Ethnic differences in *CAPN10* SNP-19 in type 2 diabetes: a North-West Indian case-control study and evidence from meta-analysis

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**Summary**

Calpain 10 (*CAPN10*) variants have been associated with the genetic susceptibility to type 2 diabetes (T2D). In the present case-control study, we analysed the distribution of SNP-19 insertion/deletion (I/D) polymorphism in a total of 607 samples: 103 T2D cases and 102 healthy controls from Brahmin; 100 T2D cases and 100 healthy controls from Bania; and 100 T2D cases and 102 healthy controls from Jat Sikh ethnic groups of the North-West Indian population. Increased frequency of I allele and II genotype was found in T2D in Brahmin ethnic group \(P = 0.003, \text{OR} = 2.83 (1.43-5.61 \text{ at 95% CI})\]. Significant correlation between II genotype and body mass index (BMI) was also observed \(P = 0.003, \text{OR} = 3.31 (1.52-7.20 \text{ at 95% CI})\]. No association for the genotypes and alleles was seen in Banias and Jat Sikhs. Our data suggest that SNP-19 I/D variation in the *CAPN10* gene is modulated by ethnicity and influences the susceptibility to T2D in the North-West Indian population. We also performed a meta-analysis of relevant studies to assess the validity of this association. Data from 13 case-control studies with 15760 samples comprising 8395 T2D cases and 7365 controls were finally analysed. Significant heterogeneity between individual studies was evident in dominant and codominant models. The results of the present meta-analysis indicate an association of T2D with carriers of DD genotype of *CAPN10* I/D polymorphism. However, further analyses on a larger sample size are required to establish a conclusive association in meta-analysis.

**1. Introduction**

Type 2 diabetes (T2D) is a global public health crisis threatening all economies especially those of the developing countries (Hu, 2011). It is characterized by three major metabolic abnormalities: impaired insulin-stimulated glucose uptake in muscle and fat, alterations in glucose-stimulated insulin secretion, and increased hepatic glucose production (American Diabetes Association, 2011). Estimates from the reports of International Diabetes Federation (IDF) predicted that by the year 2030, 552 million people will be suffering from T2D worldwide, and the projected figure for India is 101.2 million (International Diabetes Federation, 2011). The threat imposed by the escalating prevalence of the disease makes it a medical catastrophe of worldwide dimensions. T2D is a classic example of multifactorial disorder; its aetiology combines both genetic and environmental factors (Herder & Roden, 2011). T2D is most often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity and certain ethnicities (Singh, 2011). Keeping in view the prevalence and aetiology of the disease, it becomes imperative to define population-specific genetic and environmental risk factors.

Calpains are a family of calcium-activated, neutral, non-lysosomal cysteine proteases. They have been proposed to be implicated in the regulation of a variety of cellular functions and may be responsible for adipocyte differentiation (Marshall *et al.*, 2005) as well as insulin-induced down-regulation of insulin receptor substrate-1, a key mediator of insulin action (Smith *et al.*, 1996). Calpain-10 is expressed in many important tissues in glucose metabolism, including...
pancreatic islets, skeletal muscle and liver, which suggested that it might affect insulin secretion, insulin action and hepatic glucose production (Horikawa et al., 2000). Genetic variants in the CAPN10 gene are associated with elevated free fatty acids and insulin resistance (Orho-Melander et al., 2002). CAPN10 is the first gene to be linked to T2D by positional cloning (Horikawa et al., 2000). Since then due to genetic heterogeneity, the role of CAPN10 has been examined in different ethnic groups with contradictory results (Cox et al., 2004). However, these results provide a tentative molecular mechanism to explain the association between variability in CAPN10 and T2D.

