Association of ACE I/D gene polymorphism and related risk factors in impaired fasting glucose and type 2 diabetes: a study among two tribal populations of North-East India

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Abstract

Background Type 2 diabetes is a serious public health concern in India, even the indigenous tribal populations are not left unaffected. The present study aims to understand the association of major risk factors i.e. obesity, hypertension, dyslipidemia, ACE I/D polymorphism with impaired fasting glucose (IFG) and type 2 diabetes (T2D) among two different Mendelian populations of North East India.

Methods Demographic, somatometric, physiological variables along with fasting blood samples were collected from 609 individuals. The participants were screened for ACE I/D polymorphism.

Results ACE I/D polymorphism was found to follow HWE among Liangmai tribe but not among Mizo tribe. Distribution of DD genotype/D allele was found to be significantly higher for T2D among Mizo tribe. Significant association were observed between DD genotype/D allele of ACE I/D polymorphism and TC as well as LDL with both IFG and T2D only in Mizo tribe.

Conclusions The present study is an example of gene-environment interaction where DD genotype or D allele and dyslipidemia (high TC and high LDL) are posing risk for IFG and T2D both independently and in combination only among Mizo tribe with relatively less physical activity attributed to their residence in less hilly terrain however Liangmai tribe which resides in high hilly terrain shows no such association.

Keywords Impaired fasting glucose · Type 2 diabetes · ACE I/D polymorphism · Dyslipidemia · Gene-environment interaction · Tribal population

Introduction

Type 2 diabetes is a leading serious public health complication, affecting both developed and developing countries all around the world [1–3]. Type 2 diabetes was commonly regarded as a disease of affluent societies previously, but now it has encroached the rural and urban societies [4], irrespective of their ethnicity, class and socio-economic condition. Even the indigenous tribal populations of India which are undergoing epidemiological transition are affected by diabetes with prevalence ranging up to 10% [5, 6].

Diabetes increases the risk for cardiovascular and metabolic diseases thereby reducing the quality of life and increasing the risk for premature mortality [7]. The increasing prevalence of T2D is attributed by ever increasing prevalence of obesity, hypertension and physical inactivity. Studies have also shown the association of T2D with dyslipidaemia [8]. T2D is multifactorial in origin where both environmental and genetic factors contribute to its pathogenesis [9]. The gene encoding angiotensin converting enzyme has been listed as one of the widely evaluated candidate gene in association with T2D. Angiotensin converting enzyme (ACE) is a key component of renin-angiotensin system (RAS) by generating vasoconstrictor angiotensin II and degrading kinins [10]. High levels of angiotensin II have been suggested to play an important role in glucose and insulin regulation which may increase the risk of insulin resistance and diabetes [11]. ACE located on chromosome 17q23, has many polymorphisms, of which 287–base
pair Alu insertion/deletion (I/D) polymorphism in intron 16 results in three genotypes—insertion homozygote I/I, insertion/deletion heterozygote I/D, D/D deletion homozygote. [12]. The DD genotype is associated with higher levels of circulating ACE than ID and II genotype. In previous reports, D allele of ACE I/D polymorphism is found to be associated with many cardiovascular diseases [13, 14], such as Type 2 diabetes [15, 16] metabolic syndrome [17]; hypertension [18, 19]; central obesity [20] and hypertriglyceridemia [21]. The present study attempts to understand the association of major risk factors i.e. obesity, hypertension, dyslipidemia, ACE I/D polymorphism with impaired fasting glucose (IFG) and Type 2 diabetes (T2D) among two different Mendelian populations, with different gene pools. The two selected tribal populations have been reported to have different ancestral origins [22, 23], different lifestyles and inhabit different ecological niche. The Liangmai Naga tribe inhabit the villages of high hilly terrain of Tamenglong and Senapati districts while Mizo tribe inhabit the low-lying hilly terrain villages of Churachandpur districts of Manipur. The two selected study populations are reported to be different with respect to the distribution of obesity where obesity among Mizo tribe was higher as compared to that of the Liangmai population [24]. The prevalence of IFG and T2D were also reported to be significantly higher among Mizo tribe as compared to Liangmai tribe [25].

