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Interaction of antioxidant gene variants and susceptibility to type 2 diabetes mellitus

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ABSTRACT

Background: Diabetes is the seventh most common disease leading to death with a global estimate of 425 million diabetics, expected to be 629 million in 2045. The role of reactive metabolites and antioxidants, such as glutathione, glutathione peroxidase, superoxide dismutase and catalase in type 2 diabetes mellitus (T2DM) provides an opportunity for identifying gene variants and risk genotypes. We hypothesised that certain antioxidant gene-gene interactions are linked with T2DM and can model disease risk prediction.

Materials and methods: Genotyping of single nucleotide polymorphisms (SNPs) in antioxidant genes for glutathione (*GST*), glutathione peroxidase (*GPx*), superoxide dismutase (*SOD*) and catalase (CAT) was performed in 558 T2DMs and 410 age and sex matched healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), routine lab indices by standard techniques.

Results: The null/null allele combination of *GSTM1*del and *GSTT1*del increased disease risk up to 1.7-fold. The combination of SNPs in *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G and in *CAT*-21A/T, *SOD2* + 47C/T, *GPx1* + 599C/T increased the risk of diabetes 13.5 and 2.1-fold, respectively. Interaction of SNPs *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val), *CAT*-21A/T, *SOD2* + 47C/T, *GPx1* + 599C/T were significantly linked with disease risk >5 × 10^3 fold.

Conclusion: As the number of gene combinations increase, there is a rise in the odds ratio of disease risk, suggesting that gene-gene interaction plays an important role in T2DM susceptibility. Individuals who possess the *GSTM1*del, *GSTT1*del, *GSTP1* 105I/V(+313A/G), *CAT*-21A/T, *SOD2* + 47C/T and *GPx1* + 599C/T are at very high risk of developing T2DM.

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Introduction

Diabetes mellitus is a chronic disorder characterised by impaired metabolism of glucose and lipids due to defects in insulin secretion (β-cell dysfunction) or activity (insulin resistance) [1]. Characteristic features are chronic hyperglycaemia with microvascular (e.g. retina, renal glomerulus and peripheral nerves) and macrovascular (e.g. atherosclerosis, coronary artery disease, stroke) disease with more than 17.5 million deaths worldwide annually [2]. The global burden of diabetes mellitus is presently 425 million affected people with 212 million still undiagnosed.

There is evidence that oxidative stress plays a part in the development of type 2 diabetes [4]. Relevant biochemical pathways include auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes, formation of lipid peroxides and decreased ascorbic acid levels. The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT), and the amino acid glutathione contribute to the elimination of reactive oxygen species such as superoxides and hydroxyl radicals [5–10]. The role of these metabolites provides an opportunity to identify antioxidant gene variants and risk genotypes [11], whilst different variants in glutathione protect from adverse side effects in the chemoradiotherapy of cervical cancer [12].

There are several reports of risk variants of antioxidant enzyme gene variants associated with type 2 diabetes, but few of these reports consider more than one gene single nucleotide polymorphism (SNP) per investigation. We therefore hypothesised that different combinations of several antioxidant gene SNPs would be linked to a greater risk of this form of diabetes.

Materials and methods

Patient selection and clinical evaluation were performed as per previous reports [5,13]. We performed an age and sex matched case-control study with healthy subjects recruited from universities and institutes in Lucknow with due approval of institutional ethics committee (No. 1234/R-cell-10 dated 18 August 2010). Inclusion criteria for T2DM cases were age 40–70 years, presence of classic symptoms of diabetes together with gross and

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unequivocal elevation of plasma glucose, elevated glucose concentrations on more than one occasion, i.e. fasting plasma glucose: ≥7.0 mmol/L after 8 h or overnight fasting, post-prandial glucose: ≥11.1 mmol/L 2 h after consumption of meal, random blood sugar ≥ 11.1 mmol/L. Exclusion criteria were pregnant or nursing mothers, patients diagnosed of psychotic disorders or hospitalised for depression, mature onset diabetes of the young, latent autoimmune diabetes in adults, gestational diabetes and diabetes due to any type of pancreatic injury, or fluctuations in glucose readings in multiple examinations. Inclusion criteria for controls were no family history of any specific disease/allergy/infection/inflammatory response, including diabetes, normal body mass index and normal fasting plasma glucose and post-prandial glucose. Patients were prescribed with metformin, modified, if necessary, with addition of a sulphonylurea or a thiazolidinedione.