CAPN10 SNP-19 polymorphism is in the non-coding region and is believed to alter the risk by affecting its transcriptional regulation (Horikawa et al., 2000). SNP-19 significantly alters the insulin sensitivity index by synthesis of a mutant protein and/or altered transcriptional regulation, which could contribute to the diabetes risk (Raj & Ramteke, 2012). Given the unclear role of CAPN10 in diabetes pathogenesis, defining whether SNP-19 modifies diabetes risk in other populations is of critical importance. We focussed our investigation on the possible association between SNP-19 polymorphism in the CAPN10 gene and T2D in three ethnic groups of North-West India (Brahmin, Bania and Jat Sikhs).

2. Materials and methods

(i) Study participants

In the present study, a total of 607 samples (103 T2D cases and 102 healthy controls from the Brahmin community, 100 T2D cases and 100 healthy controls from the Bania community and 100 T2D cases and 102 healthy controls from Jat Sikhs) of North-West Indian population were analysed. Diagnosis of T2D was made according to the criteria given by the American Diabetes Association (2011). Informed consent as per Indian Council of Medical Research (ICMR) guidelines was obtained from all the studied subjects before withdrawing their blood samples and the study was approved by the institutional ethics committee. Sex and ethnicity matched individuals of 40 years of age or above without family history of diabetes were included as controls for the present study. Clinical and demographic details and anthropometric measurements like height, weight, waist circumference (WC), hip circumference (HC) were measured.

(ii) Genotyping

SNP-19 is a biallelic insertion/deletion (I/D) polymorphism consisting of two or three copies of a 32-bp repeat sequence. Genomic DNA was extracted from peripheral blood by standard phenol–chloroform method (Gill et al., 1985) with some modifications. This polymorphism was typed using primers as given by Evans et al. (2001). The amplified PCR products were analysed by electrophoresis on 2.5% agarose gels. DNA bands were visualized by staining gel with ethidium bromide and photographed under UV transilluminator. Allele 1 (two repeats) is 155 bp, and allele 2 (three repeats) is 187 bp in length.

(iii) Statistical analysis

Continuous data are represented as mean±standard deviation (SD). Two-tailed Student’s t-test was used to calculate the mean difference between the continuous variables. CaTS power calculator was used to determine the power of the study (Skol et al., 2006). Hardy–Weinberg equilibrium (HWE) was tested in all ethnic groups using the chi-square test. Allele frequency in T2D cases and controls was calculated by the gene counting method and categorical variables were compared by the chi-square test. Statistical significance threshold was adopted to be <0.05. Association analysis between cases and controls was performed for genotype frequencies, allele frequencies and different genetic models using chi-square analysis. Odds ratio (OR) at 95% confidence interval (CI) was determined for alleles and genetic models. Corrections for age, sex, WC, waist–hip ratio (WHR) and body mass index (BMI) were also performed using Logistic regression. Hazard ratio (HR) obtained from Cox regression models with their corresponding CI towards T2D susceptibility on the basis of BMI according to CAPN10 SNP-19 genotypes was done. The statistical analysis was performed using Statistical Package for Social Science program (SPSS version 16.0; SPSS Chicago, IL).

The meta-analysis was carried out by using Comprehensive Meta-Analysis software (version 2). The HWE was tested for all studies included in the analysis. The statistical significance of summary OR was determined with Z-test and P < 0.05 was considered statistically significant. We also analysed different genetic models (dominant, recessive and codominant models) for the polymorphism under study in meta-analysis. Heterogeneity among studies was examined with the Q-test and I² statistics. If there was no significant heterogeneity, the fixed-effect model (the Mantel–Haenszel method) was employed to estimate the summary OR. Otherwise, the random-effect model (the DerSimonian and Laird method) was adopted. We also assessed the publication bias using Egger’s regression asymmetry test with the significance level at 0.05 and funnel plot. Begg and Mazumdar test was performed to further assess the potential effects
Comparison of clinical parameters of cases and controls in three ethnic groups (Brahmins, Banias and Jat Sikhs) of North-West India