Methodology

Study design and data collection

The present study is a cross-sectional population-based study. It was conducted among two tribal populations of North-East India namely the Liangmai Naga and Mizo. Recruitment of the study participants were done from districts where the selected tribal populations predominantly reside. The Liangmai Naga tribe is supposed to be the original inhabitants of Manipur and they reside in Tamenglong and Senapati districts at an altitude between 1060 and 1788 m above sea levels [23]. As per the Mizo tribe, samples were collected from Churachandpur district of Manipur where the tribe reside after migration from the neighbouring state of Mizoram [22]. Mizo tribe people migrated from Mizoram and settle in Manipur have categorised into few sub groups based on their dialects. Surprisingly there is no mating restriction between these sub-groups. However, the samples collected in the present study is from a single sub-group called Lushai. Hence, the mendelian nature of the population is not affected. Churachandpur district resides at a relatively low altitude (914 m) above sea level. In short, for the present study, samples were collected from different altitudes which is also the natural habitation of the studied population.

A total of 609 individuals (399 Liangmai and 270 Mizo) of both sexes in the age group of 18 to 60 years unrelated up-to first cousin were recruited randomly from the two-study populations. The recruitment of the participants was done through household survey. Pre-tested interview schedule and standard techniques were employed for collecting data pertaining to demographic (name, age, sex, place of birth, education and occupation), somatometric (height, weight, waist circumference, hip circumference) and physiological variables (blood pressure) from each recruited participant. The study was approved by The Ethical committee, Department of Anthropology, University of Delhi.

Height (cm) and weight (kg) were taken from all the participants with lightweight clothing and without shoes, using Anthropometer rod and weighing machine respectively. Body Mass Index (BMI) was calculated using the formula -weight in kilogram divided by height in meter square (kg/m²). On the basis of the BMI value, participant subjects were classified as underweight (<18.5 kg/m²), normal weight (18.5–22.99 kg/m²), overweight (23–25 kg/m²) and obese (>25 kg/m²) according to Asia Pacific population criteria [26]. Waist circumference (cm) was measured at the least circumference between the lower ribs and the iliac crest. Waist circumference <90 cm for males and <80 cm for female was taken as normal [27]. Hip circumference was measured as the buttock yielding the maximum circumference. Waist-hip ratio was calculated using the formula waist (cm) divided by hip (cm) i.e. (W/H) [28]. The circumference was measured using a non-expandable steel tape. Normal WHR calculated, as was classified as <0.90 (males), <0.80 (females) [27]. Blood pressure was measured by mercury sphygmomanometer [29] on each participant thrice in an interval of not less than 5 min, and the average of the three readings was taken as final. Categorization of blood pressure has been done by AHA/ACC 2017 guidelines [30].

Intravenous blood samples (5 mL from each individual) after overnight fasting were collected from the participant subjects by trained technician and are stored at 2–8 °C in ice box and transported to the molecular laboratory, Manipur University. Aliquots of plasma were separated for glucose analysis within 6 h after the blood collection. Plasma glucose levels were measured by spectrophotometry using commercially available kits (Randox, USA). Classification of glycemic status of the recruited subjects was done by following World Health Organization standard [31]. A participant is classified as having T2D if the individual has fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL) or 2-h OGTT ≥11.1 mmol/L (200 mg/dL), impaired fasting glucose (IFG) <126– ≥110 mg/dL or 2-h OGTT ≥140–200 mg/dL and normal glucose level <110 mg/dL or 2-h OGTT, 140 mg/dL. The value of FPG was used for the classification.
of the recruited subjects to normal, impaired fasting glucose and type 2 diabetes in the present study. NCEP ATP III guidelines were used for lipids [8]. In the present study, ACE I/D polymorphism was successfully genotyped for 609 individuals (339 Liangmai; 270 Mizo).

**PCR and genotyping of ACE I/D polymorphism**

Extraction of DNA was done by using salting-out method [32]. Genotyping of ACE I/D gene polymorphism was carried out using Forward primer sequence: 5′ CTG GAG ACC ACT CCC ATC CTT TCT 3′ and reverse primer sequence: 5′ GAT GTG GCC ATC ACA TCC GTC AGAT 3′ [33]. The PCR was carried out using the Thermocycler (C-1000 Touch™, Bio-Rad, USA). Genotyping of PCR products [in base pair(bp), DD (190 bp); ID (490 bp,190 bp) and II (490 bp)].

