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# Association of Calpain-10 gene polymorphisms with Type 2 diabetes mellitus: a case-control study from a tertiary care hospital in Pakistan

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## Abstract

**Introduction** Type 2 diabetes mellitus (T2DM) is a major public health challenge, with rising prevalence in low- and middle-income countries such as Pakistan. Genetic susceptibility plays a critical role in its pathogenesis. Calpain-10 (CAPN-10), a gene implicated in insulin secretion and glucose homeostasis, has been studied for its potential involvement in T2DM. This study aimed to evaluate the association of CAPN-10 polymorphisms—SNP44 (rs2975760) and SNP43 (rs3792267)—with T2DM in a Pakistani cohort.

**Methods** This case-control study included 164 T2DM patients and 164 healthy controls (mean age ± SD: 57.2 ± 8.2 vs. 53.9 ± 6.3 years; age range: 41–82 years). The male-to-female ratio was 41.4–58.6% in cases and 37.2–62.8% in controls. Participants were enrolled using non-probability convenience sampling. Genomic DNA was extracted from whole blood, and genotyping of CAPN-10 SNPs (rs3792267 and rs2975760) was performed using PCR-RFLP. Genotype distributions were assessed for Hardy-Weinberg equilibrium. Associations with T2DM were evaluated using odds ratios (ORs) and 95% confidence intervals (CIs) via logistic regression. Chi-square tests were used for categorical comparisons, with $p < 0.05$ considered statistically significant. Analyses were conducted using SPSS version 26.

**Results** For SNP44, no significant association with T2DM was observed under dominant, heterozygous, or recessive models after Bonferroni correction (adjusted $p > 0.05$). Similarly, SNP43 showed no statistically significant association with T2DM in either dominant or recessive models (adjusted $p > 0.05$), although the AA genotype appeared more frequently among T2DM cases. These findings suggest no significant role of CAPN-10 polymorphisms in T2DM susceptibility in this population.

**Conclusion** CAPN-10 polymorphisms SNP44 and SNP43 showed no significant association with T2DM in this population, suggesting limited predictive value for disease susceptibility.

**Keywords** Type 2 diabetes mellitus, CAPN-10 gene polymorphisms, Single nucleotide polymorphisms (SNPs), Genetic susceptibility, Pakistani population

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## Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounting for approximately 90% of all cases in developed countries. It primarily affects individuals aged 45 years and older. This chronic metabolic disorder is characterized by insulin resistance in peripheral tissues and impaired insulin secretion by pancreatic β-cells, resulting in disrupted glucose homeostasis [1]. To date, over 36 genes have been associated with T2DM, among which the Calpain-10 (CAPN-10) gene has emerged as a notable candidate. CAPN-10, a member of the calpain protease family located on chromosome 2q37.3, plays a pivotal role in glucose metabolism, insulin-stimulated glucose uptake, and glycogen synthesis. Given its biological relevance, CAPN-10 is considered a potential susceptibility gene for T2DM [2].

CAPN-10 is a gene associated with type 2 diabetes mellitus (T2DM) and is predominantly expressed in insulin-sensitive tissues such as adipose tissue, skeletal muscle, and the liver. Its role in T2DM is linked to its modulation of key components within the insulin signaling pathway, thereby affecting both glucose metabolism and insulin sensitivity. The broader calpain protease family, to which CAPN-10 belongs, has been implicated in the molecular pathogenesis of T2DM and represents a potential target for therapeutic intervention. The functional relevance of CAPN-10 highlights the complex interplay between genetic factors and insulin signaling, contributing to a deeper understanding of glucose homeostasis in T2DM [3, 4].

Recent genome-wide association studies (GWAS) have identified over 1,200 genetic loci associated with the pathogenesis of T2DM, underscoring its multifactorial and polygenic nature. In a landmark study, Suzuki et al. (2024) reported 1,289 single nucleotide polymorphisms (SNPs) linked to T2DM risk, highlighting the complexity of its genetic architecture [5]. Among these, polymorphisms in the CAPN-10 gene—particularly SNP44 (rs2975760) and SNP43 (rs3792267)—have been associated with altered gene expression and function in the context of T2DM. These variants contribute to inter-individual disease susceptibility and have shown associations in diverse populations, including Mexican Americans and British/Irish cohorts [6]. Differences in allelic frequencies between diabetic and non-diabetic individuals further support the genetic contribution of CAPN-10 to disease risk. Despite the high burden and increasing prevalence of T2DM in Pakistan, research on CAPN-10 polymorphisms in this population remains limited. This gap presents a critical opportunity to explore population-specific genetic associations. Investigating the frequency and potential role of CAPN-10 variants in the Pakistani population may provide novel insights into T2DM pathophysiology and support the development of targeted prevention and management strategies [7].