<table>
<thead>
<tr>
<th>Variables</th>
<th>Jat Sikhs</th>
<th>Banias</th>
<th>Brahmins</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53±10</td>
<td>56±11</td>
<td>52±11</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>91±22</td>
<td>97±12</td>
<td>96±12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WHR</td>
<td>0.9±0.07</td>
<td>0.9±0.08</td>
<td>0.9±0.08</td>
<td>0.9±0.08</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28±6</td>
<td>30±6</td>
<td>29±6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SGP (mmHg)</td>
<td>133±27</td>
<td>126±15</td>
<td>126±15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DIAB (mg/dl)</td>
<td>259±23</td>
<td>259±23</td>
<td>259±23</td>
<td>0.9±0.08</td>
</tr>
</tbody>
</table>

Data represented as mean±SD. *P-value after Bonferroni correction ≤0.05 are considered to be significant. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RBS, random blood sugar; WC, waist circumference.

No other clinical parameter was found significant in all three ethnic groups (P<0.05). However, no significant difference in genotype and allele frequencies was seen among Banias and Jat Sikhs. Analysis under dominant model (II versus ID+DD) revealed that II genotype provided approximately 3-fold risk towards T2D susceptibility [OR=2.83 (1.43–5.61 at 95% CI), P=0.003] in the Brahmin population after correction for age, sex, WC, WHR and BMI.

BMI was categorized on the basis of normal cut-off values recommended for Asian Indians as described by Snehalatha et al. (2003). Significant difference was observed in the distribution of CAPN10 SNP-19 genotype (P=0.02) and allele frequency [OR=1.88 (1.19–2.98 at 95% CI), P=0.007] distribution of polymorphism in BMI ≥ 23 in T2D cases and controls of Brahmins (Table 2). Further analysis of various genetic models in comparison between different groups revealed that under dominant model (II versus ID+DD), I allele increases the susceptibility towards T2D in individuals with higher BMI [OR=3.31 (1.52–7.20 at 95% CI), P=0.003] after correction for age, sex, WC, WHR and BMI. No significant difference was seen in Bania and Jat Sikh groups.

When BMI was examined as a continuous variable (Fig. 1), the risk towards T2D susceptibility with increase in BMI according to the genotypes was not significantly different in Bania (P=0.31) and Jat Sikh (P=0.72) groups, as a similar trend was observed for II versus ID/DD genotype model in T2D. However, in Brahmins, II genotype tends to give nearly 1.5-fold more risk as compared with ID/DD genotype for T2D susceptibility with increase in BMI [OR = 1.47 (0.98–2.21 at 95% CI), P=0.04].

Table 1. Comparison of clinical parameters of cases and controls in three ethnic groups (Brahmins, Banias and Jat Sikhs) of North-West India

3. Results

The comparison of clinical parameters among T2D cases and controls in the three ethnic groups are given in Table 1. Significant differences were observed in age (P=0.003) in Banias; age (P=0.001) and WHR (P=0.03) in Jat Sikhs after Bonferroni correction. Random blood sugar (RBS) was seen to be statistically significant in all three ethnic groups (P<0.05).
Table 2. Allele frequency and genotypic model distribution of CAPN10 SNP19 I/D polymorphism and on the basis of BMI (≥ 23.0) in T2D and controls.

