RESULTS

The observations on the completion of objectives of the study in assessing genetic damage in hypertensive and normotensive Jat Sikhs as a function of selected genotypes have been compiled and appropriately statistically analyzed. The results are initially presented as distribution of data on demographic, obesity and disease-specific variables and on biomarkers of genetic damage, oxidative stress, and cholestrolemia. Molecular genotypes of cases and controls (normal healthy persons) have also been compared. Genetic and non-genetic factors, prevalent in the cases and control participants and contributing towards the state of hypertension, have been earmarked. Stratification by age, gender, menopause-status, obesity, disease-duration and treatment-duration has been carried out to assess for any within group differences in cases and controls, which ever appropriate. Genotypic distribution of six allelic variants of the four genes and their interactions in patients and controls have been compared and stratified for genetic damage and oxidative stress biomarkers. Association and regression analyses have also been performed for predictors of genetic damage. The results are described in detail under the appropriate sub-headings.

4.1 Demographic Variables

The study participants were all Jat Sikhs between 40-90 years (average age 61.59±0.80y of patients, 60.36±0.89y of controls) and comprised 50.50% female (59.47±1.13y) and 49.50% male (63.47±1.11y) patients with 47.00% female (59.84±1.28y) and 53.00% male (60.80±1.24y) controls being matched for age and gender representations (Table 1a). The participants were all married, non-smokers, belonged to rural area. Males were involved in agricultural practices and females were mostly housewives rendering help in the fields only during the harvesting season. Dietary patterns revealed matching habits as ~70% (71.29%females, 68.68% males) were non-vegetarian among patients and 60% (34.04% females, 83.02% males) among controls with more males in control group having non-vegetarian preference. Cooking medium matched with use of saturated fats (32.50% patients, 35.50% controls), unsaturated fats (23.50% patients, 25.50% controls) and both (44.00% patients, 39.00% controls). Alcohol drinking was a
prevalent practice among males (42.50% patients, 46.00% controls) while none of the females took alcohol. Patients and controls were matched for socio-economic status also though there was more representation of the middle class (38.50% patients, 35% controls), followed by that of the upper-middle (25.50% patients, 32.50% controls) and of lower middle class (22.50% patients, 18.00% controls) while there was almost equal representations of lower socio-economic status (7.00% vs. 8.00%). Mobile-phone usage was very less (5.50% patients, 2.00% controls) and 48.00% individuals, each among patients and controls, had residences near mobile phone base stations. Nearly 50.00% (51.50% patients, 52.50% controls) were moderately physically active. On inquiring about family history of hypertension, surprisingly 25.00% of the control group reported it while this was 47.00% among patients. In the patient group, there were 41.00% first-degree relatives, 36.78% second-degree relatives and 22.22% third-degree relative while these were 23.46%, 49.08% and 27.46%, respectively in controls.

4.2 Obesity Status of Study Group

General obesity calculated on the basis of body mass index (BMI) revealed 60.50% of patients and 77.50% of controls as obese (Table 1b). Regional adiposity (WHR and WHtR bases) was also less among patients (87.50%) than controls (90.50%) as was central obesity assessed by waist circumferences (WC) with more obese controls (91.50%) than patients (89.00%). Comparison of obesity status between patients and controls revealed that these were matched for WC, WHR and WHtR (Table 2a).

4.3 Clinical Characteristics of Patient Group

The patient group had average SBP/DBP of 145.52±1.39/86.86±0.66mmHg which differed (p≤0.001) significantly (130.00±0.38/76.79±0.32mmHg) from the values of control group (Table 1c). The age-of-onset of disease varied from 36-83y and as treatment had been initiated on diagnosis, the treatment time (1-4y) and duration of disease intervals were same, not varying between genders. Despite monodrug therapy with atenolol, stage I hypertension category (41.50%) was maximum, followed by those in the high normal (22.00%) and normal (11.00%) categories with 16.00% in stage II and 8.50% in stage III categories. Some patients also had high mean arterial pressure
(MAP) (29.00%) and pulse pressure (PP) (22.50%). Controls were normotensive (47.50%) or in the optimal category (52.50%) with MAP and PP levels normal.

Dyslipidemia was prevalent as 42.50% patients had high total cholesterol, 69.05% high LDL-C and 39.00% high TG compared to those respectively in controls (19.50%, 34.50% and 10.50%).

An additional untreated hypertensive patient group (n=05) also matched for demographic, anthropometric and disease-related variables to treated hypertensive patients.

4.4 Gender Stratification of Clinical Variables (Table 2b)

The WHtR values were significantly (p≤0.05) higher in female patients (0.62±0.01) compared to male patients (0.58±0.008) and to female controls (0.62±0.009) implying higher obesity measures in female patients.

Male and female patients differed for age with there being older male patients on the basis of average age. However there were no gender differences for duration of disease/treatment duration, age-of-onset of disease and blood pressure measurements. Controls also matched for blood pressure measurements though respective patients group had significantly higher (p<0.001) levels.