This study aims to address the existing research gap by investigating the prevalence of CAPN-10 gene polymorphisms—rs2975760 (SNP44) and rs3792267 (SNP43)—in Pakistani patients with T2DM. The objective is to evaluate potential associations between these variants and T2DM susceptibility, thereby contributing to the broader understanding of the disease's genetic etiology. The findings may yield novel insights into the molecular mechanisms underlying T2DM and support the development of genetically informed therapeutic strategies. Given the rising burden of T2DM in Pakistan, this research underscores the significance of CAPN-10 in glucose metabolism and insulin signaling, with implications for advancing personalized medicine in the prevention and management of T2DM.

## Materials and methods

This case-control study was reported according to Strengthening the reporting of observational studies in epidemiology (STROBE) checklist for case-control study.

### Study design and setting

This case-control study was conducted in Pathology Department, King Edward Medical University, Lahore and Advance Research Center for Biomedical Sciences (ARCBS).

### Sample size

A total of 328 participants were included in the study, comprising 164 healthy controls and 164 T2DM cases. The sample size was estimated using a 95% confidence level and a 10% absolute precision, with the expected genotype frequencies of 23% for the 2AA + GA genotypes in T2DM cases and 64% in the control group. This calculation ensured adequate statistical power to detect significant differences between the two groups. Participants were recruited through a non-probability convenient sampling method, with informed consent obtained from all individuals prior to inclusion in the study.

### Ethical approval

This study as approved by the Institutional Review Board (IRB) of King Edward Medical University, Lahore vide letter no. 482/RC/KEMU. Written informed consent was obtained from all participants prior to sample collection, and the study was conducted in accordance with the Declaration of Helsinki.

### Participants selection criteria

#### *Inclusion criteria*

Participants included in this study were adults aged 18 years or older, both male and female, who had been

previously diagnosed with Type 2 Diabetes Mellitus (T2DM) by a healthcare professional. The diagnosis of T2DM was confirmed based on the American Diabetes Association (ADA) criteria [8], which include fasting plasma glucose ≥ 126 mg/dL, 2-hour plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test, or HbA1c ≥ 6.5%. Controls were recruited from among attendants of diabetic patients at Mayo Hospital. Only individuals who were willing to participate and provided informed consent were included in the study.

#### *Exclusion criteria*

T2DM patients with any of the following conditions were excluded from the study: (1) Participants with additional metabolic conditions, such as obesity, hyperlipidemia, or metabolic syndrome, which could confound the results of the genetic analysis, were excluded; (2) Patients with chronic liver diseases, including cirrhosis, liver cancer, or viral hepatitis, were excluded to avoid potential interference in glucose metabolism and insulin signaling, which could alter the genetic associations being studied; (3) Individuals diagnosed with CHF were excluded due to the impact of cardiovascular disease on metabolic function, which could affect insulin resistance and glucose regulation; (4) Participants with autoimmune conditions such as rheumatoid arthritis, lupus, or multiple sclerosis were excluded, as these diseases can influence glucose metabolism and insulin sensitivity; (5) Patients with current or previous infections, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), or any active bacterial or viral infections, were excluded due to their potential impact on insulin resistance and inflammatory markers; (6) Any participant with a history of cancer was excluded to ensure that genetic factors linked to cancer would not interfere with the study of T2DM-specific genetic polymorphisms; (7) To avoid genetic bias, controls were screened to ensure non-relatedness to T2DM cases, based on self-report and verbal family pedigree inquiries. First- and second-degree relatives of cases were excluded.

### Data collection

Informed written consent was obtained from all participants prior to sample collection. The consent process ensured that participants were fully informed regarding the study's objectives, procedures, and any potential risks. Confidentiality of all personal and medical information was strictly maintained, and data were used exclusively for research purposes.