Whole blood samples from all subjects were collected in plain and 0.5M EDTA vials after written consent was obtained. Blood and serum were analysed for standard biochemical indices by routine methods. Anthropometric and biochemical parameters of cases (n = 558) and controls (n = 410) are shown in Table 1.

SNPs in selected glutathione genes were *GSTM1*del, *GSTT1*del, *GSTP1* 105I/V(+313A/G) (rs1695), and in antioxidant genes *CAT* −21A/T (rs7943316) in catalase, *SOD2* + 47C/T (rs4880) in superoxide dismutase and *GPx1* + 599C/T (rs3811699) in glutathione peroxidase were genotyped (Table 2) by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP). The primers and restriction enzymes were designed by Primer3 and NEBcutter respectively (ver. online). Initially, 100 normal healthy control subjects were genotyped in order to determine the Minor Allele Frequency (MAF) of each SNP in the study population. The sample size was calculated using QUANTO software (ver 3.0). Allele and genotype frequencies with carriage rates of polymorphic alleles in patient and control groups were evaluated by Pearson's chi-square (χ^2) and Fisher's exact test. The Hardy-Weinberg equilibrium at individual loci was assessed by χ^2 statistics using SPSS (v 21.0). Data are presented as mean with SD. Differences were considered statistically significant if $P < 0.05$.

Gene-gene interaction analysis was performed in different antioxidant genes with SNPs (MAF ≥ 0.02) showing significant association with type 2 diabetes in genotyping studies ($P \leq 0.05$) [5,13] (Table 2). The analysis of linkage disequilibrium (LD) between SNPs was carried out by SHEsis software (ver. Online). Haploview was used to analyse the patterns of LD and LOD (Log Odds) in genetic data by estimating haplotype frequencies [13,14]. Pairwise Linkage Disequilibrium (LD) based on D′ statistics and correlation coefficient (r^2) of frequencies was performed for gene-gene interactions.

Table 1. Comparison of anthropometric and biochemical parameters of cases and controls.

Parameters	Controls (n = 410)	T2DM cases (n = 558)
Anthropometric parameters		
Age (Years)	50.2 ± 6.3	49.7 ± 11.2
Body mass index (kg/m^2)	23.6 ± 1.6	23.8 ± 4.2
Biochemical parameters		
Fasting plasma glucose	4.1 ± 0.3	9.9 ± 4.0
Post-prandial glucose	7.1 ± 0.6	15.4 ± 6.2
Total cholesterol	3.7 ± 0.7	5.2 ± 1.1
Triglycerides	1.5 ± 0.6	1.3 ± 0.3
High-density lipoprotein	1.59 ± 0.30	1.20 ± 0.28
Low-density lipoprotein	1.47 ± 0.76	3.23 ± 1.44
Creatinine	92 ± 10	117 ± 11.5

All lab units mmol/L, except creatinine (μmol/L). Data mean ± SD. All differences $P < 0.001$ except age ($P = 0.09$).

Table 2. Location of SNPs in antioxidant genes, MAF values and sample size.

Gene	SNPs	Location	MAF
GSTM1	Deletion (N/P*)	16 kb whole gene deletion	0.244*
GSTT1	Deletion (N/P*)	54 kb whole gene deletion	0.239*
GSTP1	rs1695	+313A/G	0.06*
CAT	rs7943316	−21A/T	0.14*
SOD2	rs4880	+47C/T	0.21**
GPx1	rs3811699	+599C/T	0.16**

MAF, minor allele frequency; N, null; P, present. Sample size for genotyping (Controls/Cases) *201/204, **210/207

Results

Most anthropometric and biochemical measures in controls and cases showed significant differences (Table 1). The sample size for each SNP genotyped is shown in Table 2. The P values and ORs of significant gene-gene interactions are summarised in Table 3. The results of gene-gene interaction were analysed to assess allele combinations responsible for predisposition to diabetes are shown in Figure 1 and Table 4. Abbreviations for allele genotypes in genes are: N = Null (-/-); P = Present (+/+); V = Valine (Val); I = Isoleucine (Ile); A = *CAT*-21A;