**Statistical analysis**

Hardy–Weinberg Equilibrium (HWE) was calculated to determine the variation in the distribution of alleles and genotypes within the population. Chi square test was used to see the differences in the distribution of the genotype and the categorical variables. Logistic regression analysis was used to determine risk after adjustment with confounders. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, for Windows version 20.0). Statistical significance was taken at p value ≤ 0.05 for all the statistical tests.

**Results**

In the present study, the two populations were found to be significantly differing with respect to the distribution of ACE I/D rs4646994 polymorphism where individuals with DD genotype were found to be significantly higher among the Mizo tribe. ACE I/D rs4646994 polymorphism follows HWE among the Liangmai tribe, but not in Mizo tribe. However, D allele frequency is higher among the Liangmai tribe i.e., D-0.52 as compared to that of the Mizo tribe i.e., D-0.4. The distribution of ACE I/D rs4646994 polymorphism as per fasting blood glucose levels i.e. normoglycemia, impaired fasting glucose (IFG) and type 2 diabetes (T2D) indicate no significant differences between the case and control groups in Liangmai tribe. Whereas in DD carrying individuals among Mizo tribe are found to be higher in percentage among the T2D group as compared to their respective controls with a suggestive p-value (Table 1).

Odds ratio analysis after controlling for age reveals more than two-fold significant increased risk of DD genotype against II genotype (co-dominant model) [OR 2.10, 95% CI (1.10–4.39)] and I allele (II + ID—recessive model [OR 2.18 (1.16–4.09)] for T2D only among Mizo tribe and not among Liangmai tribe (Table 2).

Distribution of individuals with abnormal lipids which includes total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) among cases (i.e. IFG and T2D) and their respective controls (normoglycemia) shows that individuals with high total cholesterol (TC) and high low-density lipoprotein (LDL) are significantly higher among both the cases i.e. IFG and T2D as compared to that of their respective controls only among Mizo tribe. Whereas no such differences were observed between cases and controls with respect to the distribution of dyslipidemia among Liangmai tribe (Fig. 1a, b). No differences were observed in the distribution of obesity among the cases and their respective controls in both the population. Odds ratio analysis of obesity, hypertension, and lipids variable also reveal more than two-fold significant increased risk for TC for both IFG (OR 2.22, 95% CI 1.14–4.32) and T2D (OR 2.53; 95% CI 1.51–4.23) in Mizo tribe. LDL also posed two-fold significant increased risk for IFG (OR 2.10; 95% CI 1.10–3.91) and one-fold increased risk for T2D (OR 1.04; 95% CI 1.02–1.06) in Mizo tribe (Fig. 2b, d). Odds ratio analysis between lipids and ACE I/D polymorphism showed no association between the two in both Liangmai and Mizo tribe (Supplementary Table 1).

Gene-environment interactions analysis (Table 3) shows more than twofold significant increased risk for both IFG and Type 2 Diabetes when there is a combination of D allele with high TC and high LDL only among the Mizo tribe and not among the Liangmai tribe.

**Discussion**

As reported in one of our previously published paper on the same cohort, percentage of individuals with higher age was significantly higher among the Type 2 diabetes group as compared to their respective controls among both Liangmai and Mizo groups. However, no such difference was observed between the IFG cases and their respective controls in both the studied population groups. Studies associating ACE ID polymorphism with either IFG and T2D show variable results, where some show positive association [10–12, 14–16, 34–36] while some have also reported no association [37–39] as well as some negative association [40–42]. These discrepancies in the association are usually attributed to the limitation of differing ethnicities and study designs [43]. To overcome such limitation, in the present study, both cases and controls are derived from two different Mendelian populations and the association analysis was done in both
the population groups separately. The two studied Mendelian populations not only have different gene pools because of relatively restricted mating rules within the population, they also inhabit different ecological niche i.e., Liangmai tribe inhabit relatively higher hilly terrain and Mizo tribe inhabit a lower altitude hilly terrain. Differences in the genetic and environmental background must have led to the differential distribution of complex phenotype like obesity, hypertension, IFG, T2D and dyslipidemia in the two selected population groups [44].