Also statistically significantly (p<0.001) increased lipid levels were prevalent in patients compared to levels in controls though no gender differences were there in both groups. On the other hand, HDL-C levels were significantly (p≤0.0001) decreased in males patients (34.49±1.09 mg/dl), females patients (33.05±0.84 mg/dl) and total patient group (33.77±0.69 mg/dl) when compared to levels in control males (48.35±1.39 mg/dl), control females (49.02±1.43 mg/dl) and in the total group (48.66±0.99 mg/dl). TC/HDL, LDL/HDL and TG/HDL levels were also significantly higher (p≤0.001) in total patient group as well as in male and female patients compared to the values in respective controls.

Overall, except for higher WHtR values in female versus male patients, disease-duration, age-of-onset of disease, blood pressure measurements and lipid levels showed no gender differences in patients or in controls.
4.5 Metabolic Syndrome

The patient and control groups were also examined for the presence of metabolic syndrome, a state which increases future cardiovascular risk. According to the Adult Treatment Panel (Grundy et al., 2005), metabolic syndrome is indicative if three of the five variables of increased waist circumference (≥120cm in males and ≥88cm in females), hypertriglyceridaemia (≥1.7mmol/l), low HDL-C (<1.03mmol/l), hypertension (≥130mmHg systolic blood pressure and/or ≥85mmHg diastolic blood pressure) and increased glucose levels (≥5.6mmol/l) are present in an individual. According to International Diabetes Federation (IDF, 2005), simultaneous occurrence of elevated WC (≥94cm for non-Hispanic men, ≥90cm for Mexican-American men and ≥80cm in women) with two other variables is considered as metabolic syndrome. For the present study, 53.50% among patients had metabolic syndrome with only 4% among controls considering WC (cut-off >85cm for males and >80cm for females) (Snehlata et al., 2003). As diabetic patients were not included in the study participants so the condition of glucose levels was excluded. According to combination of WC, HDL-C and blood pressure maximum patients (28.50%; 17.17% males, 39.60% females) followed by WC, TG and BP measurements (19.00%; 18.18% males, 19.80% females) were found to have metabolic syndrome. While maximum patient males (18.18%) were observed to have metabolic syndrome (Table).

4.6 Genetic Damage and Oxidative Stress

Genetic damage parameters (Table 2c) assessed as per cent tail DNA (4X), tail moment (3X), Olive tail moment (6X), damage index (8X) and damage frequency (5X) were significantly (p≤0.001) increased in the patient group. Male patients had however significantly higher (p≤0.001) damage parameters of damage index (DI=191.61±2.67) and damage frequency (DF=93.07±0.53) compared to values in female patients (119.50±2.63 and 90.79±0.76, respectively). Patients also had significantly increased (p≤0.001) total oxidant status (6X), lipid peroxidation measured as malondialdehyde levels (10X) as well as calculated oxidative stress index (OSI, 17X) while total antioxidant capacity (TAC) was significantly reduced (p≤0.05) in male patients (1.13±0.12 nmol Trolox Equ./L), female patients (0.82±0.06 mmol Trolox equ./L) and
total patients (0.97±0.07 mmol Trolox equ./L) when compared to levels in control males (4.58±0.16 mmol, Trolox equ./L), control females (4.81±0.16 mmol Trolox Equ./L) and total control group (4.69±0.12 mmol Trolox Equ./L). Male patients had however significantly higher (p≤0.05) TAC compared to female patients with no other significant gender differences within patients and controls.

Higher levels of per cent tail DNA, TM, OTM and DF were observed in untreated hypertensive patients as compared to treated hypertensive patients though the difference did not reach statistical significance. Levels of total antioxidant capacity was however significantly higher and of total oxidative stress and oxidative stress index significantly reduced in untreated patients when compared to respective values in treated hypertensive patients.

As genders did not differ significantly for obesity measures and levels of blood pressure, lipids, genetic damage and oxidative stress in the patient group (also not in the control group), the data on these parameters were pooled for further analysis.

4.6.1 Principal Component Factor Analysis (PCA) for best Genetic Damage Parameters in Patients and Controls

In order to discern the best parameter of genetic damage assessment in the present study group, PCA was performed for all the five parameters separately in the patient and control group.

In patients, factor loadings for genetic damage parameters of per cent tail DNA, tail moment, Olive tail moment, damage index and damage frequency in patients, loaded two factors from original five variables explaining a variance of 77.36%. Factor one loaded with per cent tail DNA, tail moment and Olive tail moment explaining 47.20% of total variance while factor two was loaded with damage index and damage frequency explaining 30.15% of the variance.

In the control group also, two factors loaded for genetic damage end-points explaining a total variance of 77.82%. Factor one had high loading of damage index and damage frequency explaining the 39.30% of total variance while factor two loaded with per cent tail DNA, tail moment and Olive tail moment explaining the variance of 38.52%.
On total study group, PCA revealed only one factor for genetic damage parameters explaining 68.32% variance. All the genetic damage parameters had factor loadings more than 0.4 so explaining that all the genetic damage parameters are valuable to explain the genetic damage results.

