

Susceptibility of NPPA and IL6 with Type 2 Diabetes and Hypertension in Punjab, Pakistan

Andleeb Batool

GC University, Lahore, Pakistan, bandleeb@yahoo.com

Riffat Iqbal

GC University, Lahore, Pakistan

Maryam Mukhtar

GC University, Lahore, Pakistan

Hong Xue

Hong Kong University of Science and Technology, Clear Water Bay Kowloon, Hong Kong

Follow this and additional works at: <https://corescholar.libraries.wright.edu/jbm>



Part of the [Biodiversity Commons](#), and the [Biology Commons](#)

Recommended Citation

Batool, A., Iqbal, R., Mukhtar, M., & Xue, H. (2015). Susceptibility of NPPA and IL6 with Type 2 Diabetes and Hypertension in Punjab, Pakistan, *Journal of Bioresource Management*, 2 (3).

DOI: 10.35691/JBM.5102.0028

ISSN: 2309-3854 online

This Article is brought to you for free and open access by CORE Scholar. It has been accepted for inclusion in *Journal of Bioresource Management* by an authorized editor of CORE Scholar. For more information, please contact library-corescholar@wright.edu.

Susceptibility of NPPA and IL6 with Type 2 Diabetes and Hypertension in Punjab, Pakistan

© Copyrights of all the papers published in Journal of Bioresource Management are with its publisher, Center for Bioresource Research (CBR) Islamabad, Pakistan. This permits anyone to copy, redistribute, remix, transmit and adapt the work for non-commercial purposes provided the original work and source is appropriately cited. Journal of Bioresource Management does not grant you any other rights in relation to this website or the material on this website. In other words, all other rights are reserved. For the avoidance of doubt, you must not adapt, edit, change, transform, publish, republish, distribute, redistribute, broadcast, rebroadcast or show or play in public this website or the material on this website (in any form or media) without appropriately and conspicuously citing the original work and source or Journal of Bioresource Management's prior written permission.

SUSCEPTIBILITY OF NPPA AND IL6 WITH TYPE 2 DIABETES AND HYPERTENSION IN PUNJAB PAKISTAN

Andleeb Batool^{1*}, Riffat Iqbal¹, Maryam Mukhtar¹, Hong Xue²

¹Department of Zoology, G.C. University, Lahore

²Applied Genomic Centre and Division of Life Sciences, Hong Kong University of Science and Technology, Clear Water Bay Kowloon, Hong Kong

Email: andleeb@yahoo.com

ABSTRACT

Type 2 Diabetes (T2D) and Hypertension are the major health issues affecting quality of life of young ages throughout the world, especially the third world countries facing more complications with diabetes due to poor disease management. The present study was conducted to explore the association of genetic polymorphism with T2D and hypertension in the Punjabi population. The case control study was conducted comprising of 288 patients (118 male, 170 female) and 170 controls (104 male, 66 female). The selected genes along SNPs were NPPA (rs5064 G>A, rs5063 C>T) and IL6 (rs1800796 C>G). DNA was amplified by Nested PCR and sequencing was performed for genotyping. The rs5063 and rs5064 from NPPA was not associated with hypertension and not involved in the predisposition of diabetes ($p < 0.05$). Moreover, rs1800796 (IL6) showed an association ($p < 0.001$) with diabetes (OR = 0.394 (0.265, 0.584)). SNPs analysis with demographic data confirmed that rs1800796-CC ($p = 0.008$) is significantly associated with positive family history of T2D. Risk of T2D development associated with IL6 was confirmed, whereas NPPA was not associated with hypertension.

Keywords: NPPA, IL6, T2D, SNPs

INTRODUCTION

Type 2 Diabetes (T2D) and hypertension have emerged as major health problems worldwide, and they are increasing rapidly in most Asian countries (Rees *et al.*, 2011). Hypertension is a quantitative trait that varies among subjects according to environmental factors for a short duration and diurnal patterns for longer-term changes (Lewington *et al.*, 2002). It has been reported that T2D is 4 to 6-fold more common in South Asians than in Europeans (Rees *et al.*, 2011). According to the International Diabetes Federation, T2D prevalence is higher in Asian countries and the top five countries with a diabetic population were India (50.8 million), China (43.2 million), Pakistan (7.1 million), Japan (7.1 million) and Indonesia (7 million) (Yang *et al.*, 2010).