Diagnosed T2DM cases were recruited from the diabetic clinic at Mayo Hospital, Lahore. All cases had been previously diagnosed by a specialist physician according to the American Diabetes Association (ADA) criteria. Healthy control participants were recruited from the attendants of patients, provided they had normal HbA1c levels and no history of metabolic disorders, including T2DM. Both cases and controls underwent identical consent and sampling procedures. Peripheral blood samples were collected from all participants for subsequent genetic analysis.

### Quantitative variables

The primary outcome of the study was the presence of Type 2 Diabetes Mellitus (T2DM), confirmed through clinical diagnosis by a specialist physician based on the American Diabetes Association (ADA) diagnostic criteria, which include fasting blood glucose levels ≥ 126 mg/dL, HbA1c levels ≥ 6.5%, or a 2-hour plasma glucose level ≥ 200 mg/dL during an oral glucose tolerance test (OGTT). Secondary outcomes included the frequencies of CAPN-10 gene polymorphisms (rs2975760 and rs3792267) among T2DM cases and healthy controls. The exposures analyzed were the CAPN-10 gene polymorphisms, assessed through genotyping techniques. Predictors included the genetic variants rs2975760 and rs3792267, along with demographic factors such as age and gender. Potential confounders were identified as Body Mass Index (BMI), HbA1c levels, fasting blood glucose levels, and histories of metabolic disorders, infectious diseases, or other chronic conditions. Age and gender were also considered potential effect modifiers, as they could influence the association between CAPN-10 polymorphisms and T2DM. All variables were clearly defined to ensure standardization of data collection and analytical procedures.

### Data measurement

Genomic DNA was isolated from peripheral blood samples to genotype the CAPN-10 polymorphisms. Approximately 5.0 mL of blood was collected in EDTA-coated tubes, and DNA extraction was performed using a commercially available kit following the manufacturer's protocol. PCR amplification was then conducted using sequence-specific primers targeting the regions flanking SNP43 (rs3792267) and SNP44 (rs2975760). Amplification was carried out in a thermal cycler (T100, Bio-Rad, USA) under standardized cycling conditions: initial denaturation at 95 °C, primer annealing at 60 °C, and extension at 72 °C. The expected amplicon sizes were 245 bp for SNP43 and 166 bp for SNP44. Post-amplification, enzymatic digestion was performed to identify genotypes using restriction fragment length polymorphism (RFLP) analysis. For SNP43, the **NdeI** enzyme was used, while **BstUI** was used for SNP44. The resulting restriction fragments were indicative of specific genotypes based on known cleavage patterns. (Table 1)

Digested products were resolved using gel electrophoresis. Initial PCR products were confirmed using 2.5%

**Table 1** Primer sequences and restriction enzymes used for the analysis of CAPN-10 gene polymorphisms

| SNP | Primer Sequence 5′ → 3′ | PCR Product | Restriction Enzyme | Restriction Product (bp) |
|---|---|---|---|---|
| SNP-43 (rs3792267) G→A | **F**: gct ggc tgg tga cat cag tgc<br>**R**: acc aag tca agg ctt agc ctc acc ttc ata | 245 bp | NdeI | **GG**: 245<br>**GA**: 245, 223, 31<br>**AA**: 223, 31 |
| SNP-44 (rs2975760) T→C | **F**: gca ggg cgc tca cgc ttg ccg<br>**R**: gca tgg ccc cct ctc tga ttc | 166 bp | BstUI | **TT**: 166<br>**TC**: 166, 145, 21<br>**CC**:145, 21 |

SNP: Single Nucleotide Polymorphism; PCR: Polymerase Chain Reaction; bp: Base Pair; NdeI: A restriction enzyme used for digesting DNA at specific sites; BstUI: A restriction enzyme used for DNA fragment analysis

agarose gel electrophoresis with DNA gel stain, while post-digestion fragments were analyzed via 10% polyacrylamide gel electrophoresis (PAGE). Gel imaging was performed under UV illumination using a gel documentation system (SYNGENE Gel Doc System, UK), and genotypes were inferred based on the fragment size patterns. This standardized approach ensured the comparability of measurements across both T2DM cases and control groups. While PCR-RFLP is a well-established and cost-effective genotyping method suitable for resource-limited settings, it has lower specificity and sensitivity compared to techniques such as real-time PCR, SNP arrays, or next-generation sequencing. These limitations are acknowledged and should be considered when interpreting the results (see Fig. 1).