Further, in the present study, significant differences were observed between the two tribal populations w.r.t the distribution of ACE I/D polymorphism where DD genotype was significantly higher among Mizo population. Further, when the association was observed between IFG, T2D, dyslipidemia and ACE I/D polymorphism, there seems to be variability in the association. In the present study, DD genotype is found to be significantly associated with T2D among Mizo tribe. The present result, among Mizo tribe is in concordance with studies conducted by Vishwanathan et al. 2001; Bhavani et al. 2006 in South India and Raza et al. 2013 in North India [15, 45] and also with studies conducted among Japanese, Saudi Arabian, Egyptian population, [14, 46, 47] where positive association between DD genotype and T2D were reported. A large meta-analysis of 24 reports by Zhou et al. validated the association of D allele with Type 2 diabetes in Caucasian and East Asian populations [47] while no association between DD genotype and Type 2 diabetes among Liangmai tribe is in concordance.

### Table 1: Distribution of ACE I/D rs4646994 polymorphism with impaired blood glucose (IFG) and Type 2 diabetes among Liangmai and Mizo population of Manipur

| Geno-type | Liangmai | Mizo |
|-----------|---------|------|
| II N (%)  | 72 (21.20) | 66 (21.7) |
| ID N (%)  | 179 (52.8) | 157 (51.6) |
| DD N (%)  | 88 (26.0) | 81 (26.6) |

| χ² (p-value) between Normoglycemia and IFG | χ² (p-value) between Normoglycemia and T2D | χ² (p-value) between the distribution of ACE I/D polymorphism between Liangmai and Mizo tribe |
|-------------------------------------------|-------------------------------------------|-------------------------------------------|
|                                       |                                           |                                           |

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| χ² (p-value) between Normoglycemia and IFG | χ² (p-value) between Normoglycemia and T2D |
|-------------------------------------------|-------------------------------------------|
|                                       |                                           |

### Table 2: Odds ratio (OR) analysis of ACE I/D rs4646994 polymorphism with IFG and type 2 diabetes

| Model | Liangmai | Mizo |
|-------|---------|------|
|       | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Dominant (II/ID + DD) |
| N vs IFG  | 2.21 (0.49–9.89) | 0.29 | 1.08 (0.48–2.45) | 0.83 |
| N vs T2D | 0.94 (0.29–3.02) | 0.91 | 1.43 (0.82–2.49) | 0.20 |
| Co-dominant (II/DD) |
| N vs IFG  | 1.63 (0.28–9.17) | 0.58 | 1.02 (0.33–3.15) | 0.97 |
| N vs T2D | 0.65 (0.14–3.04) | 0.58 | 2.10 (1.10–4.39) | 0.02* |
| Co-dominant (II/ID) |
| N vs IFG  | 2.52 (0.54–11.58) | 1.2 | 0.46–2.75 | 0.79 |
| N vs T2D | 1.07 (0.32–3.58) | 0.90 | 1.04 (0.55–1.97) | 0.89 |
| Recessive (II + ID/DD) |
| N vs IFG  | 0.78 (0.25–2.45) | 0.68 | 0.96 (0.33–2.75) | 0.95 |
| N vs T2D | 0.64 (0.17–2.32) | 0.48 | 2.18 (1.16–4.09) | 0.01* |

*a*Age adjusted

*p*-Value < 0.05 significant
with studies conducted by Chmaisse et al. among Lebanese population and Pirozzi et al. among Brazilian Type 2 patients [48, 49]. Reporting the association between IFG/T2D and dyslipidemia condition, only high TC and high LDL were found to pose a significant risk for IFG and T2D only among Mizo tribe and not among Liangmai tribe. However, ACE I/D polymorphism is not found to be associated with any of the considered lipid variables in both the Liangmai and Mizo tribes. As per previous reports individuals with obesity, hypertension, and abnormal lipid variables, are significantly higher among Mizo tribe making this tribe prone to cardiovascular diseases [26]. Further DD genotype which is also found to be associated with T2D is found to be more frequent in Mizo tribe, which indicates that major contributors of T2D in Mizo tribe could possibly be through ACE I/D polymorphism in light of other risk factors. However, obesity is not found to be associated with IFG and T2D, hypertension is not found to be associated with IFG.