These observations therefore indicate that in patients the assessed indices (per cent tail DNA, TM and OTM) contributed more while in controls the derived (DI and DF) indices contributed almost equally. In the total group all the parameters contributed; per cent tail DNA was the best index of genetic damage for the present study group.

4.7 Molecular Genetic Investigations

Molecular genetic investigations studied the genotypic status of CYP2D6 *2 (rs16947), CYP2D6 *4(rs3892097), CYP2D6*10 (rs1065852) and GST (T1, M1 and P1; rs1695) genes in patients and controls in order to find whether these polymorphisms have an association with disease-status, and with genetic damage levels.

4.7.1 Genotypic and Allelic Frequencies

The patient and control groups matched for genotype frequencies (Table 5a) of CYP2D6 (*2, *4 and *10) and GST (T1 and M1) genes (p>0.082 for all cases) but varied significantly (p=0.0001) for the GST P1 genotypes, as the homozygous variant (GG genotype) was in higher frequency (35.50%) as was the heterozygous (AG, 43.00%) genotype compared to the respective frequencies of 14.00% and 26.50% in controls.

Crude odds ratio analysis of genotype frequency between patients and controls revealed that individuals with the heterozygous genotype (GA) of CYP2D6 *4 gene had ~1.6 fold higher likelihood (OR=1.594; 95% CI= 1.0516-2.4181; p=0.028) and individuals with homozygous mutant genotype (AA) of CYP2D6 *4 gene had ~3 fold higher likelihood (OR=3.2121; 95% CI=1.5368--6.7136; p=0.0019) for being hypertensive. Similarly, individuals with the heterozygous genotype (CT) of CYP2D6 *10 had ~2 folds higher likelihood (OR=2.4140; 95% CI= 1.5165-3.8427; p=0.0002) for hypertension. In the case of GST P1, the heterozygous genotype (AG) had ~4.50 fold (OR=4.4906; 95% CI= 1.7548-7.3201; p=0.0001) higher likelihood for hypertension.
However, on adjustment for gender, age, alcohol consumption, socioeconomic status, BMI, family history of disease, TAC and TOS levels, this significance was lost.

The allelic distribution (Table 5b) of CYP2D6 *4 and GST P1 were observed to be significantly different between patients and controls ($\chi^2=11.098; \ p=0.0009$ and $\chi^2=71.391; \ p<0.0001$, respectively), indicating a higher minor allele frequency in patients (0.3775 for CYP2D6*4 minor allele and 0.57 for GST P1 minor allele).

A significant deviation from Hardy-Weinberg equilibrium was observed for the CYP2D6*2 allele in both, patient and control ($p=0.0001$ for both) groups (Table 5b). The allelic frequencies of CYP2D6*10 and GST P1 were also not in Hardy-Weinberg equilibrium only in the control group ($p=0.000$ for both). There was a lack of Hardy-Weinberg equilibrium for the CYP2D6*2 alleles in patients and controls.

### 4.7.2 Linkage Disequilibrium (LD) and Haplotype Analysis

Linkage disequilibrium is the non-random association of different alleles. Haplotype analysis is performed to check whether any specific haplotype shows significant association with disease.

Analysis of LD plot and of haplotypes was made using Haploview programme. The p-values less than 0.05 were considered as significant implying an association between the genes.

The analysis revealed that the variants CYP2D6 *2 (rs16947), CYP2D6 *4(rs3892097), CYP2D6*10 (rs1065852) and GST P1 (rs1695) were not in linkage with each other. However no such haplotype was generated and so none of the six SNPs had the tendency to be inherited together in the group under study.

### 4.7.3 Models of Inheritance

These comprise the additive (homozygous wild compared to homozygous mutant), dominant (homozygous recessive and heterozygous compared to homozygous wild), co-dominant (heterozygous compared to homozygous wild) and recessive (homozygous wild compared to homozygous recessive) models of inheritance.
Analysis for the different models (Table 31) revealed that the additive model of inheritance is the best fit model for CYP2D6*2 (OR=7.02; 95% CI=4.01-12.28; p=0.000) and for GST P1 (OR=7.02; 95% CI=4.01-12.28; p=0.000) and the dominant model of inheritance for CYP2D6*4 (OR=1.08; 95% CI=1.21-2.68; p=0.004) and CYP2D6*10 (OR=1.76; 95% CI=1.17-2.66; p=0.007) variants. The results revealed 1.08-7.02 fold higher risk associated with the disease.