<table>
<thead>
<tr>
<th></th>
<th>I-allele (%)</th>
<th>D-allele (%)</th>
<th>II versus ID+DD</th>
<th>ID versus II+DD</th>
<th>DD versus ID+II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brahmins</strong></td>
<td></td>
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<tr>
<td>T2D cases (103)</td>
<td>59.2</td>
<td>40.8</td>
<td>*P = 0.003</td>
<td></td>
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<tr>
<td>Controls (102)</td>
<td>45.6</td>
<td>54.4</td>
<td><strong>P = 0.006</strong></td>
<td><strong>OR = 2.69 (1.39–5.20)</strong></td>
<td><strong>OR = 0.69 (0.48–1.19)</strong></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>OR = 1.73 (0.87–3.43)</strong></td>
</tr>
<tr>
<td>Overweight/Obese (79)</td>
<td>61.4</td>
<td>38.6</td>
<td>*P = 0.009</td>
<td>OR = 2.63 (1.26–5.50)</td>
<td>OR = 0.73 (0.38–1.39)</td>
</tr>
<tr>
<td>Controls (71)</td>
<td>45.8</td>
<td>54.2</td>
<td><strong>P = 0.003</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>OR = 1.88 (1.19–2.98)</strong></td>
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<tr>
<td><strong>Baniyas</strong></td>
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<td></td>
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<tr>
<td>T2D cases (100)</td>
<td>51.0</td>
<td>49.0</td>
<td>*P = 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (100)</td>
<td>59.0</td>
<td>41.0</td>
<td><strong>P = 0.12</strong></td>
<td><strong>OR = 0.61 (0.34–1.10)</strong></td>
<td><strong>OR = 0.75 (0.32–1.77)</strong></td>
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<td></td>
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<td></td>
<td><strong>OR = 1.28 (0.73–2.26)</strong></td>
</tr>
<tr>
<td>Overweight/Obese (87)</td>
<td>53.4</td>
<td>46.6</td>
<td>*P = 0.91</td>
<td>OR = 1.04 (0.54–2.0)</td>
<td>OR = 1.50 (0.82–2.76)</td>
</tr>
<tr>
<td>Controls (86)</td>
<td>47.7</td>
<td>52.3</td>
<td></td>
<td><strong>P = 0.28</strong></td>
<td><strong>OR = 1.26 (0.83–1.92)</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td><strong>OR = 1.70 (0.87–3.33)</strong></td>
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<tr>
<td><strong>Jat Sikhs</strong></td>
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<tr>
<td>T2D cases (100)</td>
<td>61.0</td>
<td>39.0</td>
<td>*P = 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (102)</td>
<td>61.3</td>
<td>38.7</td>
<td><strong>P = 0.95</strong></td>
<td><strong>OR = 1.23 (0.69–2.21)</strong></td>
<td><strong>OR = 0.67 (0.39–1.17)</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td><strong>OR = 0.59 (0.24–1.44)</strong></td>
</tr>
<tr>
<td>Overweight/Obese (81)</td>
<td>58.0</td>
<td>42.0</td>
<td>*P = 0.75</td>
<td>OR = 1.11 (0.58–2.12)</td>
<td>OR = 0.66 (0.36–1.21)</td>
</tr>
<tr>
<td>Controls (87)</td>
<td>60.9</td>
<td>39.1</td>
<td><strong>P = 0.59</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>OR = 0.89 (0.57–1.37)</strong></td>
<td><strong>OR = 0.48 (0.19–1.23)</strong></td>
</tr>
</tbody>
</table>

*P < 0.05 is significant, **P Corrected for Age, Sex, BMI, WC and WHR.

(i) Meta-analysis results

Literature search identified 117 studies related to the calpain-10 gene. Data from 13 articles that investigated the association between the CAPN10 I/D polymorphic variant and T2D met the inclusion criteria and were included in the meta-analysis. Total number of cases enrolled in these studies was 15760 comprising 8395 T2D cases and 7365 controls (Online Supplementary Table S1 at http://journals.cambridge.org/ghr).