Table 3. Combinations of genetic variants in different antioxidant genes

Genetic variantsin different genes	Significant interactions	Odds Ratio (95% CI): P value
*GSTM1*del, *GSTT1*del	N N	1.74 [1.08–2.80] p=0.001
*GSTM1*del, *GSTT1* del, *GSTP1*+313A/G (105Ile/Val)	N N V	13.47 [1.75–103.48] P<0.001
CAT-21A/T, *SOD2*+47C/T, *GPx1*+599C/T	T C C	2.06 [1.32-3.21 P=0.001
GSTM1 del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val)	P N I A	0.45 [0.21–0.95] P=0.002
*GSTM1*del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *SOD2*+47C/T	P N I T	0.31 [0.09–1.06] P=0.049
*GSTM1*del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *GPx1*+599C/T	P N I T	0.27 [0.08–0.90] P=0.023
GSTM1 del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *CAT*-21A/T, *SOD2*+47C/T, *GPx1*+599C/T	N P I A C T P P I T C T	2.53 [0.96-6.63] P=0.05 5083.35 [303.11–85250.92] p<0.001

Abbreviations for allele genotypes in genes are as follows: N=Null (-/-); P=Present (+/+); V=Valine (Val); I=Isoleucine (Ile); A=*CAT*-21A; T= *CAT*-21T; T=*SOD2*+47T; C= *SOD2*+47C; T= *GPx1*+599T; C= *GPx1*+599C.