4.7.4 Metabolic Phenotypes based upon CYP2D6 (*2, *4 and *10) allelic variants

Based CYP2D6 upon gene variants, patients and control group individuals (Table 5c) were classified into four metabolic phenotypes viz. ultra-rapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM) phenotypes (Blake et al., 2013). On the basis of enzyme activity, the present study participants were categorized as extensive (CYP2D6*2), poor (CYP2D6*4), intermediate (CYP2D6*10) metabolizers (Kaiser et al., 2002; Ishiguro et al., 2004). Depending upon the genotyping results of the CYP2D6*2, *4 and *10 variants present in the study participants, metabolic phenotypes were assigned to each participant. On considering singly, for those with CYP2D6*2 (AA/AG) variants the phenotype assigned was extensive metabolizers (EM) while those with CYP2D6*2 (GG) with intermediate metabolizers (IM). Similarly participants with CYP2D6*10 (CC/CT) genotypes were EMs and with TT genotype are IMs. Homozygous wild CYP2D6*4 (GG) genotypes are EMs, GA genotypes were IMs and AA genotypes were poor metabolizers (PM). The present study participants had nine genotype combinations: six were IM metabolizers {CYP2D6*2 (AG/GG)/ CYP2D6*4 (GA/AA)/ CYP2D6*10 (CT/TT) combinations of heterozygous and homozygous variants} and extensive metabolizers (CYP2D6*2 AA/ CYP2D6*4 GG/ CYP2D6*10 CC combinations). The individual variations of CYP2D6*2 and CYP2D6*10 and combined CYP2D6*2, CYP2D6*4 and CYP2D6*10 phenotypes revealed no significant differences for frequency of individuals with respective EM and IM phenotypes between patients (5.50% and 94.50%) and controls (10.00 and 90.00%, respectively). However, significant differences were observed for EM (38.50 vs. 53.00%) vs. IM (47.50% vs. 41.00%) and EM (38.50 vs. 53.00%) and PM (14.00 vs. 6.00%) phenotypes in patients and controls based upon CYP2D6*4 variants.
4.7.5 Principal Component Factor Analysis (PCA) for Genetic Variants

To find the best molecular genetic variants that predict(s) maximum variance for disease-status, principal component factor analysis was performed considering the six allele variants in the patient group (Table 6). The analysis reduced these to three factors explaining a cumulative variance of 57.53% for disease. The first factor was highly loaded with CYP2D6*4 gene variant explaining 19.79% of the variance. The second factor had high loadings of CYP2D6*10 and almost explained similar (19.14%) of variance while the third factor loaded with the GST M1 variant with 14.89% variance explained.

4.8 Stratification of Data on Genetic Damage, Oxidative Stress and Lipid Levels

Stratification of the parameters as a function of different variables (age, disease-related, obesity-related and menopausal status in females) provides information about the differential effects.

4.8.1 Age

Among patients and controls 46.50% each were between 40-60y of age with 53.50% each in the >60-90y group (Table 7a-7b). Genetic damage, oxidative stress and lipid levels in these groups were therefore analyzed for any differential levels. Among various genetic damage parameters, only the damage index was observed to be significantly (p≤0.05) higher in >60y patients (190.23±2.57 AU) than in those who had lower age (180.03±2.88 AU). In parallel age-groups, patients had significantly higher (p<0.0001) genetic damage as compared to controls. Though in patients >60y, total oxidant status (6.78±0.19µmol H₂O₂ equivalent/l), oxidative stress index (18.85±3.63) and MDA (10.75±0.33µmol/l) levels were higher in 40-60y (6.36±0.20 µmol H₂O₂ equivalent/l, 14.87±1.96 and 10.21±0.43 µmol/l, respectively) while total antioxidant capacity decreased with age (0.99±0.10 mmol trolox equivalent/l vs. 0.96±0.09 mmol trolox equivalent/l). A similar non-significant trend was also observed in case of controls for total oxidant status, oxidative stress index and malondialdehyde levels. In both patient and control groups also there was no effect of age on coronary index (TC/HDL), artherogenic index (LDL/HDL) and TG/HDL ratios being non-significantly different. Lipid levels increased with age in patients while decreased in controls.
4.8.2 Hypertension Categories

Stratification of genetic damage, oxidative stress and lipid profile parameters by hypertension categories (Table 8a and 8b) revealed highest genetic damage in individuals in hypertension category III. The genetic damage parameters of per cent tail DNA, tail moment and Olive tail moment were significantly higher (p≤0.05) in hypertension stage III category individuals as compared to levels in high normal and hypertension stage I category individuals. In controls, per cent tail DNA, tail moment and Olive tail moment were higher in high normal category individuals. Hypertension patients in normal and high normal categories had significantly (p≤0.001) higher genetic damage and total oxidative stress as compared to respective control group individuals. Dyslipidemia state was also more in hypertensive patients as compared to the normal control group individuals.

4.8.3 Duration of Treatment

Stratification on the basis of treatment-/disease-duration (Table 9a-9b) revealed a significant (p<0.05) increase in total antioxidant capacity and TG/HDL ratio (p<0.05) with increased duration of treatment. Also though DNA damage parameters of per cent tail DNA, tail moment and Olive tail moment increased with increase in treatment duration, yet these failed to reach statistical significance.

