Sirtuin 1 rs1467568 and rs7895833 in South African Indians with early-onset coronary artery disease

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Abstract

Background: Sirtuin 1 (SIRT1), a class III histone deacetylase, has been identified as a candidate molecule affecting the epigenetic mechanisms of cardiovascular disease (CVD). Previous studies have shown that some SIRT1 single-nucleotide polymorphisms (SNPs) are associated with body mass index, diabetes, blood pressure, cholesterol metabolism and coronary artery calcification. We investigated two A>G SIRT1 SNPs, rs1467568 and rs7895833, in young South African (SA) Indians with coronary artery disease (CAD) and compared them to Indian and black controls.

Methods: For rs1467568, a total of 287 subjects were recruited into this study (104 CAD patients, 99 age-, gender- and race-matched controls, and 84 age- and gender-matched black controls). For rs7895833, a total of 281 subjects were recruited into this study (100 CAD patients, 99 age-, gender- and race-matched controls, and 82 age- and gender-matched black controls). All patients were male, of Indian ethnicity, stable CAD and dyslipidaemia, all of which are well-established risk factors for developing early-onset CAD.

Results: The variant allele for both SNPs was found at a higher frequency in the total Indian group compared to the total black population (rs1467568: 41 vs 18.5%, respectively, \( p < 0.0001 \), OR = 3.190, 95% CI: 2.058–4.094; and rs7895833: 41 vs 22%, respectively, \( p < 0.0001 \), OR = 2.996, 95% CI: 1.850–4.004). No difference was seen in the distribution of both SNPs between CAD patients and either race-matched controls, and compared them to Indian and black controls.

Conclusion: Both SNP variant alleles occurred more frequently in SA Indians than in SA blacks. A larger study group and confirmed on angiography, mean age 37.5 years; range 24–45. All subjects were genotyped using TaqMan SNP genotyping assays. For rs7895833, a total of 281 subjects were recruited into this study (100 CAD patients, 99 age-, gender- and race-matched controls, and 82 age- and gender-matched black controls). All patients were male, of Indian ethnicity, stable CAD and dyslipidaemia, all of which are well-established risk factors for developing early-onset CAD.

Keywords: sirtuin 1, rs1467568, rs7895833, single-nucleotide polymorphism, premature coronary artery disease, South African Indians

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Sirtuins are a class of NAD+-dependent proteins involved in a wide range of biological processes such as aging, transcription, apoptosis and inflammation.1 Sirtuin 1 (SIRT1) is located in the nucleus and cytoplasm, and plays an important role in epigenetic regulation by deacetylating a range of transcription factors to control downstream gene expression.2 The targets of SIRT1 include Forkhead box O (FOXO)1, (FOXO)3, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1\( \alpha \)), tumour suppressor p53, nuclear factor-kappa B (NF-kB), Notch, hypoxia-inducible factor (HIF) 1\( \alpha \), liver X receptor (LXR), farnesoid X receptor (FXR) and sterol regulatory element-binding protein (SREBP)1c.3

Recent studies have demonstrated a protective role of SIRT1 in atherosclerosis, the underlying process of coronary artery disease (CAD).4 SIRT1 performs an anti-inflammatory function by downregulating the expression of several pro-inflammatory cytokines by interfering with the NF-kB signalling pathway. By deacetylating NF-kB, SIRT1 suppresses the expression of lectin-like oxidised low-density lipoprotein receptor-1 (Lox-1), a scavenger receptor for oxidised low-density lipoproteins (oxLDL), therefore preventing foam cell formation.5 SIRT1 controls the activity of LXR, an important regulator of lipid homeostasis and inflammation.6 Activation of LXR results in expression of ATP-binding cassette (ABC) transporter ABCA1, which regulates the removal of cholesterol into high-density lipoproteins (HDL), a process known as reverse cholesterol transport (RCT). Dysfunctional RCT could lead to accumulation of cholesterol, thus stimulating foam cell production and the progression of atherosclerosis.4,5 Given the important role of SIRT1 in cardiovascular disease, research on genetic variation in the SIRT1 gene has become of interest.