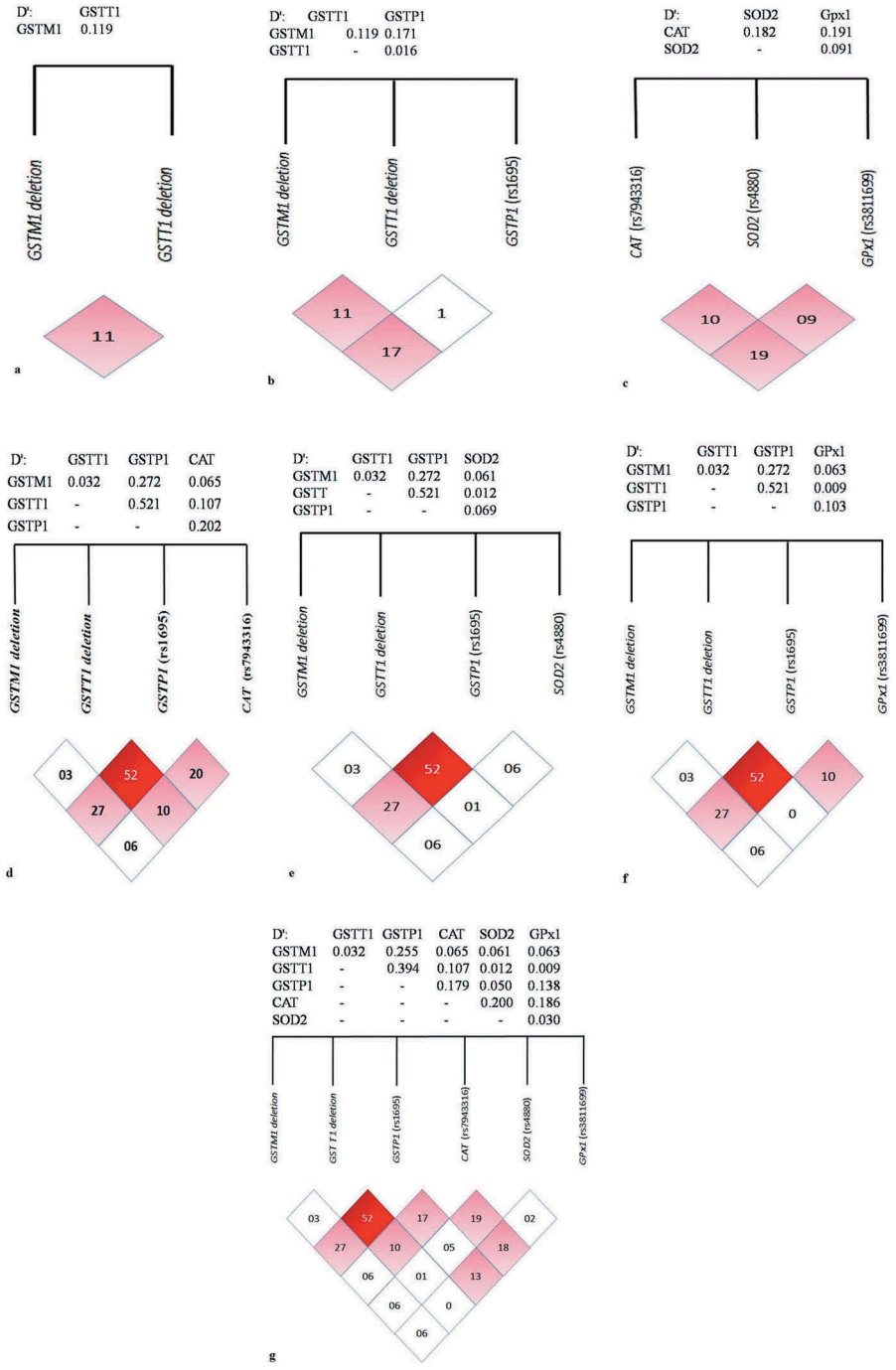

Figure 1. (a–g): Linkage disequilibrium (LD) of combination of antioxidant gene variants (Combinations 1–7) in North Indian population. Pairwise LD is represented as pink squares for little LD and red squares for moderate LD. (a): *GSTM1* del, *GSTT1* del; (b): *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val 'or' rs1695); (c): *CAT*-21A/T (rs7943316), *SOD2* + 47C/T(rs4880), *GPx1* + 599C/T (rs3811699); (d): *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val, rs1695), *SOD2* + 47C/T(rs4880); (e): *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val, rs1695), *GPx1* + 599C/T (rs3811699); (f): *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val rs1695), *CAT*-21A/T(rs7943316); (g): *GSTM1*del, *GSTT1*del, *GSTP1* + 313A/G (105Ile/Val, rs1695), *CAT*-21A/T (rs7943316), *SOD2* + 47C/T(rs4880), *GPx1* + 599C/T (rs3811699).

T = *CAT*-21T; T = *SOD2* + 47T; C = *SOD2* + 47C; T = *GPx1* + 599T; C = *GPx1* + 599C.

The interaction of deletion SNPs *GSTM1*del and *GSTT1*del showed four different combinations. The Null/Null (NN) combination showed significant association with diabetes (OR [95% CI] 1.74 [1.08–2.80] P = 0.001) (Figure 1(a)). Gene-gene interaction analysis of *GSTM1*del, *GSTT1*del and *GSTP1* 105I/V(+313A/G) polymorphisms showed seven different combinations, of which NNV showed highly significant association (OR 13.47 [1.75–103.48], P = 0.001) with T2DM (Figure 1(b)). The *CAT*-21A/T, *SOD2* + 47C/T and *GPx1* + 599C/T SNPs showed eight different combinations, of which seven were relevant, of which the TCC* combination showed highly significant association (OR 2.06 [1.32–3.21], P < 0.001) (Figure 1(c)).

Table 4. Combinations (A-G) of allelic variants in different antioxidant genes in T2DM cases and controls.