4.8.4 Age-of-onset of Disease

Age-of-onset of disease did not show any significant effect on genetic damage, oxidative stress and lipid profile parameters. However damage index significantly increased with age-of-onset of disease (Table).

4.8.5 Obesity Status

All the assessed biomarkers were separately stratified according to obesity categories of BMI, WC, WHR and WHtR.

4.8.5.1 Body Mass Index (BMI)- Among patients, general obesity was prevalent in 60.50% and on stratification for genetic damage, as such no trends for significant increase was observed within categories except that the high normal category had significantly (p≤0.05) higher per cent tail DNA (17.13±1.58) and Olive tail moment
(37.56±4.11) compared to respective values in the obese patients (13.24±0.78 and 27.56±2.01) (Table 11a and 11b). Similarly, total antioxidant capacity levels (1.25±0.23 mmol Trolox Equivalent/l) were also significantly (p≤0.05) higher in high normal patients than those in obese patients (0.78±0.06 mmol Trolox Equivalent/l). Lipid levels also generally did not significantly vary within patients and controls in different categories though significantly increased dyslipidemia was observed in most categories of patients compared to those in controls. Levels of TC, HDL-C and LDL-C were also significantly higher (p≤0.01) in patients in normal category compared to those in the below normal category while obese patients had significantly higher (p≤0.01) LDL/HDL (atherogenic index) compared to that in the below normal patients.

4.8.5.2 Waist-to-Height Ratio (WHR)- Within the patient group, obese patients had increased genetic damage and oxidative stress levels compared to non-obese patients (Table 12a and 12b) though significant increase was only observed for per cent tail DNA (non-obese hypertensive patients vs. obese hypertensive patients). Non-obese and obese controls also did not differ for levels of genetic damage and oxidative stress markers. However obese patients vs. obese controls and non-obese patients vs. non-obese controls had highly significant (p≤0.001) increased genetic damage and oxidative stress levels.

4.8.5.3 Waist Circumference (WC)- On considering obese and non-obese patient categories on the basis of WC cut-offs, a similar trend was observed with no significant difference for genetic damage and oxidative stress levels despite being higher in obese patients (Table 13a, 13b). However, per cent tail DNA was significantly higher (p≤0.05) in obese (14.79±0.65%) compared to that in non-obese (10.51±1.63%) patients. In controls, no differences were significant though patients and controls differed with significantly raised levels in both non-obese and obese patients.

4.8.5.4 Waist-to-Hip Ratio (WHR)- With 94% obese patients and 90% obese controls on the basis of WHR, patients and controls did not differ for genetic damage, oxidative stress and lipid levels between the obese and non-obese categories except that non-obese patients had significantly higher VLDL-C, TG and TG/HDL levels compared to levels in obese categories (Table 15a-15b). Differences were however significant (p≤0.001) for respective categories between patients and controls.
4.8.6 Menopausal Status

Female participants in patient and control groups were placed in premenopausal (≤45y, 17.82% vs. 23.40%), peri-menopausal (46-54y, 14.85% vs. 11.70%) and post-menopausal (>54y, 67.33% vs. 64.89%) categories.

In pre-menopausal state, menstruation cycle is normal and periods are regular (Meeta et al., 2013). The transition zone between pre-menopausal and post-menopausal stages is known as peri-menopausal stage, and the period after one year of last menstruation cycle is considered as the post-menopausal period.

Genetic damage and oxidative stress did not differ as a function of menopausal status and there was no increasing/decreasing trend with respect to various parameters (Table 16a – 16b). The per cent tail DNA level was highest in peri-menopausal patients and in post-menopausal controls while tail moment, Olive tail moment and damage frequency were maximum in peri-menopausal patients while post-menopausal patients had highest damage index. In controls, tail moment was highest in peri-menopausal, Olive tail moment in post-menopausal and damage index and damage frequency highest in pre-menopausal females. The comparison between patients and controls in all the three categories however revealed significantly higher (p≤0.001) genetic damage in patients.

Though none of the biomarkers of oxidative stress varied significantly in different categories, yet among the patients, the total antioxidant capacity levels were highest in pre-menopausal group while total oxidant status, oxidative stress index and MDA levels were highest in post-menopausal females. Levels of total oxidant status, oxidative stress index and MDA were significantly (p≤0.001) higher in all patient categories when compared to similar control categories while total antioxidant capacity was significantly reduced.

The levels of lipid markers also did not significantly vary in patients in different menopausal categories while TC and LDL-C levels were highest in peri-menopausal patients and VLDL-C and TG levels were highest in pre-menopausal patients. In controls, except for HDL-C levels which were highest in peri-menopausal participants and TC, LDL-C, VLDL-C, and TG levels were highest in pre-menopausal females.
However, TC/HDL, LDL/HDL and TG/HDL levels were significantly (p≤0.001) higher in all the categories of patients as compared to respective categories in controls.