Figure 2 shows the results between CAPN10 I/D polymorphic variant and the risk of T2D. Lesser heterogeneity between studies was observed in overall analysis (P = 0.21, I² = 20.6%). Presence of I-allele in meta-analysis shows no association [fixed effect OR = 0.94 (0.89–1.01 at 95% CI), P = 0.09]. All studies are also suggesting the neutral effect of I-allele on T2D except two studies (Baroudi et al., 2009; Ezzidi et al., 2010) providing protective role (P = 0.048 and P = 0.0005, respectively) whereas the present study on the Brahmin ethnic group conferred a significant risk for T2D [OR = 1.73 (1.17–2.56 at 95% CI), P = 0.006 (Supplementary Table S2 at http://journals.cambridge.org/ghr)]. Under dominant model analysis (Fig. 3), carriers of I-allele is not suggestive of any conclusive role for T2D [random effect OR = 0.89 (0.77–1.02 at 95% CI), P = 0.10] with evidence of between study heterogeneity (P = 0.000, I² = 70.24%). Co-dominant model (Fig. 4) analysis gave a non-significant association [random effect OR = 1.04 (0.94–1.15 at 95% CI), P = 0.39; F = 41.83%, P = 0.04]. However, recessive model (Fig. 5) DD carriers provided increased risk for T2D susceptibility [fixed effect OR = 1.09 (1.01–1.2 at 95% CI), P = 0.03; F = 4.14%, P = 0.01] (Supplementary Table S3 at http://journals.cambridge.org/ghr).

To assess the publication bias, we calculated the Egger’s regression asymmetry tests which showed no evidence of publication bias in all three genetic models (dominant: P = 0.38; recessive: P = 0.81; and codominant: P = 0.26). Begg–Mazumdar test results suggested a low probability of publication bias in all genetic contrasts (dominant: P = 0.71; recessive: P = 0.90; and codominant: P = 0.65). No publication bias was seen on analysing the funnel plots for allelic contrasts (Fig. 6).

4. Discussion

CAPN10 has been established as an important candidate gene for T2D susceptibility by positional cloning and variants in the gene have been associated with an increased risk of T2D in several populations (Horikawa et al., 2000; Tsuchiya et al., 2006; Adak et al., 2010; Ezzidi et al., 2010). SNP-19 polymorphism has been reported to influence insulin sensitivity and mRNA level of CAPN10 (Nakamura et al., 1998; Elbein et al., 2002). However, reports from
Fig. 1. HR of T2D by BMI in (A) Brahmins (B) Banias and (C) Jat Sikhs stratified by CAPN10 II versus ID/DD model.

Fig. 2. Forest plot of OR estimates with corresponding 95% CI of the allele contrast (CAPN10 I versus D) and predisposition of T2D. The OR estimate of each study is marked with a symbol. The CIs are displayed as a horizontal line through the respective symbols. The horizontal axis is plotted on a log scale. ‘Baroudi 2009a and 2009b’ are Arabs and Berbers of Djerba Island, respectively; ‘Present study a, b and c’ are Brahmin, Bania and Jat Sikh groups of the present study.
Indian population give a heterogeneous picture owing to its diverse ethnicity. Therefore, we evaluated the relationship between \textit{CAPN10} SNP-19 and T2D in three ethnic groups of Punjab. To the best of our knowledge, association between \textit{CAPN10} SNP-19 polymorphism has not been reported yet in these endogamous groups of North-West India.

The distribution of ancestral I-allele of the studied polymorphism in controls varied from 0.55 to 0.69 (Supplementary Table S2 at http://journals.cambridge.org/grh). Frequency of I-allele is reported to be lesser in Brahmins (0.46) than the rest of Asians (Cassell \textit{et al.}, 2002; Adak \textit{et al.}, 2010; Bodhini \textit{et al.}, 2011). The north-western Indian region has experienced several waves of immigrations like invasions from the Middle East and Central Asia which added to the complexity of genetic diversity (Mehra, 2010). Therefore, ethnic heterogeneity seems to play an important role in the distribution of allele frequency across different population groups.

