

Multivariable linear regression model for subcutaneous fat in the female population

Dependent Variable	Independent Variable	b value	p value
Log Subcutaneous Fat (n = 178)* Unadjusted $r^2 = 0.14$ p <0.0001	Age	0.31	< 0.0001
	Genotype coding[a]	0.20	0.007
	Smoking[b]	-0.09	0.222

*[a]Genotype coding, 1=CC and 0=CA/AA; [b]smoking code, 1=smoker and 0=non-smoker, *smoking data unavailable for two participants*

DISCUSSION

The 121Q (C) variant of rs1044498 has been shown to be associated with obesity (9, 21), insulin resistance (10, 22) and type 2 diabetes (11, 23). However, these associations are not seen in all studies (13, 14). Within the South African Black population studied, the C allele was found to be the predominant allele (frequency=0.89). This data correlates with data generated by the 1000 genomes project where, in the African population, the frequency of the C allele was 0.89 (24). The allele frequency data for this locus in the African population, within both the 1000 genomes project and the current study, is markedly different to that seen in South Asian, East Asian and European populations where the C allele frequency is 0.13, 0.10 and 0.13 respectively (24).

Despite the high frequency of the C allele in our population, our study showed no association of this allele with obesity or insulin resistance. This is similar to studies by Morrison et al. and Lyon et al. who found

no association of the K121Q polymorphism with insulin resistance or obesity, respectively, in African American individuals (25, 26). There are conflicting results which show an association with obesity and insulin resistance and the presence of the C allele (9, 10, 21, 22). Our results may differ from the results seen in other studies due to the ethnic differences between the populations investigated.

Although this study failed to show an association between obesity and the rs1044498 C allele, an association was found between this allele and higher levels of subcutaneous fat mass. Further analysis showed that the C allele was linked to higher subcutaneous fat mass in females but not males. Multivariable regression analysis was then performed to determine the main contributors to subcutaneous adiposity in both the total, male and female populations. The total population model (Table 6) explained 38 % of the variance in subcutaneous fat deposition. Unsurprisingly female gender was the biggest contributor to subcutaneous fat levels. It has previously been shown that women have more subcutaneous fat than their male counterparts (27). However, the association of the C allele at rs1044498 with subcutaneous adiposity in the total study population was weak (p=0.057). Within the female population model (Table 7) the significant association between subcutaneous fat levels and genotype remained (p=0.007), however, no association was seen in the male population. The gender-specific association of polymorphisms is not uncommon (28-30). A study by Tanyolac and colleagues found gender differences in the association of the K121Q ENPP-1 polymorphism with obesity (31). A study by Wan et al showed that Chinese females possessing the C allele were more likely to be obese than those carrying the A allele (32). Whilst our female population did not show any genotype effect for BMI, visceral fat or waist-to-hip ratio, the K121Q C allele was associated with increased subcutaneous adiposity. The mechanism by which the K121Q C allele may contribute toward gender-specific abdominal subcutaneous fat levels is not known. However, a possible mechanism may be via interactions with sex steroids, but there is no data in the literature linking ENPP-1 function or expression to these molecules.

The main limitation of this study is the relatively small sample size. Although we did have sufficient data to observe a significant effect of the K121Q C allele on subcutaneous fat, we did not observe any significant effect on BMI or insulin sensitivity, and this may be due to a lack of power. In addition, we looked at only one variant in the ENPP-1 gene and it is therefore possible that the K121Q locus is not the site of the causal variant but may be in linkage disequilibrium with such a variant.

CONCLUSION

In conclusion, within the South African Black population the CC genotype at the K121Q locus is the most frequent, being found in 78.1 % of the cohort. This is in contrast to studies carried out in the European population showing that AA is the dominant genotype. The presence of the CC genotype within the South African Black population was not associated with BMI or cardiometabolic disease, but it was related to greater subcutaneous adiposity within females only. Further gene association studies are required to determine whether the K121Q locus harbours the true causal variant and mechanistic studies are needed to analyse the gender-specific effects of the variant on subcutaneous adipose tissue mass.

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