### Statistical analysis

Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous variables (e.g., age, BMI, HbA1c, fasting glucose) were expressed as mean ± standard deviation (SD), and categorical variables (e.g., sex, genotype) were presented as frequencies and percentages. The association between CAPN-10 gene polymorphisms—SNP44 (rs2975760) and SNP43 (rs3792267)—and T2DM was evaluated using chi-square tests under various genetic models: dominant (e.g., TC + CC vs. TT), recessive (CC vs. TC + TT), and additive. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Genotypic distributions were assessed for Hardy-Weinberg equilibrium (HWE) in the control group using the chi-square goodness-of-fit test; a p-value > 0.05 indicated conformity with HWE. Bonferroni correction was applied to account for multiple comparisons, and adjusted significance thresholds were reported accordingly. Subgroup analyses were performed to assess interactions between CAPN-10 variants and demographic or clinical variables, including age, sex, and BMI. Potential confounders were controlled

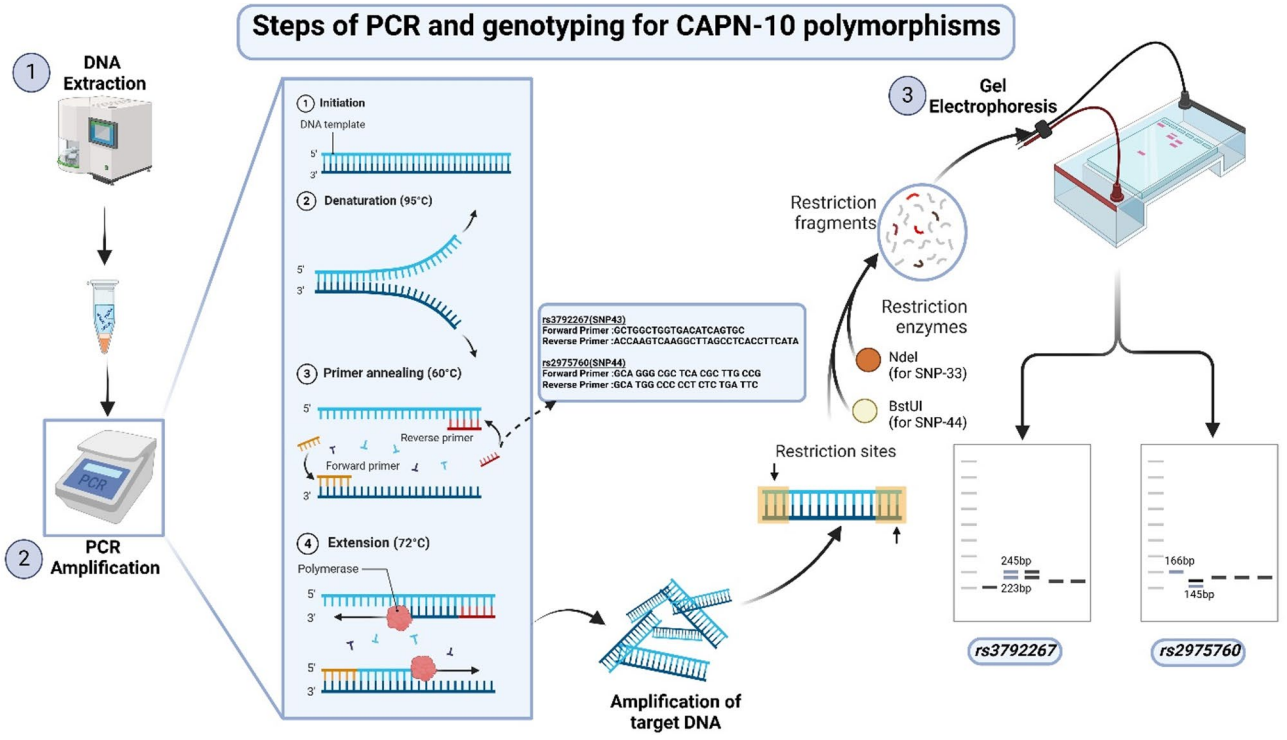


**Fig. 1** Steps of PCR and genotyping for CAPN-10 polymorphisms. Schematic representation of the four-step process used to genotype SNP43 and SNP44: (1) DNA extraction from whole blood, (2) PCR amplification of target regions, (3) enzymatic digestion with NdeI (SNP43) and BstUI (SNP44), and (4) gel electrophoresis for genotype identification based on fragment size