4.8.7 Metabolic Syndrome and Genetic Damage

Genetic damage was also stratified according to presence and absence of metabolic syndrome (MS) in patient and control groups. Though in patients with MS (53.50%), there were increased levels of per cent tail DNA (14.74±0.80), tail moment (31.23±2.14), Olive tail moment (31.23±2.14) and damage frequency (92.06±0.59) compared to those not having MS (53.50%), yet the increase did not reach statistical significance (Table 17). In the control group however, those without the MS had significantly (p≤0.05) higher levels of damage index and damage frequency than controls with the metabolic syndrome probably because of very less very less number of controls as only 4.00% of controls had MS.

4.8.8 Association of Genetic Polymorphisms with Genetic Damage and Oxidative Stress Levels

On stratifying the genetic damage and oxidative stress levels for the CYP2D6 *2, *4 and *10 and GST T1, M1 and P1 genotypes (homozygous wild, variant and heterozygous) among patients, only the CYP2D6*4 heterozygous genotype (GA) showed significantly (p=0.05) higher damage index and homozygous variant (AA) with decreased DI. Such an association was also observed in controls for CYP2D6 *2 heterozygous genotype (AG), homozygous wild (AA) and heterozygous (GG) genotypes of CYP2D6*4 and heterozygous (CT) genotype of CYP2D6 *10 with genetic damage in controls (Table 18a-23b)

4.8.9 Metabolic Phenotypes and Genetic Damage

Based upon metabolizer phenotypes the drug concentration in plasma may range from less than therapeutic levels in UMs to very high toxic concentration in PMs which can result in adverse drug reaction (Nasare et al., 2014). The increased concentration of drug in the plasma may react with DNA causing damage to the DNA molecule (Deavall et al., 2014).
On stratification for metabolic phenotypes on the basis of CYP2D6 *2, *4 and *10 genotypes, in patients damage index was significantly (p≤0.05) higher in intermediate metabolizers (according to CYP2D6 *4 genotype) as compared to that in extensive metabolizers. Similarly in the control group according to CYP2D6*4 genotypes, extensive metabolizers had significantly higher (p≤0.05) per cent tail DNA and Olive tail moment when compared to poor metabolizers. Olive tail moment was also significantly higher in extensive metabolizers as compared to levels in intermediate metabolizers (Table 24).

4.10 Modulators of Genetic Damage and Oxidative Stress in Patients and Controls

The Pearson’s correlation, linear followed by multivariate regression analyses and combined multivariate regression analysis and analysis of variance (ANOVA) were performed on patient data, control data and combined (patient and control) data in order to identify variables that can modulate genetic damage and oxidative stress.

4.10.1 Patient Group

Determinants of genetic damage and oxidative stress evaluated by correlation analysis (25a to 25c) were alcohol-intake, physical activity, BMI, SBP, DBP, PP, MAP, family history and lipid profile (TC, HDL, LDL, VLDL).

On linear univariate regression analysis and analysis of variance, association of genetic damage parameters was observed with BMI, PP, HDL, TC/HDL, LDL/HDL ratio, TAC and family history. However on univariate followed by multivariate regression analysis only PP, HDL, TC/HDL and LDL/HDL ratios were retained.

As no factors act independently in causing genetic damage, hence to study the combined effect on dependent variables, combined multivariate regression analysis was also performed. The analysis revealed physical activity, SBP, PP, TC, HDL, TC/HDL, LDL/HDL and TAC as predictors of genetic damage but not BMI.

4.10.2 Control Group

Correlation analysis revealed significant association of dietary pattern, cooking medium used, mobile phone usage, socioeconomic status, BMI, WHtR, TC, HDL-C, LDL-C, VLDL-C, TG, TC/HDL and TG/HDL (Table 26a to 26).
Univariate analysis predicted cooking medium used, BMI, mobile phone usage, HDL-C, LDL-C, VLDL-C, TG and TG/HDL. On multivariate regression analysis cooking medium used, BMI, mobile phone usage, HDL-C, LDL-C, VLDL-C, TG and TG/HDL were retained. The combined multivariate regression analysis however revealed cooking medium used, socioeconomic status, BMI, HDL-C, LDL-C, TG/HDL and TOS as genetic damage predictors. Analysis of variance also showed same association as shown by linear regression analysis.

4.10.3 Total Study Group

Overall correlation analysis revealed that blood pressure measurements (SBP, DBP, MAP, PP), lipid levels (TC, HDL-C, LDL-C, VLDL-C, TG, TC/HDL, LDL/HDL), obesity measures (HC, WC, BMI), oxidative stress parameters (MDA, TAC, TOS), socioeconomic status, diet and family history of the disease showed highly significant (p≤0.001) association with genetic damage parameters.

Univariate regression analysis predicted SBP, DBP, MAP, PP, BMI, TC, TG, HDL-C, LDL-C, VLDL-C, TC/HDL, LDL/HDL, MDA, TAC, TOS as factors influencing genetic damage on subsequent multivariate analysis SBP, DBP, BMI, PP, TG, TAC and TOS were retained. However combined multivariate regression analysis exhibited a reduction gender, diet, alcohol-intake, BMI, DBP, family history of the disease and oxidative stress parameters were revealed as predictors of genetic damage. Analysis of variance also revealed the significant association of blood pressure and lipid parameters with genetic damage.