Genetic variations such as single-nucleotide polymorphisms (SNPs) in the SIRT1 gene have been associated with inflammation, body mass index, type 2 diabetes, blood pressure and dyslipidaemia, all of which are well-established risk factors for CAD.7,8 Coronary artery disease remains a leading cause of mortality worldwide, with an unusually high prevalence of early-onset disease among the Indian population. South African (SA) Indians have a much higher prevalence of CAD compared to SA blacks.9 There are currently no studies on SIRT1 SNPs in SA Indians with CAD. We therefore investigated the SIRT1 A>G SNPs, rs1467568 and rs7895833 in young SA Indians with CAD and compared them to Indian and black controls.
Methods
A total of 287 subjects were recruited into the SIRT1 rs1467568 study (104 CAD patients, 99 age-, gender- and race-matched controls, and 84 age- and gender-matched black controls) following institutional ethical approval (BE067/14). The inclusion criteria for CAD patients were: Indian ancestry and unrelated adult males aged < 45 years, and stable CAD confirmed on angiography. The exclusion criteria for controls included an acute coronary syndrome/vascularisation procedure in the preceding three months, chronic renal or liver disease, malignancy and known inflammatory or infectious disease.

Blood samples were obtained following an overnight fast. A full pathology report of clinical markers was assessed by routine laboratory testing at the Global Clinical and Viral Laboratory (Durban, South Africa), a South African national accreditation system (SANAS) certified laboratory. The following parameters were tested: haematology (Roche Sysmex 1800XT), chemistry (Beckman Coulter DXC600), endocrinology and high-sensitivity C-reactive protein (hsCRP) (Siemens Centaur XP) and serology (BD Biosciences FACS Calibur), as per international standards to obtain levels of total cholesterol, HDL-C, LDL-C, triglycerides, fasting glucose, two-hour glucose, fasting insulin, glycosylated haemoglobin, sodium, potassium, bicarbonate, chloride, urea, creatinine, glomerular filtration rate, CD4, CD8, CD45 and CD3 count. The physical measurements of weight, height, abdominal circumference, waist circumference and patient history were conducted by the cardiologist (Dr S Khan).

Genomic DNA was extracted from the whole blood sample of each patient and control, according to the method described by Sambrook et al.11 Cells were transferred to 600-μl lysis buffer [0.5% sodium dodecyl sulphate (SDS), 150 mM NaCl, 10 mM ethylenediaminetetra-acetic acid (EDTA), 10 mM Tris–HCl (pH 8.0)]. To this, RNase A (100 μg/ml; DNase-free) was added to the solution and incubated at 37°C for one hour. Proteinase K (200 μg/ml) was then added and incubated for three hours at 50°C.

Protein contaminants were then precipitated by adding 5 mM 0.1% potassium acetate before centrifugation at 5 000 × g for 15 min. Supernatants containing genomic DNA were transferred to fresh tubes and extracted with 100% isopropanol on ice, and thereafter washed with 70% ethanol. DNA samples were dissolved in 10 mM Tris and 0.1 mM EDTA (pH 7.4, 4°C). DNA concentration was used to genotype all subjects for both SNPs. The TaqMan® MGB probes consist of target-SNP based on the change in fluorescence of the dyes associated with the probes. The TaqMan® genotyping assay contains two primers for amplifying the SNP of interest, 2 × TaqMan® genotyping master mix, nuclease-free water, and a 10-ng genomic DNA template. The experiment was done using the Applied Biosystems® ViIA™ 7 Real-Time PCR system.

Statistical analysis
The Hardy–Weinberg equilibrium was used to test for deviation of allele/genotype frequency. All other statistical analyses were performed with Graphpad prism software (version 5.0). Allele and genotype frequencies were calculated using the Fisher’s exact and chi-squared tests, respectively. The comparison of biochemical measures between the wild type and variant genotypes was done with a non-parametric t-test. Results are expressed as mean ± standard error. A p-value less than 0.05 was considered statistically significant.