**Table 2** The demographic and clinical characteristics of the T2DM cases and healthy controls

| Characteristics | T2DM cases ($n = 165$) | Healthy controls ($n = 164$) | $p$ value |
|---|---|---|---|
| Age (years) (Mean ± SD) | 41–82 (57.19 ± 8.22) | 41–60 (53.93 ± 6.32) | $p = 0.0003$ |
| BMI (kg/m$^2$) (Mean ± SD) | 27.5 ± 4.1 | 25.8 ± 3.9 | $p = 0.0306$ |
| HBA1c (Mean ± SD) | 183.95 ± 51.54 | 175.06 ± 33.05 | $p < 0.0001$ |
| Fasting blood glucose level (mg/dl) (Mean ± SD) | 88.72 ± 23.1 | 82.61 ± 20.6 | $p = 0.01$ |
| Males/females (%) | 68 (41.4%) / 96 (58.6%) | 61 (37.2%) / 103 (62.8%) | $p = 0.3461$ |

T2DM: Type 2 diabetes mellitus; SD: Standard deviation; BMI: Body-mass index

**Table 3** Genotypic distribution of SNP44 (rs2975760)

| Comparison | OR | 95% CI | Unadjusted $p$-value | Bonferroni-adjusted $p$-value |
|---|---|---|---|---|
| TC + CC vs. TT | 1.07 | 0.86–1.37 | 0.752 | 1.000 |
| TC vs. TT + CC | 1.10 | 0.63–1.43 | 0.176 | 1.000 |
| CC vs. TC + TT | 1.43 | 0.70–3.03 | 0.063 | 0.379 |

**Table 4** Genotypic variations of SNP43 (rs3792267)

| Comparison | OR | 95% CI | Unadjusted $p$-value | Bonferroni-adjusted $p$-value |
|---|---|---|---|---|
| GA + AA vs. GG | 1.54 | 0.77–1.58 | 0.740 | 1.000 |
| AA vs. GG + GA | 2.05 | 1.14–3.68 | 0.403 | 1.000 |

using multivariate logistic regression models adjusting for age, gender, BMI, and relevant clinical parameters.

Missing data were addressed using multiple imputation to enhance statistical robustness. Participants with incomplete key variables were excluded from subgroup analyses but retained in primary analyses if core data were available. Cases and controls were matched on age and gender to reduce confounding bias.Sensitivity analyses were conducted by excluding outliers and re-evaluating models with and without imputed data to validate the robustness of findings. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

## Results

This study assessed the association between two key polymorphisms, **SNP44 (rs2975760)** and **SNP43 (rs3792267)**, of the **CAPN-10 gene** with **Type 2 Diabetes Mellitus (T2DM)** in a cohort of Pakistani participants. The sample included 164 T2DM patients and 164 healthy controls. The results are presented below in terms of demographic, clinical characteristics, and genotypic frequencies.

### Demographics and clinical characteristics

The mean age of T2DM cases was 57.19 ± 8.22 years, significantly higher than the controls (53.93 ± 6.32 years, $p = 0.0003$). BMI was also significantly higher in cases (27.5 ± 4.1 kg/m$^2$) compared to controls (25.8 ± 3.9 kg/m$^2$, $p = 0.0306$). T2DM cases had significantly elevated HbA1c levels (183.95 ± 51.54) compared to controls (175.06 ± 33.05, $p < 0.0001$). Similarly, fasting blood glucose levels were higher in cases (88.72 ± 23.1 mg/dL) compared to controls (82.61 ± 20.6 mg/dL, $p = 0.01$). Gender distribution did not differ significantly between groups, with males comprising 41.4% of cases and 37.2% of controls ($p = 0.3461$). (Table 2)