4.11 Genetic Damage and Genetic Polymorphisms

4.11.1 Patients

In patient group GST M1 genotype was significantly associated with Per cent tail DNA and tail moment as revealed by correlation and combined multivariate regression analysis. While linear regression analysis revealed the association of GST M1 with tail moment only.

4.11.2 Controls

After correlation analysis GST P1 is significantly associated with total antioxidant capacity. No association was observed with linear regression analysis and combined multivariate regression analysis.
4.11.3 Total Study Group

In the total group correlation, regression and ANOVA revealed significant association of CYP2D6*4 and GST P1 genotype with per cent tail DNA, OTM, DI and DF.

4.12 Principal Component Factor Analysis (PCA) for prevalent Disease-Risk Factors

Hypertension is a multifactorial disease which has strong environmental influence in combination with its polygenic nature being influenced by both, genetic and life style factors such as obesity, dietary pattern, physical inactivity alcohol intake and smoking. Hence these are good predictors of hypertension and being inter- and co-related, are equally responsible in inducing hypertension. Therefore, principal component factor analysis was performed to extract independent factors from the large number of inter-correlated factors (Goodman et al., 2005; Sundaram et al., 2010). Such a method of data-reduction has been used for identification of clustering of risk factors in cardiovascular diseases (Cox et al., 2008; Kaur et al., 2012).

PCA performed for 40 variables including genetic damage as probable risk factors for hypertension (Table 28a) and also 35 variables excluding the genetic damage parameters (Table 28b).

4.12.1 PCA with Genetic Damage Parameters

On performing PCA for disease-status, from a total of 40 variables, 16 explained 77% of the total variance for the disease. Factor one had high loading of lipoproteins explaining the largest portion of variance (8.50%) comprising LDL-C, TC/HDL, LDL/HDL which are strong indicators of atherosclerosis. Factor two predominantly loaded with blood pressure-related variables (SBP, DBP, MAP, PP) explaining 8.34% of variance while 6.12% of variance from factor three comprised genetic damage parameters of percent tail DNA, tail moment and Olive tail moment. The factors 4 and 5 reflect dyslipidemia in hypertensive patients, loading high on lipid parameters with 5.15% of variance. The factor 6 loaded the obesity traits of WC and WHtR. Genetic determinants of CYP2D6 (*10) and GST (M1) also explained 3.88% of the total variance at factor 11, with oxidative stress index (OSI) and GSTT1 genotype at factor
12 with 3.20% of variance and 3.03% variance from drug-treatment/duration and the CYP2D6*2 genotype at factor 13.

4.12.2 PCA Excluding Genetic Damage Parameters

As genetic damage parameters are directly not included in hypertension risk factors, so PCA was also performed for disease-status including all the risk factors but excluding genetic damage parameters.

A total of 15 factors were loaded from original 36 variables explaining a total of 78.43% variance which was higher than for 16/40 variables (77%). The factor loadings however loaded same as loaded when genetic damage parameters were included in the analysis. Factor one (9.53%) loaded with lipoprotein-related parameters, factor two (9.41%) with blood pressure-related variables, factors three (6.61%) and four (6.58%) with lipid profile parameters and factor five with obesity-related parameters. Oxidative stress parameters loaded at factor eight explaining 4.65% of the variance. The factor 11 was loaded with socioeconomic status and CYP2D6*10 genotype explaining 3.33% variance.

4.13 Multifactor Dimensionality Reduction (MDR) for Disease-Status

One of the objectives of the present study was to identify polymorphism of CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) genes, which may present an increased risk for hypertension and genetic damage. However in complex diseases like hypertension, such a relationship is difficult to characterize, as both, genetic and environmental factors play an important role in disease-pathophysiology. Multifactor dimensionality reduction (MDR) can reduce the dimensionality of multifactor data into only one dimension and enables detection of gene-gene and gene-environment interactions. The MDR analysis was hence performed for gene-gene interactions (CYP2D6 and GST) as well as for gene-environment interactions by considering CYP2D6 and GST genes, disease-specific risk factors and disease-specific information. This study is the first of its kind to explore complex gene-gene and gene-environment interactions prevalent in essential hypertensive patients belonging to Punjabi Jat Sikh population sub-group.
4.13.1 Gene-Environment Interactions (including genetic damage parameters)

The MDR analysis comprising a total number of 40 genetic and non-genetic factors revealed that the combinations of MDA with OSI as well as DF, MDA and TAC were risk combinations for hypertension (Table 29a). The best combination model with high Testing accuracy TA=0.96 and Cross Validation CV consistency=10/10 comprised DF, MDA and TAC revealing highest risk (OR=1315.28; 95% CI=294.93-5865.75; p<0.0001) for hypertension. On the basis of MDA and OSI, there were, 85.50% (n=171) low-risk individuals in controls with only one (0.50%) among patients. High-risk individuals for hypertension are those with higher than median values of MDA and OSI which comprised 99.50% (n=199) of patients and 14.50% (n=29) in controls. The best three-factor model (MDA, DF and TAC) further revealed that hypertensive patients 99.00% of hypertensive patients and 14.50% of controls had more than median values of MDA and DF and less than median value of TAC being at increased risk for hypertension (Table 21a and 21b; Fig 21c-21d).