Results

SIRT1 rs1467568
The genotype distribution complied with the Hardy–Weinberg equilibrium in the CAD patients and Indian controls (chi-squared p = 0.233 and p = 0.941, respectively), but not in the black control group (chi-squared p < 0.05).

No significant difference was observed in the distribution of the SIRT1 rs1467568 alleles between the CAD patients and Indian controls (41 vs 40% respectively, p = 0.9196, OR = 1.040, 95% CI: 0.6998–1.545). The Indian controls presented with a higher frequency of the variant allele compared to the black controls (40 vs 18.5%, respectively, p < 0.0001, OR = 2.996, 95% CI: 1.850–4.853). The variant allele was found at a higher frequency in the total Indian group compared to the total black population (41 vs 18.5%, respectively, p < 0.0001, OR = 3.057, 95% CI: 1.974–4.733) (Table 1).

SIRT1 rs7895833
The genotype distribution complied with the Hardy–Weinberg equilibrium in the CAD patients, Indian controls and black controls (chi-squared p = 0.970, p = 1.000 and p = 0.164, respectively).

No significant difference was observed in the distribution of the SIRT1 rs7895833 alleles between CAD patients and Indian controls (40.5 vs 41%, respectively, p = 0.9188, OR = 0.9629, 95% CI: 0.6457–1.436). The Indian controls presented with a higher frequency of the variant allele compared to the black controls (41 vs 22% respectively, p < 0.0001, OR = 2.513, 95% CI: 1.578–4.004). The variant allele was found at a higher frequency in the total Indian group compared to the total black population (41 vs 22%, respectively, p < 0.0001, OR = 2.466, 95% CI: 1.620–3.755) (Table 1).

Phulukdaree and co-workers reported biochemical measures of CAD patients and healthy controls in 2012.12 As expected, in our study, CAD patients presented with more conventional risk factors, such as higher body mass index (BMI), higher total and LDL cholesterol and triglyceride levels, and a higher prevalence of type 2 diabetes mellitus than the control groups. No association between the SIRT1 SNPs and biochemical measures were found in the CAD patients (Table 2), Indian controls (Table 3) and black controls (Table 4).
Table 1. SIRT1 rs1467568 and rs7895833 genotype and allele frequencies in CAD patients and controls

| SIRT1 rs1467568 Genotypes | CAD patients (n = 104) | SA Indian controls (n = 99) | Total SA Indians (n = 203) | SA black controls (n = 84) |
|---------------------------|------------------------|-----------------------------|---------------------------|---------------------------|
| Genotypes                 | n, (%)                 | n, (%)                      | n, (%)                    | n, (%)                    |
| AA                        | 40 (38.46)             | 36 (36.36)                  | 76 (37)                   | 62 (73.81)                |
| AG                        | 42 (40.38)             | 46 (46.46)                  | 84 (43)                   | 13 (15.48)                |
| GG                        | 22 (21.15)             | 17 (17.17)                  | 39 (19)                   | 9 (10.71)                 |
| SIRT1 rs7895833 Genotypes | (n = 100)              | (n = 99)                    | (n = 199)                 | (n = 82)                  |
| Genotypes                 | n, (%)                 | n, (%)                      | n, (%)                    | n, (%)                    |
| AA                        | 36 (36)                | 34 (34.34)                  | 70 (35)                   | 47 (57.32)                |
| AG                        | 47 (47)                | 48 (48.48)                  | 95 (48)                   | 34 (41.46)                |
| GG                        | 17 (17)                | 17 (17.17)                  | 34 (17)                   | 1 (1.22)                  |
| Alleles                   |                         |                             |                          |                          |
| A                         | 122 (59)               | 118 (60)                    | 240 (59)                  | 137 (81.5)                |
| G                         | 170 (41)               | 166 (41)                    | 336 (41.5)                | 17 (1.22)                 |
| Table 2. Characteristics of CAD patients according to the SIRT1 rs1467568 and SIRT1 rs7895833 genotypes