Haplotype analysis of \textit{CAPN10} variants (SNP-43, -19 and -63) have implicated the role of SNP-19 II genotype towards increased risk of T2D in Tunisians (haplotype 121/221) [OR = 2.38 (1.05–5.48 at 95\% CI), \(P = 0.02\)] and Polish populations (haplotype 121/121) [OR = 1.93 (1.03–3.54 at 95\% CI), \(P = 0.03\)] (Malecki \textit{et al.}, 2002; Kifagi \textit{et al.}, 2008). On the contrary, a study conducted on Tunisian Arab population supported the association of SNP-19 DD genotype with risk of T2D (Ezzidi \textit{et al.}, 2010). Another study on Arab and Berber populations suggested association of DD genotype with high risk of T2D in Arab sub-group only (Baroudi \textit{et al.}, 2009). Reports from South Indians (Cassell \textit{et al.}, 2002), Spanish (Saez \textit{et al.}, 2008), Asian (Song \textit{et al.}, 2007) and Swedish (Einarsdottir \textit{et al.}, 2006) populations fail to support the association of this variant with T2D, which is in agreement with the results of present study observed for Bania and Jat Sikh groups. Obesity is considered as a key risk factor that has contributed to the rising prevalence of T2D (McCarthy, 2010). Our results in the Brahmin group were in concordance with a study on Japanese population (Shima \textit{et al.}, 2003), which also associated SNP-19 II genotype with increased BMI. The control population in the Bania and the Jat Sikh groups also presented with higher BMI owing to their sedentary lifestyle, higher energy intake and reduced energy expenditure.
Thus, the present study indicates the importance of different ethnic/endogamous groups in differential roles of genetic polymorphisms to disease susceptibility under the effect of different environmental conditions.

In the present study, the consistency of genetic effects of SNP19 I/D polymorphism across populations from different ethnicities was investigated through meta-analysis. No other meta-analysis has been done individually on this polymorphic variant till now. The overall results from the meta-analysis indicated that CAPN10 I allele is not associated with T2D while analysis under dominant and codominant models showed significant heterogeneity. The studied variant has been associated with either no significant effect or a protective role towards T2D in certain subgroups (Tunisian Arabs and Scandinavians) (Rasmussen et al., 2002; Ezzidi et al., 2010); but is associated with an increased risk in the Brahmin ethnic group in the present study. The power of individual studies is usually low which affects the rate of false discovery. Therefore, meta-analysis improves the power and reduces the rate of false discovery. The present meta-analysis supported an association of T2D with carriers of DD genotype of CAPN10 I/D polymorphism. However, SNP19 is an intronic variant with comparatively lesser functional implications. Therefore, further studies screening the role of functionally relevant variants in the promoter and coding regions of CAPN10 gene in linkage disequilibrium with SNP19 are required to be undertaken on a larger sample size to establish the effect of CAPN10 polymorphisms on the aetiology of the disease.

5. Conclusions
The present investigation suggests that II genotype in CAPN10 SNP-19 locus appears to increase the risk towards T2D susceptibility in Brahmins but not in Banias and Jat Sikhs suggesting population specific risk towards T2D. Comprehensive meta-analysis indicates that there is insufficient evidence to demonstrate a strong association between CAPN10 SNP-19 polymorphism and risk of T2D. However, there seems to be a significant association between DD genotype and T2D predisposition. The exact role of the variant still remained to be an unresolved issue. Therefore, further large-scale, better designed population-based studies are needed to ascertain the impact of this variant on diabetes risk.
Fig. 5. Forest plot of OR estimates with corresponding 95% CI of the genotype model (CAPN10 DD versus II+ID) and predisposition of T2D. The OR estimate of each study is marked with a symbol. The CIs are displayed as a horizontal line through the respective symbols. The horizontal axis is plotted on a log scale. ‘Baroudi 2009a and 2009b’ are Arabs and Berbers of Djerba Island, respectively; ‘Present study a, b and c’ are Brahmin, Bania and Jat Sikh groups of the present study.

Fig. 6. Evaluation of publication bias using a funnel plot. No significant funnel asymmetry was observed which could indicate publication bias. The horizontal line in the funnel plot indicates the random effects summary estimate, while the sloping lines indicate the expected 95% CIs for a given standard error, assuming no heterogeneity between studies. Each circle represents a study.
We would like to thank the patients and controls for taking part in this study.

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Statement of Interest
None.

Ethical standards
The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary material
The online data can be found available at http://journals.cambridge.org/grh.

References