Many studies suggested that hypertension is heritable, although environmental factors and obesity were sustainably contributing to the high incidence of hypertension (Luft, 2001; Haslam and James, 2005; Kearney *et al.*, 2005, Tobin *et al.*, 2005). Tobin *et al.* estimated that the chance of hypertension heritability in a population based study is about 65% (Tobin *et al.*, 2005). The incidences of Hypertension varied across the world. Its highest incidences were reported in Poland (68.9% in men and 72.5% in women) and lowest incidences were reported in rural areas of India (3.4% in men and 6.8% in women) from 1980 to 2003 (Kearney *et al.*, 2004). Hypertension is more common in urban areas (22.7%) as compared to rural areas (18.1%) in Pakistan (Jafar *et al.*, 2003). As the incidences of

hypertension and T2D increase worldwide, it is important to determine the genetic risk factors of hypertension (Cowley, A.W. 2006). T2D and hypertension are multifactorial diseases; undoubtedly both genetic as well as environmental factors contribute to the development of disease (Rees *et al.*, 2011).

A Natriuretic Peptide Precursor A gene (*NPPA*; chromosome location 1p36.21) containing rs5064 was reported to be associated with hypertension, which is involved in the predisposition of diabetes. The Interleukin-6 (*IL6*; chromosome location 7p21) gene was reported to be involved in insulin resistance and increased the risk of T2D development (Conen *et al.*, 2007; Meiner *et al.*, 2008; Yin *et al.*, 2013).

Pakistanis are more vulnerable to T2D and hypertension because of the rapid urbanization, susceptible life style (more indoor activities) and cousin marriages. The population of Pakistan consists of vast heterogeneity due to diverse geographical area and ethnic groups. Diversity within our population depicted a different genetic makeup in relevance to Europeans and Americans (Shera *et al.*, 2007). There is no study available on selected genes in our population. Family history is a major risk factor in diabetes development in the Punjabi population of Pakistan (Zafar *et al.*, 2007; Ralph and Coop, 2013). Three single nucleotide polymorphisms (SNPs) are selected from *NPPA* and *IL6*. The aim of the current study was to report the genetic association of selected SNPs with T2D and Hypertension.

MATERIAL AND METHODS

Ethical Approval

The current study was approved by the Board of Advanced Research, G.C. University, Lahore, Pakistan and Diabetic center of Jinnah Hospital Lahore, Pakistan.

A written consent was taken from patients and controls before sampling.

Subjects

A total of 458 Punjabi subjects (case = 288; controls = 170) were included in the study. The cases were diagnosed with T2D by a physician according to WHO criteria. All control subjects were healthy and had a negative family history of T2D. Demographic characteristics such as age, age of diagnosis, Body Mass Index (BMI), hypertension and complications of the subjects were studied.

DNA Isolation

The blood samples (3 ml) were collected in EDTA coated tubes and stored at 4°C for DNA extraction. DNA extraction was performed by Fermentas DNA purification kit (#K0512). DNA samples were stored at -20°C for further genetic analysis.

Nested PCR

Primers for Polymerase Chain Reaction (PCR) along annealing temperatures are listed in Table 1. DNA fragments of interest were amplified by using nested PCR. DNA amplification was carried out in a 20 µl reaction mixture consisting of 50-60ng of genomic DNA, 2.5 mM magnesium chloride (MgCl₂, Sigma: M2670), 200 µM of each deoxyribonucleotide triphosphate (dNTPs: promega USA U1515), 125 nM of each primer and 1U *Taq*DNA polymerase (Amersham Bioscience, Uppsala, Sweden). PCR cycle conditions were as follows: 5 min for denaturation at 95°C, 30 cycles of the three steps of 95°C for 30 sec, 30 sec at 56°C, 2 min at 72°C, and then final extension at 72°C for 8 min. PCR products were purified by manual method (ethanol) which is as follows: PCR products were precipitated with chilled 100% ethanol and

then washed with 70% ethanol. After washing, pellets were dried and dissolved in

Table 1: The primer sequences used for PCR along with optimized conditions.