| SIRT1 rs1467568 genotype | Wild type (AA) | Variant (AG+GG) | p-value |
|--------------------------|---------------|-----------------|---------|
| BMI (kg/m²)              | 27.52 ± 0.81  | 28.57 ± 0.55    | ns      |
| Total cholesterol (mmol/l) | 5.73 ± 0.32  | 5.17 ± 0.20     | ns      |
| LDL (mmol/l)             | 3.70 ± 0.29   | 3.27 ± 0.21     | ns      |
| HDL (mmol/l)             | 0.98 ± 0.04   | 0.89 ± 0.03     | ns      |
| Triglycerides (mmol/l)   | 2.41 ± 0.28   | 2.37 ± 0.18     | ns      |
| Fasting glucose (mmol/l) | 6.48 ± 0.50   | 6.27 ± 0.33     | ns      |
| Fasting insulin (μU/ml)  | 16.97 ± 2.21  | 15.54 ± 1.12    | ns      |
| HBA1c (%)                | 6.63 ± 0.33   | 6.61 ± 0.24     | ns      |
| hsCRP (mg/l)             | 9.83 ± 2.58   | 6.97 ± 0.98     | ns      |
| IL-6 (pg/ml)             | 2.80 ± 0.90   | 2.45 ± 0.59     | ns      |
| Table 3. Characteristics of Indian controls according to the SIRT1 rs1467568 and SIRT1 rs7895833 genotypes

| SIRT1 rs1467568 genotype | Wild type (AA) | Variant (AG+GG) | p-value |
|--------------------------|---------------|-----------------|---------|
| BMI (kg/m²)              | 25.88 ± 0.93  | 26.65 ± 0.69    | ns      |
| Total cholesterol (mmol/l) | 5.32 ± 0.16  | 5.54 ± 0.13     | ns      |
| LDL (mmol/l)             | 3.47 ± 0.13   | 3.86 ± 0.12     | ns      |
| HDL (mmol/l)             | 1.04 ± 0.07   | 0.91 ± 0.03     | ns      |
| Triglycerides (mmol/l)   | 1.79 ± 0.22   | 1.92 ± 0.27     | ns      |
| Fasting glucose (mmol/l) | 5.59 ± 0.34   | 5.38 ± 0.16     | ns      |
| Fasting insulin (μU/ml)  | 15.91 ± 1.96  | 16.72 ± 1.66    | ns      |
| HBA1c (%)                | 5.78 ± 0.21   | 5.65 ± 0.11     | ns      |
| hsCRP (mg/l)             | 4.58 ± 0.60   | 7.95 ± 1.71     | ns      |
| IL-6 (pg/ml)             | 2.16 ± 0.79   | 2.86 ± 0.63     | ns      |

Discussion

Indian populations throughout the world show early-onset CAD, one to two decades earlier than other ethnic groups. South African Indians have the highest mortality rates due to CAD, while black South Africans have a very low prevalence of the disease. Increasing evidence has shown that SIRT1 is involved in CAD by regulating a number of key metabolic and physiological processes. SIRT1 serves as an anti-atherosclerotic factor by mediating endothelial nitric oxide synthase (eNOS) and improving endothelial dysfunction, regulating inflammation, reversing cholesterol transport and reducing the risk of CAD. Several SNPs have been identified in SIRT1, a candidate molecule involved in the epigenetic regulation of CAD. To date, there are only a few human genetic association studies regarding SIRT1 SNPs and CAD. Our study was the first investigation of SIRT1 rs1467568 and rs7895833 in SA Indian CAD patients. We observed that the variant alleles of both SIRT1 SNPs occurred more frequently in SA Indians compared to SA blacks. We did not observe any difference in allele frequencies between CAD patients and control groups.