### Genotypic distribution SNP44 (rs2975760)

The association between SNP44 (rs2975760) and T2DM was evaluated under multiple genetic models, including dominant (TC + CC vs. TT), heterozygous (TC vs. TT + CC), and recessive (CC vs. TC + TT) contrasts. Genotype distributions among controls were in Hardy-Weinberg equilibrium ($p = 0.216$), indicating appropriate population structure for analysis. The odds ratio (OR) for the dominant model (TC + CC vs. TT) was 1.07 (95% CI: 0.86–1.37), with an unadjusted p-value of 0.752 and a Bonferroni-adjusted p-value of 1.000. For the heterozygous model (TC vs. TT + CC), the OR was 1.10 (95% CI: 0.63–1.43), with an adjusted p-value of 1.000. Under the recessive model (CC vs. TC + TT), the OR was 1.43 (95% CI: 0.70–3.03), with an unadjusted p-value of 0.063 and a Bonferroni-adjusted p-value of 0.379. Although some comparisons indicated elevated odds ratios, none of the associations were statistically significant following correction for multiple testing. These findings suggest that SNP44 (rs2975760) is not significantly associated with T2DM susceptibility in this population. (Table 3)

### Genotypic variations of SNP43 (rs3792267)

For SNP43 (rs3792267), genotype distributions among controls also conformed to Hardy-Weinberg equilibrium ($p = 0.174$). Association analyses were conducted using dominant (GA + AA vs. GG) and recessive (AA vs. GA + GG) models. Under the dominant model, the OR for T2DM was 1.54 (95% CI: 0.77–1.58), with an unadjusted p-value of 0.740 and a Bonferroni-adjusted p-value of 1.000. The recessive model yielded an OR of 2.05 (95% CI: 1.14–3.68), with an unadjusted p-value of 0.403 and an adjusted p-value of 1.000. (Table 4) Despite the elevated point estimate for the AA genotype in the recessive model, no statistically significant associations were observed after correction for multiple comparisons. Thus, these results do not support a significant role of SNP43 (rs3792267) in T2DM predisposition within this

cohort. The complete genotypic and allelic distributions for both SNPs are presented in Table 5.

## Discussion

This study examined the association between SNP44 (rs2975760) and SNP43 (rs3792267) polymorphisms of the *CAPN-10* gene and type 2 diabetes mellitus (T2DM) in a Pakistani population. Significant differences in age, BMI, HbA1c, and fasting blood glucose levels between T2DM cases and healthy controls were observed, consistent with established metabolic markers of the disease and supporting the validity of the sample. Gender distribution did not differ significantly between groups, indicating a balanced representation of male and female participants. Although the TC and CC genotypes of SNP44, and the GA and AA genotypes of SNP43, were more frequent among T2DM cases, no statistically significant associations were found following Bonferroni correction. These findings suggest that, in this population, SNP44 and SNP43 are not independently associated with increased T2DM risk, despite prior reports of CAPN-10 involvement in glucose metabolism and insulin signaling [9].

These findings underscore the importance of population-specific genetic research. Although *CAPN-10* polymorphisms have been linked to T2DM in certain populations, the current study suggests that SNP44 and SNP43 may not significantly contribute to T2DM susceptibility in the Pakistani population [10]. It is plausible that gene–environment interactions—such as region-specific lifestyle, dietary patterns, and environmental exposures—modulate the phenotypic expression of these variants, thereby diminishing their detectable effect in this cohort.

Previous studies investigating the association of *CAPN-10* SNPs with T2DM across different populations have reported inconsistent results, reflecting the complex nature of genetic predisposition to the disease. While associations between SNP44 and SNP43 and T2DM have been observed in Hispanic and select Asian populations, studies in European cohorts have failed to demonstrate significant associations [11].

**Table 5** Genotypic distribution and allelic frequencies (Final sample: 164 T2DM cases, 164 healthy controls)

| Genotype | T2DM Cases | (%) | Controls | (%) |
|---|---|---|---|---|
| **SNP44 (rs2975760)** | | | | |
| TT | 48 | 29.3 | 59 | 36.0 |
| TC | 82 | 50.0 | 66 | 40.2 |
| CC | 34 | 20.7 | 39 | 23.8 |
| **SNP43 (rs3792267)** | | | | |
| GG | 32 | 19.5 | 49 | 29.9 |
| GA | 81 | 49.4 | 85 | 51.8 |
| AA | 51 | 31.1 | 30 | 18.3 |

Early studies conducted in European populations provided initial evidence that certain *CAPN-10* polymorphisms may influence susceptibility to T2DM. For example, the T allele of SNP44 (rs2975760) was associated with an increased risk of T2DM in cohorts of Spanish and Finnish ancestry. However, subsequent investigations in other European populations produced inconsistent findings, with several studies failing to replicate this association [12]. These discrepancies highlight the complexity of genetic contributions to T2DM and underscore the need for further investigation across diverse ethnic groups [10].