Gene	SNP ID	1 st PCR	Nested PCR	PCR Product (bp)	*Temp (°C)
NPPA	rs5063	F: CCCCCTTCTTCATTTCGGCT	F: ACTTGTGGGGGCACGACCTCAT	568	60
	rs5064	R: TGTGACCTTGGTGGCTCGGT	R: CTGTGACAAGCCCTGCGGGATG		
IL6	rs1800796	F: ACAATAGCCGCCGGGAAGTCCT R: TGCGAGGTGCCTTTGGAGCA	F: CACAGTTGCTGCAGGGCAGTTC R: AGCCAGACATTCAACAAGAGCACC	674	60

bp = Base Pairs; * = Annealing Temperature; F = Forward Primer; R = Reverse Primer.

DEPC water. It was stored at 4°C for genotyping.

Direct DNA sequencing

Direct sequencing of the nested PCR product was executed for SNP genotyping (Lo *et al.*, 2007). Sequencing reaction contained 0.75 µl of Big Dye (ABI, 1201096), 14.5 µl Milli Q H₂O, sequencing buffer 3.00 µl (ABI 1203142), 240 nM primer and 1.5 µl of PCR product. The sequencing reaction was initially denatured at 96°C for 1 min followed by 25 cycles, and each cycle was of 10 sec at 96°C, 5 sec at 50°C, and 4 minutes at 60°C. After the cleanup of the post-sequencing mixture with ethanol, the air-dried sequencing sample was dissolved by adding 10µl of Hi-Deionized formamide (Applied Biosystems Inc.). It was denatured at 95°C for 5 min and chilled to 4°C prior to sequencing (Model 3130 Genetic Analyzer, Applied Biosystems).

Statistical Analysis

The distributions of demographic characteristics among studied subjects were presented in numbers and percentages (%). First, the selected SNPs were analyzed by

the Hardy-Weinberg Equilibrium (HWE) using

the Genepop software on the web. All allelic and genotypic frequencies in case-control analysis were compared and calculated by using the Chi square test (χ^2) as well as by SHEsis: <http://analysis.bio-x.cn/myAnalysis.php>. Associations between age of diagnosis, gender, hypertension and genotypes by logistic regression (SPSS version 18) *P* values less than 0.05 were considered to be significant. Changes in amino acid sequences were determined by aligning sequences on Mega 6 software.

RESULTS

A total of 458 subjects were included in the study with 20 and 22 years minimum age of diabetes diagnosis for males and females respectively (Table. 2). Certain SNPs were associated with T2D. The selected SNPs did not deviate from the Hardy- Weinberg equilibrium ($p > 0.05$), but rs1800796 did ($p > 0.01$). From the three studied SNPs, only rs1800796 [$p - 0.0001$, OR- 0.394 (95% CI 0.265-0.584)] was associated with T2D in single site analysis (Table 3).

Table 2: Demographic characteristics of samples used in the study.

Characteristics	Case (n = 288)		Control (n = 170)	
	Male	Female	Male	Female
Number	118	170	104	66
Age of diagnosis (y)	42.86±1.123	41.18±0.824	-	-
Minimum age of diagnosis (y)	20	22	-	-
Family history	74 (62.71%)	110(64.70%)	Negative	Negative

Hypertension	53(44.91%)	91 (53.53%)	Negative	Negative
--------------	------------	-------------	----------	----------

Table 3: Allelic and Genotypic frequency of genetic variants in T1D and control samples used in the study.

SNP	Patients n (%)	Controls n (%)	χ^2 Value	<i>p</i> Values
rs5063				
Genotype				
CC	154 (84.6%)	104 (83.9%)	1.79	0.408
CT	24 (13.2%)	14 (11.3%)		
TT	4 (2.2%)	6 (4.8%)		
Allele				
C	332 (91.2%)	222 (89.5%)	0.49	0.482
T	32 (8.8%)	26 (10.5%)		
rs5064				
Genotype				
AA	5 (2.6%)	1 (1.0%)	1.19	0.552
AG	38 (19.6%)	17 (17.0%)		
GG	151 (77.8%)	82 (82.0%)		
Allele				
A	48 (12.4%)	19 (89.5%)	1.077	0.299
G	340 (87.6%)	181 (10.5%)		
rs1800796				
Genotype				
CC	91 (34.2%)	44 (57.9%)	18.07	0.001*
CG	77 (29.3%)	22 (28.9%)		
GG	95 (36.1%)	10 (13.2%)		
Allele				
C	259 (49.0%)	110 (71.9%)	25.43	0.007*
G	267(0.8%)	42 (27.5%)		

*: Represents Significance $p < 0.01$

In analysis of genotype frequency shown in Table 3, Genotype CC of rs1800796 was increasing the risk of diabetes development in comparison to CG and GG genotypes, and rs1800796 is significantly associated with diabetes onset ($p < 0.001$). However rs5063 ($p = 0.408$) and rs5064 ($p = 0.552$) did not depict association with hypertension at the genotype level.