4.13.2 Gene-Environment Interactions (excluding genetic damage parameters)

This analysis also revealed that lipid peroxidation and oxidative stress (MDA-OSI and MDA-TAC-TOS models) posed increased risk for hypertension (Table 29b). The best model with testing accuracy of 0.955 and CV consistency of 10/10 was MDA-TAC-TOS with 1138.5 times higher risk (OR= 1138.5, 95% CI=258.22-5019.68; p<0.0001) for developing hypertension. The best model revealed that 99% of the patients and 8% of controls with MDA and TOS values more than median and TAC values less than median were at highest risk for developing hypertension (Fig21c to 21d).

4.13.3 Gene-Gene Interactions

Genetic variation in several gene pathways affects blood pressure and the pathways may interact with each other. Statistical significance was checked empirically by performing the permutation test and the MDR analysis on the case-control data on the six functional polymorphisms of CYP2D6 and GST provided evidence for significant epistatic interactions for hypertension viz. of CYP2D6*4, CYP2D6*10 and GST P1 and CYP2D6*4, CYP2D6*10 and GST P1. The best model based upon testing accuracy and CV consistency exhibited interaction of CYP2D6*4, CYP2D6*10 and GST P1 indicating
~7.4 times higher (OR=7.3889; 95% CI=4.7417-11.5141; p<0.0001) disease-risk. The two-loci combinations in individuals with one or both mutant alleles for CYP2D6*4 and GST P1 were at increased risk of developing hypertension (22% patients and 16.50% controls) and in the three-loci genotypic combination, 12% patients and 2% controls with heterozygous genotypes for CYP2D6*4, CYP2D6*10 and GST P1 were at highest risk for disease and are depicted in Fig 22a.

**4.14 Multifactor Dimensionality Reduction (MDR) for Genetic Damage End-points**

MDR analysis was performed for the best gene-environment and gene-gene combinations that predict the various genetic damage parameters.

**4.14.1 Gene-Environment Interactions**

After adjustment for age, gender, dietary pattern, alcohol consumption, family history of disease, BMI, TAC and TOS, MDR analysis revealed that the combination of CYP2D6*10, GST P1 and socioeconomic status was the best predictor (TA=0.6815; CV=9/10) for percent tail DNA (OR=5.9492; 95% CI=4.4559-7.9429; p<0.0001) and tail moment (TA=0.6753; CV=6/10; OR=5.311; 95% CI=4.3298-6.5149; p<0.0001). About 17% (16.50%) participants belonging to upper-middle class and with homozygous wild genotypes of CYP2D6*10 and GST P1 genes were at maximum risk for increased genetic damage followed by 11.50% belonging to the lower-middle class having the same genotypic status. For increased Olive tail moment, 18.25% with OTM greater than median values and having homozygous wild genotype of CYP2D6*10, and heterozygous genotype of GST P1 belonging to upper-middle class were at maximum risk for genetic damage (Table 30b; fig 23a-23i).

The best predictor model for both, damage index and damage frequency included combination of GST P1, BMI and TOS (TA=0.872; CV=10/10 and TA=0.8602; CV=8/10, respectively). The results revealed that individuals in the high normal BMI category with homozygous variant genotype of GST P1 and having TOS levels more than the median value were at maximum risk for increased damage index and damage frequency.
4.14.2 Gene-Gene Combinations

The MDR analysis when performed on CYP2D6 and GST variants separately. CYP2D6*2, *4 and *10 for genetic damage parameters revealed that the combination of heterozygous genotypes of CYP2D6*4, CYP2D6*10 and homozygous wild genotype of CYP2D6*2 is at highest risk of genetic damage. While For GST gene variants the combination of heterozygous genotype for GST P1, present GST T1 present and GST M1 null genotypes were at maximum risk for the genetic damage.

The MDR analysis comprising genotypic status of CYP2D6 and GST variants with genetic damage parameters revealed that three loci combination of CYP2D6*4, CYP2D6*10 and GST P1 was the best combination for per cent tail DNA (testing accuracy=0.6175; CV consistency=10/10), tail moment (testing accuracy=0.5475; CV consistency=6/10), Olive tail moment (testing accuracy=0.5925; CV consistency=10/10), damage index (testing accuracy=0.7025; CV consistency=10/10) and damage frequency (testing accuracy=0.7025; CV consistency=10/10). For all the genetic damage parameters, the individuals with heterozygous genotypes of CYP2D6*4, CYP2D6*10 and GST P1 were at increased risk for increased levels of genetic damage (Table 30a; Fig 22c to 22l).