Beyond Europe, *CAPN-10* polymorphisms have also been examined in Asian populations. While some studies have reported associations between SNP44 and increased T2DM risk in Chinese, Japanese, and Korean cohorts, others have not found significant associations, reflecting inter-population variability. Similar inconsistency has been observed in studies of SNP43 (rs3792267), where associations with T2DM risk have been identified in certain Asian subgroups, but not in others [12]. These mixed results reinforce the importance of conducting population-specific research to better understand the genetic architecture of T2DM across different ethnic backgrounds.

Research in Hispanic/Latino populations has also yielded inconsistent results regarding the role of *CAPN-10* polymorphisms in T2DM. While some studies have reported associations between SNP44 and T2DM risk in Mexican American and Puerto Rican populations, others have found no significant correlations. Similarly, investigations of SNP43 in these cohorts have demonstrated both positive and negative findings [13, 14] Studies conducted in African and Middle Eastern populations have been limited and inconclusive. While some African cohorts have shown significant associations between *CAPN-10* variants and T2DM, other studies have failed to replicate these findings [15].

Variation in allelic frequencies and genotype–phenotype associations of *CAPN-10* polymorphisms across populations is particularly noteworthy, as it may provide insight into population-specific genetic risk profiles for T2DM. Allelic frequencies represent the distribution of genetic variants within a population and are shaped by evolutionary, demographic, and genetic factors [16]. Differences in these frequencies may reflect historical patterns of migration and admixture. Furthermore, genotype associations can identify specific alleles that may confer increased or decreased risk of T2DM [17].

The identification of genetic variants associated with T2DM susceptibility offers significant potential for the advancement of personalized medicine. Incorporating genetic markers into clinical risk stratification could enable earlier identification of high-risk individuals and

support targeted preventive strategies. Moreover, pharmacogenomic insights derived from genotype data may inform more precise therapeutic decisions, optimizing treatment efficacy and minimizing adverse effects [18, 19].

From a public health perspective, characterizing the distribution and impact of *CAPN-10* variants in diverse populations is critical to reducing the global burden of T2DM. Such knowledge can inform the development of population-specific screening and prevention programs, particularly in high-risk regions [20]. The integration of genetic data into clinical care may enhance early diagnosis, improve disease management, and ultimately contribute to better health outcomes at the population level [21].

Despite notable progress, significant gaps remain in the genetic understanding of T2DM. Genetic heterogeneity across populations and limited knowledge of gene–environment interactions hinder the identification of universally applicable markers and individualized prevention strategies [22]. While research has largely focused on common variants with modest effects, rare variants with potentially greater impact remain underexplored due to technical and financial constraints [10, 23, 24].

The *CAPN10* gene encodes calpain-10, a calcium-dependent cysteine protease involved in various cellular processes, including insulin secretion and action. Polymorphisms such as SNP-43 (rs3792267) and SNP-19 (rs3842570) have been studied for their potential functional impact. For instance, SNP-19, a 32-base pair insertion/deletion in intron 6, may influence gene expression or alternative splicing, thereby affecting insulin sensitivity [25]. Associations between these polymorphisms and T2DM have been reported in diverse populations. In a Bangladeshi cohort, the 2R/3R genotype of SNP-19 was significantly associated with increased T2DM risk [25]. Similarly, in a Tunisian Arab population, the 2R allele of SNP-19 and the 111 haplotypes (comprising SNP-43, SNP-19, and SNP-63) were linked to higher T2DM susceptibility [26]. However, these associations have not been consistently replicated across all ethnic groups, reflecting the influence of genetic heterogeneity and underscoring the importance of population-specific research to elucidate the role of *CAPN10* polymorphisms in T2DM pathogenesis [27].