Haplotypes analysis of rs5063 and rs5064 indicated that CA, CG, TA and TG were not significantly associated with hypertension and T2D onset ($p > 0.05$),

whereas the rest of the haplotypes were not associated with T2D development.

Protein alignment analysis on Mega 6 software showed a change in the nucleotide at the rs5063 polymorphic site, causing the change of alanine into threonine shown in Figure 1, (a). Mutation at rs5064 leads to the change of glutamine into arginine (Figure 1: (b). Similarly, in the case of the 1800796 polymorphic site, the change in the single nucleotide leads to the change of proline into arginine (Figure 1: (c).

The genotype CC of rs1800796 (*IL6*) was associated with a family history of

diabetes ($p = 0.008$) and was also gender specific in patients ($p = 0.002$).

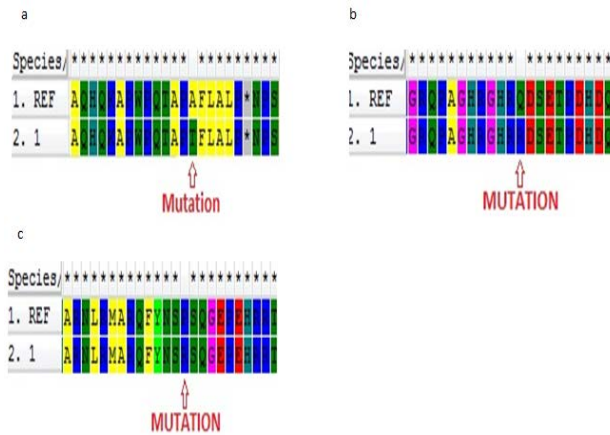


Figure 1: (a) Amino acid alignment of rs5063; (b) Amino acid alignment of rs5064; (c) Amino acid alignment of rs1800775.

DISCUSSION

Diabetes is a multifactorial disease; genetic as well as environmental factors play a crucial role in the onset of diabetes. The minimum age of diagnosis was 20 for males and 22 for females in the investigated population. The survey found that a majority of the diabetic men and women had a family history of diabetes, with 62.7% and 64.7% respectively. Positive family history was reported as a strong risk factor for the onset of diabetes and an indicator for the risk of disease development (Zafar *et al.*, 2007; Ralph and Coop, 2013).

Out of the three studied genetic variants, one exhibited a significant association with the disease (Table 3). To the best of our knowledge, this is the first study in any of Pakistani populations to report an association between the disease and the SNPs in the selected genes.

Family history is a strong predictor for the onset of T2D. It is a complex disease. A number of genes interact with each other

and multiple genetic variants are involved in the development of diabetes. Province Punjab of Pakistan is a multicultural and multiethnic region with specific traditions from generation to generation. The caste system is strong and within caste or cousin marriages are very common. This tradition narrows down the gene pool with little genetic recombination. Even this mechanism sometimes disturbs the allele and genotype frequency in the population that ultimately leads to the violation of Hardy-Wienberg Equilibrium. The genome-wide associations throughout the world identified a number of loci associated with T2D.

Chromosome 1 was previously reported as a susceptible loci linked with T2D in different populations. Our genetic data provided no association between rs5063 and rs5064 in the *NPPA* gene with hypertension in diabetics [$p > 0.01$]. The rs5064 form *NPPA* was reported to be associated with hypertension in a follow-up study of Americans (Tun-Sen *et al.*, 2013). Although rs5063 was not significantly associated with diabetes and hypertension, it is first time reported in Pakistani population and not reported in any ethnicity throughout the world.

Only rs1800796 in *IL6* remained significantly associated with T2D after a number of tests performed. SNP rs1800796 in *IL6* was found to be significantly associated with the development of T2D [$p = 0.001$, OR = 0.394 (0.265, 0.584)] in south Asians (especially Pakistanis). The rs1800796-G effective risk allele contributed to the onset of diabetes in the Punjabi population, but its C allele was protective against diabetes with 0.719 frequencies in controls. Elevated *IL6* level in plasma was found to be considerably associated with hypertension in females (Cheung *et al.*, 2011). It is controversial in various ethnic groups worldwide that rs1800796-C/G genotype has been the risk for the onset of

T2D. In line to our study, Yin *et al* (2013) evidently found the significant association between the risk of disease and rs1800796 [OR = 1.29 (1.09-1.52), $p = 0.002$] in the Chinese population. It is suggested that -572 G allele increased the risk of T2D in the corresponding population (Yin *et al.*, 2013).