Previous studies have shown that some of the SIRT1 SNPs are associated with BMI and obesity, glucose tolerance and diabetes, blood pressure, cholesterol metabolism and coronary artery calcification, all of which contribute to the CAD phenotype. We examined the possible association between rs1467568 and rs7895833 in SIRT1 and BMI, and levels of total cholesterol, LDL, HDL, triglycerides, fasting glucose, fasting insulin, HbA1c, hsCRP, or IL-6 in CAD patients and control groups, but did not observe any association.

The Rotterdam study investigated SIRT1 variation (assessed by three tagging SIRT1 SNPs: rs7895833, rs1467568 and rs497849) in relation to BMI and risk of obesity in 4573 participants, including 413 individuals with prevalent and 378 with incident type 2 diabetes mellitus (T2DM). In homozygous carriers with prevalent T2DM, the SIRT1 haplotype I had 1.9 times (95% CI: 1.1–3.2) increased risk of CVD mortality compared to non-carriers.
An intended replication study (Erasmus Rucphen family study) was carried out involving 2,347 participants. Both studies observed that the minor alleles of rs7895833 (G allele) and rs1467568 (A allele) were associated with lower BMI and a 13–18% decreased risk of obesity in two independent Dutch populations. In another study, the A allele of rs7895833 was associated with increased risk of obesity and hypertension in Japanese men.22

Recent studies investigated the association between SIRT1 SNPs (rs7895833, rs7069102, rs144124002 and rs2273773) and CAD in a Turkish population. While rs7069102, rs2273773 and rs144124002 were significantly associated with increased risk for CAD, they found no association between rs7895833 and CAD.21,22 Shimoyama et al. reported that SIRT1 rs7069102 and rs2273773 were associated with abnormal cholesterol metabolism and coronary artery calcification, respectively, in Japanese haemodialysis (HD) patients. The study also found that the A allele frequency of SIRT1 rs7895833 and G allele frequency of rs7069102 were significantly lower in HD patients compared to controls, suggesting an impact on survival.19

The allele frequencies of rs7895833 and rs1467568 show ethnic variation, and this is a possible reason for differing disease patterns among populations. The frequency of the rs7895833 A allele was relatively low (0.29) in Japanese compared to Dutch, Turkish and Caucasian subjects who had similar allele frequencies (0.80, 0.85 and 0.80, respectively).13,17,21 The A allele of rs1467568 (reported as the protective allele) showed marked difference in frequency between European (0.25) and Japanese (0.80, 0.85 and 0.80, respectively).15,17,23 The A allele of SIRT1 gene promoter in myocardial infarction. Biochim Biophys Res Commun 2012; 426(2): 232–236.

Conclusion
Both SNP variant alleles occurred more frequently in SA Indians than in SA blacks, but no difference was found between CAD patients and controls. This study is limited by sample size and a larger study may be required to fully assess the functional significance of these polymorphisms.

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Table 4. Characteristics of black controls according to the SIRT1 rs1467568 and SIRT1 rs7895833 genotype

| SIRT1 rs1467568 genotype | SIRT1 rs7895833 genotype |
|--------------------------|--------------------------|
| **Wild type (AA)**       | **Wild type (AA)**       |
| **Variant (AG+GG)**      | **Variant (AG+GG)**      |
| p-value                  | p-value                  |
|--------------------------|--------------------------|
| BMI (kg/m²)              | 25.53 ± 0.54             | 25.58 ± 0.63 |
| Total cholesterol (mmol/l)| 4.12 ± 0.12              | 4.30 ± 0.13 |
| LDL (mmol/l)             | 2.62 ± 0.10              | 2.79 ± 0.12 |
| HDL (mmol/l)             | 1.05 ± 0.045             | 1.08 ± 0.05 |
| Triglycerides (mmol/l)   | 0.99 ± 0.072             | 0.99 ± 0.06 |
| Fasting glucose (mmol/l) | 4.80 ± 0.084             | 4.87 ± 0.11 |
| Fasting insulin (µIU/ml) | 7.69 ± 0.67              | 9.21 ± 1.73 |
| HBA1c (%)                | 5.83 ± 0.062             | 5.87 ± 0.07 |
| hsCRP (mg/l)             | 6.64 ± 1.86              | 7.81 ± 2.42 |

BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, HBA1c = glycated haemoglobin, hsCRP = high-sensitivity C-reactive protein.
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**Should the findings of the TASTE and TOTAL trials change clinical practice?**

The TOTAL and TASTE trials were undertaken to evaluate whether mechanical thrombus aspiration should routinely accompany primary percutaneous coronary intervention (PCI) for STEMI.