Allele combination	Cases (freq %)	Controls (freq %)		Odds Ratio [95%CI] P value
A: *GSTM1*del, *GSTT1*del				
N N*	50 (12.3)	30 (7.5)		1.74 [1.08-2.80] p=0.021
N P*	72 (17.7)	88 (21.9)		0.77 [0.54-1.09] p=0.138
P N*	64 (15.7)	66 (16.4)		0.95 [0.66-1.39] p=0.800
P P*	220 (54.2)	218 (54.2)		0.10 [0.76-1.32] p=0.991
B: *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G(105Ile/Val)				
N N I*	37 (9.0)	29 (7.3)		1.31 [0.79-2.18] p=0.294
N N V*	13 (3.3)	1 (0.0)		13.47 [1.75-103.48] p=0.001
N P I*	58 (14.6)	73 (18.3)		0.76 [0.52-1.11] p=0.161
N P V*	14 (3.5)	15 (3.8)		0.94 [0.45-1.97] p=0.861
P N I*	54 (13.6)	62 (15.6)		0.86 [0.58-1.27] p=0.439
P P I*	193 (48.7)	199 (50.0)		0.95 [0.72-1.26] p=0.722
P P V*	27 (6.8)	19 (4.8)		1.46 [0.80-2.67] p=0.218
C: *CAT*-21A/T, *SOD2*+47C/T, *GPx1*+599C/T				
A C C*	50 (19)	92 (25)		0.69 [0.46-1.04] p=0.073
A C T*	50 (19)	66 (18)		1.06 [0.69-1.61] p=0.800
A T C*	26 (11)	57 (15)		0.66 [0.41-1.09] p=0.102
A T T*	28 (12)	41 (11)		1.07 [0.64-1.77] p=0.809
T C C*	52 (22)	43 (18)		2.06 [1.32-3.21] p=0.001
T C T*	23 (9)	33 (9)		1.07 [0.61-1.87] p=0.082
T T C*	16 (7)	29 (7)		0.88 [0.47-1.65] p=0.682
D: *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G, *CAT*-21A/T				
N N I A*	03 (2)	13 (5)	0.188	0.41 [0.11~1.61]
N P I A*	16 (13)	27 (9)	0.360	1.36 [0.70~2.63]
N P I T*	8 (6)	14 (5)	0.629	1.25 [0.51~3.07]
P N I A*	9 (7)	40 (14)	0.033	0.45 [0.21~0.95]
P N I T*	7 (6)	16 (6)	0.969	0.98 [0.39~2.46]
P P I A*	51 (40)	102 (36)	0.485	1.17 [0.75~1.82]
P P I T*	26 (20)	50 (18)	0.549	1.18 [0.69~2.01]
E: *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *SOD2*+47C/T				
N N I C*	03 (2)	11 (4)	0.296	0.48 [0.12~1.94]
N P I C*	19 (15)	25 (9)	0.101	1.70 [0.90~3.24]
N P I T*	5 (4)	16 (10)	0.558	0.74 [0.27~2.02]
P N I C*	13 (10)	36 (13)	0.424	0.76 [0.39~1.50]
P N I T*	3 (2)	20 (7)	0.049	0.31 [0.09~1.06]
P P I C*	52 (41)	96 (34)	0.226	1.31 [0.85~2.04]
P P I T*	25 (20)	56 (20)	0.902	0.97 [0.57~1.65]
F: *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *GPx1*+599C/T				
N N I C*	1 (1)	9 (3)	0.138	0.24 [0.03~1.88]
N N I T*	4 (3)	7 (2)	0.726	1.25 [0.36~4.35]
N P I C*	16 (13)	24 (8)	0.216	1.53 [0.78~2.99]
N P I T*	8 (6)	17 (6)	0.973	1.02 [0.43~2.42]
P N I C*	13 (10)	33 (12)	0.614	0.84 [0.43~1.66]
P N I T*	3 (2)	23 (8)	0.023	0.27 [0.08~0.90]
P P I C*	46 (36)	91 (32)	0.496	1.17 [0.75~1.82]
P P I T*	31 (24)	61 (21)	0.638	1.13 [0.70~1.86]
G: *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G (105Ile/Val), *CAT*-21A/T, *SOD2*+47C/T, *GPx1*+599C/T				
N P I A C T*	10 (8)	08 (3)	0.050	2.53 [0.96~6.63]
N P I A T C*	04 (3)	7 (3)	0.988	0.99 [0.28~3.47]
N P I T C C*	5 (5)	7 (3)	0.447	1.54 [0.51~4.67]
P N I A C C*	6 (5)	27 (9)	0.036	0.38 [0.15~0.96]
P P I A C C*	17 (14)	34 (12)	0.898	0.96 [0.51~1.813]
P P I A C T*	13 (10)	32 (11)	0.319	0.70 [0.35~1.41]
P P I A T C*	17 (14)	19 (7)	0.090	1.82 [0.91~3.65]
P P I A T T*	4 (3)	17 (6)	0.122	0.42 [0.13~1.30]
P P I T C C*	10 (8)	32 (11)	0.115	0.55 [0.26~1.17]
P P I T C T*	13 (10)	0 (0)	<0.001	5083.35 [303.11~85250.92]

Abbreviations for allele genotypes in genes are as follows: N=Null (-/-); P=Present (+/+); V=Valine (Val); I=Isoleucine (Ile); A=*CAT*-21A; T= *CAT*-21T; T=*SOD2*+47T; C= *SOD2*+47C; T= *GPx1*+599T; C= *GPx1*+599C. For clarity, underpowered analyses are not shown.