CONCLUSION

Conclusively, two SNPs in *NPPA* and one in *IL6* genes were studied in a Pakistani population by performing a case control analysis. We confirmed that the SNPs in *IL6* were associated with the risk of T2D. Consequently, these finding may be helpful in advanced clinical practices and public health genomics. Further studies are required in the Pakistani population with the prospective of genetics and environmental factors at large scale to reduce the risk of diabetes.

Acknowledgment

We are thankful to Prof. Dr Javed Akram Ex-CEO, Jinnah Hospital Lahore for allowing blood sampling from diabetes patients at Diabetes center.

REFERENCES

Cheung BM, Ong KL and Tso AW (2011). Relationship of plasma interleukin-6 and its genetic variants with hypertension in Hong Kong Chinese. *American Journal of Hypertension*. 24, 1331-1337.

Conen D, Glynn JR, Buring EJ, Ridker MP and Zee RY (2007). Natriuretic Peptide Precursor A Gene Polymorphisms and Risk of Blood Pressure Progression and Incident Hypertension. *Hypertension*. 50, 1114-1119.

Cowley AW (2006). The genetic dissection of essential hypertension. *Nat Rev Genet*. 7, 829-40.

Haslam DW and James WP (2005). Obesity. *Lancet*. 366, 1197-209.

Jafar TH, Levey AS, Jafary FH, White F, Gul A, Rahbar MH, Khan AQ, Hattersley A, Schmid CH and Chaturvedi N (2003). Ethnic subgroup differences in hypertension in Pakistan. *Journal of Hypertension*. 21(5), 905-912.

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK and He J (2005). Global burden of hypertension: analysis of worldwide data. *Lancet*. 365, 217-23.

Kearney PM, Whelton M, Reynolds K, Whelton PK and He J (2004). Worldwide prevalence of hypertension: a systematic review. *Journal of Hypertension*. 22(1), 111-119.

Lewington S, Clarke R, Qizilbash N, Peto R and Collins R (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 360, 1903-1913.

Luft FC (2001). Twins in cardiovascular genetic research. *Hypertension*. 37, 350-6.

Meiner V, Friedlander Y and Milo H (2008). Cholesteryl ester transfer protein (CETP) genetic variation and early onset of non-fatal myocardial infarction. *Annals of Human Genetic*. 72, 732-741.

Ralph P and Coop G (2013). The geography of recent genetic ancestry across Europe. *PLoS Biology*. 10, 1371.

Rees DS, Hydrie MZ, Shera AS, Kumar S and O'Hare JP (2011). Replication of 13 genome-wide associations (GWA)-validated risk variants for

- type 2 diabetes in Pakistani populations. *Diabetologia*. 54, 1368-1374.
- Shera AS, Jawad F and Maqsood A (2007). Prevalence of diabetes in Pakistan. *Diabetes Research and Clinical Practice*. 76, 219-222.
- Tobin MD, Raleigh SM, Newhouse S, Braund P, Bodycote C and Ogleby J (2005). Association of WNK1 gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. *Circulation*. 112, 3423–9.
- Tun-Jen H, Hwang Y, Can-Hong L, Hua-Mei C and Lin E (2013). Association of the C825T polymorphism in the *GNB3* gene with obesity and metabolic phenotypes in a Taiwanese population. *Genes and Nutrition*. 8, 137–144.
- Yang W, Lu J, Weng J, Jia W and Ji L (2010). China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. *The New England Journal of Medicine*. 362, 1090–1101.
- Yin WY, Sun QQ and Zhang BB (2013). Association between the interleukin-6 gene -572 C/G polymorphism and the risk of type 2 diabetes mellitus: a meta-analysis of 11, 681 subjects. *Annals of Human Genetics*. 77, 106-114.
- Zafar J, Bhatti F and Akhtar N (2011). Prevalence and risk factors for diabetes mellitus in a selected urban population of a city in Punjab. *Journal of Pakistan Medical Association*. 61, 40- 47.
-