*CAPN-10* has emerged as a biologically plausible candidate gene, particularly in Mexican American populations where certain polymorphisms have been linked to T2DM risk [28] However, data on *CAPN-10* variants in the Pakistani population are scarce, despite the country's escalating T2DM burden [29]. Addressing this gap may enhance our understanding of T2DM pathogenesis and support tailored interventions. Moreover, such research has global implications, contributing to the broader effort to map genetic diversity and advance precision medicine in diabetes care [24, 30, 31]. Population-specific differences in the association between *CAPN10* polymorphisms and T2DM have been observed globally, with varying results reported in Hispanic, Asian, African, and European cohorts (Table 6).

**Table 6** Summary of *CAPN10* SNP associations with type 2 diabetes mellitus (T2DM) across populations

| Population | SNPs Studied | Association with T2DM |
|---|---|---|
| Pakistani (current study) | SNP44, SNP43 | No significant association [9] |
| Mexican American | SNP44, SNP43 | Mixed results (positive in some studies) [13] |
| Puerto Rican | SNP44, SNP43 | Mixed results [14] |
| Spanish | SNP44 | Positive (initial studies) [12] |
| Finnish | SNP44 | Positive (initial studies) [12] |
| Chinese | SNP44 | Mixed results [12] |
| Japanese | SNP44 | Mixed results [12] |
| Korean | SNP44 | Mixed results [12] |
| European (general) | SNP44, SNP43 | No consistent association [12] |
| Bangladeshi | SNP19 | Positive (2R/3R genotype) [25] |
| Tunisian Arab | SNP19, SNP43, SNP63 | Positive (2R allele, 111 haplotype) [26] |
| African | CAPN10 (varied) | Mixed/limited evidence [15] |
| Middle Eastern | CAPN10 (varied) | Limited/inconclusive [15] |

### Study limitations

This study has several limitations. First, the sample size of 328 participants may have limited the statistical power to detect modest genetic associations, potentially contributing to the non-significant findings. Second, the study was conducted in a single Pakistani population, which may limit the generalizability of results to other ethnic groups. Third, key environmental and lifestyle factors—such as diet, physical activity, socioeconomic status, and degree of urbanization—were not assessed, despite their known influence on T2DM risk. Another limitation is the absence of a formal power analysis at the design stage. While the sample size of 328 participants offers a reasonable basis for preliminary analysis, it may be underpowered to detect small to moderate genetic effects typically observed in complex diseases such as T2DM. Lastly, the analysis was restricted to two polymorphisms (SNP44 and SNP43) within the *CAPN-10* gene, excluding other potentially relevant genetic variants. Future research should incorporate larger, multi-ethnic cohorts and a broader panel of genetic markers to comprehensively elucidate the genetic architecture of T2DM.

## Conclusion

No statistically significant association was observed between *CAPN-10* SNP44 or SNP43 polymorphisms and T2DM in this Pakistani cohort. While minor genotype distribution trends were noted, these findings were not supported after correction for multiple testing. Future

research incorporating larger, multi-ethnic samples and genome-wide analyses is warranted to elucidate the genetic basis of T2DM more comprehensively.

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**Author contributions**
H.F: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigations, Data curation, Visualization, Supervision, Writing - Original Draft, Writing - Review & Editing; M.N.S: Methodology, Software, Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; N.C.: Methodology, Software, Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; S.Q: Methodology, Software, Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; R.D: Software, Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; S.K: Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; H.J: Validation, Formal analysis, Investigations, Data curation, Visualization, Writing - Original Draft, Writing - Review & Editing; A.J.G: Writing - Original Draft, Writing - Review & Editing; U.A.N: Writing - Original Draft, Writing - Review & Editing.

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**Data availability**
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. Data sharing complies with institutional and ethical guidelines to ensure participant confidentiality.

## Declarations

**Ethics approval and consent to participate**
This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of King Edward Medical University, Lahore (Approval No. 482/RC/KEMU). Written informed consent was obtained from all participants prior to enrollment. Participants were informed of the study's objectives, procedures, potential risks, and their right to withdraw at any time without consequences.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Use of generative AI and AI-assisted technologies**
ChatGPT-4o (OpenAI) was used solely for improving the grammar, readability, and clarity of the manuscript. No AI tools were used for data analysis, interpretation, or generation of scientific content. All intellectual content and scientific conclusions were formulated by the authors.

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