Sixteen different combinations of *GSTM1*del, *GSTT1*del, *GSTP1* 105I/V(+313A/G) and *CAT*-21A/T SNPs were modelled, of which seven combinations were relevant. The PNIA* combination showed significant association (OR 0.45 [0.21–0.95] $P = 0.002$) (Figure 1(d)). The *GSTM1*del, *GSTT1*del, *GSTP1* 105I/V(+313A/G) and *SOD2* + 47C/T SNPs provided 16 different combinations, of which seven combinations were relevant, of which the PNIT* combination showed a weak but significant association (OR 0.31 [0.09–1.06] $P = 0.049$) with T2DM (Figure 1(e)) whilst the *GST* polymorphisms with *GPx1* + 599C/T showed 15 different combinations of which eight were relevant, with the PNIT* combination showing significant association (OR 0.27 [0.08–0.90] $P = 0.023$) (Figure 1(f)). *GST* polymorphisms with *CAT*-21A/T, *SOD2* + 47C/T and *GPx1* + 599C/T showed 12 different combinations, of which 10 were relevant. The NPIACT combination was of borderline significance (OR 2.53 [0.96–6.63] $P = 0.05$) but the PPITCT combination showed a highly significant association (OR 5083.35 [303.11–85,250.92] $P < 0.001$) (Figure 1(g)).

Discussion

The increasingly high prevalence of T2DM and related complications brings a major healthcare burden, and epidemiological data clearly indicate the role of environmental factors in diabetes onset, particularly obesity [3,9]. Increased reactive oxygen species, mainly superoxide production, leads to factors involved in insulin resistance and post-diabetic complications. There are several biochemical parameters which can be used as biomarkers [10] one of them being antioxidants (glutathione, GPX, SOD, CAT, etc.) [14]. Association of different pathway genes have been studied in context to T2DM pathogenesis viz. inflammatory mediators such as interleukins, tumour necrosis factor-α (TNF-α), adiponectin [15–20], and the macrophage Ox-LDL receptor CD36 [21–25], in addition to the antioxidants [5,13]. Antioxidant gene polymorphism studies are a comprehensive tool for understanding the stress-sensitive pathways [26]. The effects of individual polymorphisms in susceptible genes either may be absent or negligible but the cumulative effects as observed in gene-gene interaction studies provide information regarding risk genotypes/haplotypes for predicting the disease [7,8,27].

We hypothesised links between variants in different antioxidant genes and the presence of T2DM. Linkage disequilibrium (LD) was defined as non-random association of alleles at two or more loci, which may or may not be on the same chromosome, being the occurrence of combinations of alleles or genetic markers in a population. The interaction of *GSTM1*(-/-), *GSTT1*(+/+) and *GSTP1*(A/A) together showed a significantly higher association with 2.43 times risk of T2DM [28]. In the present study interaction of *GSTM1*del/*GSTT1*del, NN* showed significant association with 1.74 times risk while interaction of *GSTM1*(-/-), *GSTT1*(-/-) and *GSTP1*(I/V), i.e. NNV allele combination increased the risk up over 13-fold. An association of *SOD2* + 47C/T [29] polymorphism with type 1 diabetes mellitus has been reported [30–32]. Although *SOD2* + 47C/T alone showed only 0.64 times risk for T2DM and *GPx1* + 599C/T showed no significant association [5], their interaction with *CAT*-21A/T, i.e. TCC allele combination showed significant association with a doubling of the risk of diabetes. No significant disease risk combination was observed during analysis of four SNPs in different genes. However, interaction of all gene variants taken together viz. *GSTM1*del, *GSTT1*del, *GSTP1* 105I/V (+313A/G), *CAT*-21A/T, *SOD2* + 47C/T and *GPx1* + 599C/T showed an extremely high risk of developing T2DM in individuals possessing PPITCT allele combination.

Thus, we conclude that combined effect of antioxidant gene variants results in increased T2DM susceptibility. This could be validated in individuals from families who have not been diagnosed of T2DM but have shown certain abnormal biochemical parameters such as raised glucose and BMI. There is considerable variation amongst various ethnic populations around the world. Therefore, it is essential to perform such genetic studies in different populations in order to develop prognostic regimens and alternate treatment strategies for this disease. This paper represents an advance in biomedical science because it points to a combination of SNPs in antioxidant genes that together bring a very high risk of type 2 diabetes mellitus.

Summary table

What is known about this subject:

- Oxidative stress is one of the major causes of insulin resistance and impaired glucose metabolism.
- The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and glutathione variants (GSTM1, GSTT1 and GSTP1) contribute to the elimination of reactive oxygen metabolites.

What this paper adds:

- The combination of SNPs, NNV* in *GSTM1*del, *GSTT1*del, *GSTP1*+313A/G increases the risk of diabetes up to 13-fold.
- Interaction of selected antioxidant genes brings a highly significant link with diabetes, with an odds ratio of $> 5 \times 10^3$.

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Disclosure statement

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