Evaluating the evidence prior to the two trials, Dr David Kettles from East London, South Africa, observed that it’s been known for a long time that thrombus is the enemy of good cath lab outcomes. ‘In STEMI, epicardial flow does not equal reperfusion. Distal embolisation is often a problem and angioplasty and stenting can contribute to this. Fifteen per cent of patients undergoing primary PCI have visible distal emboli, and myocardial perfusion after primary PCI is the strongest predictor of mortality.’ He was speaking at AfricaPCR 2016.

Aspiration is one of multiple approaches to deal with distal embolisation of thrombus and atherosclerotic debris. Many studies have suggested that manual thrombus aspiration improves ST-segment resolution and various surrogate markers.

‘It’s pathophysiologically plausible and relatively simple. It makes PCI easier and may have short- and long-term benefits. But what about the risk of complications? While the literature tends to downplay these, there is very possibly a higher stroke risk in real-world settings. It’s possible the trials prior to TASTE and TOTAL underestimated the risks. So we needed these randomised, controlled trials powered for mortality.’

Reviewing the two trials, Dr Hellmuth Weich, from Cape Town, South Africa, observed that TASTE was a multicentre, randomised, controlled trial evaluating all-cause mortality at 30 days in patients undergoing primary or rescue PCI for STEMI, either with or without thrombus aspiration. Patients were randomised after angioplasty.

There was virtually no difference in outcomes between the two arms at 30 days (2.8 vs 3.0%), with comparable findings at one year. ‘While it was a good trial, it may have been underpowered. There was very low mortality overall and there might possibly have been a selection bias, given that patients were randomised post angioplasty.’

TOTAL was a bigger trial than TASTE, and subjects were randomised prior to angioplasty. Its primary endpoint was a composite of cardiovascular death, recurrent myocardial infarction, class IV heart failure and cardiogenic shock at six months.

Once again, there was no significant difference in outcomes between the two arms of the trial. Of concern, however, was an increase in stroke in the aspirated arm that continued up to six months after the procedure.

Dr Weich noted in conclusion that there is therefore no evidence to suggest that manual thrombus aspiration be undertaken routinely. While the trials were interesting, it should also be kept in mind that the patient populations were not the same as those in Africa.

In the discussions that followed, the feeling was that the trials don’t tell interventional cardiologists not to undertake aspiration, just not to do it routinely. Patient selection is therefore an important concern, as is technique, in order to prevent stroke. ‘Pay careful attention to the guiding catheter and start aspiration 2 cm proximal to the lesion’, said Dr Kettles. ‘Employ multiple slow-passage techniques – at least two or three passes. Withdraw the aspirate catheter under aspiration and aspirate the guide thereafter.’ Another important determinant is the size and extent of the thrombus.

Summing up the key learnings, Dr William Wijns, chairman of PCR, made the following points:

- There is no evidence of benefit of systematic mechanical aspiration during PCI based on primary or secondary efficacy measures.
- There’s a possible safety signal in the form of increased stroke rates; this is a hypothesis-generating finding.
- Operators may still choose thrombus aspiration in individual cases in order to facilitate procedural technique, bearing in mind that it will have no benefit in respect of endpoints such as mortality.
- ‘There is an open door for its selective use, for example in cases of significant thrombus burden. Unmet needs remain, and we require better tools to remove thrombus efficaciously while protecting the myocardium. We also need to bear in mind that TOTAL and TASTE are not fully relevant to Africa, where the dominant treatment of STEMI is pharmaco-invasive and most patients are late presenters.’

*Source: AfricaPCR 2016*