**Table 3**: Gene (ACE I/D)-environment (dyslipidemia and hypertension) interactions in IFG and T2D

| Variables | Liangmai | Mizo |
|-----------|----------|------|
|           | N vs IFG | N vs T2D | N vs IFG | N vs T2D |
| ACE I/D   | OR (95% CI) | p value | OR (95% CI) | p value |
| II vs ID+DD | 1.72 (0.64–4.5) 0.2 | 1.35 (0.46–3.82) 0.52 | 2.51 (1.10–5.68) < 0.005* | 2.56 (1.45–4.53) < 0.005* |
| TG        | 0.99 (0.34–2.86) 0.9 | 2.29 (0.85–6.13) 0.09 | 1.22 (0.49–2.99) 0.66 | 1.01 (0.99–1.02) 0.06 |
| HDL       | 1.19 (0.45–3.10) 0.71 | 0.81 (0.29–2.25) 0.69 | 0.55 (0.15–1.97) 0.36 | 0.77 (0.31–1.61) 0.49 |
| LDL       | 1.38 (0.47–4.03) 0.5 | 0.77 (0.21–2.76) 0.69 | 2.36 (1.03–5.44) 0.04* | 2.48 (1.41–4.37) < 0.005* |
| VLDL      | 0.91 (0.34–2.86) 0.98 | 2.29 (0.85–6.13) 0.09 | 1.22 (0.49–2.99) 0.66 | 1.36 (0.76–2.46) 0.29 |
| HTN       | 1.06 (0.43–2.76) 0.89 | 1.22 (0.43–3.16) 0.81 | 1.07 (0.46–2.47) 0.86 | 1.3 (0.73–2.28) 0.36 |

Data represented as TC-total cholesterol, TG-triglyceride, HDL-high-density lipoprotein, LDL-low-density lipoprotein, VLDL—very low-density lipoprotein, HTN-hypertension

*Age adjusted

*p-value < 0.05 significant
and T2D whereas dyslipidemia only in terms of high TC and high LDL are found to be associated with IFG and T2D.

D allele in combination with high TC as well as in combination with high LDL was found to pose more than two-fold significant increased risk for IFG and T2D only in Mizo tribe. As the frequency of abnormal TC and abnormal LDL is very high in this population [44] with a very high frequency of DD genotype as 43.8%, one can consider Mizo tribe to be at risk for IFG and T2D.

Further Liangmai Tribe inhabiting hilly terrain is expected to have high physical activity and so the effect of DD genotype in the causation of IFG and Type 2 diabetes is nullified which is not the same in case of Mizo tribe. The frequency of abnormal TC and dyslipidemia (high TC and high LDL) are posing risk for IFG and T2D both independently and in combination only among Mizo tribe with relatively less physical activity attributed to their residence in less hilly terrain. But no such association is found among the Laingmai tribe which resides in high hilly terrain.

The limitation of the present study includes smaller sample size and restriction to one genetic marker. Studies on tribal populations, with a larger sample size and other candidate gene associated with IFG and Type 2 diabetes are required to validate the results of the present study.

**Conclusion**

The present study shows a differential association of the major risk factors—obesity, hypertension, dyslipidemia, ACE I/D polymorphism with IFG and T2D among the two studied populations. The study indicates that individuals with DD genotype and dyslipidemia in Mizo population are prone to IFG and Type 2 diabetes, which makes this population at-risk for future cardiovascular adversities. Based on the present result, in developing countries like India, where there is an increasing burden of lifestyle diseases like T2D, community specific genetic association studies should be carried out with larger sample sizes in different geographical region.

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Data availability The data that supports the finding of this study are available from the corresponding author upon reasonable request.

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Code availability Not applicable.

Declarations

Conflict of interest All the authors declared that they have no conflict of interest.

Ethical approval The Ethical committee, Department of Anthropology, University of Delhi, India approved the study.

Consent to participate The written informed consents were obtained from all the subject participants before conducting the study.

Consent to publication Not applicable.

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