

Effect of natriuretic peptides (BNP) gene T-381C polymorphism on the levels of BNP and NT-proBNP in patients with cardiovascular disease

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Abstract : Objectives: The study was designed to consider the impact of BNP gene T-381C polymorphism on the plasma human level of BNP and NT-proBNP and compare the results that will obtain with healthy control.

Design and methods: The present study was performed on (70) patients, (35) of them with ACS and the other (35) with HF. The study also included (22) subjects have been taken as control group. Whole blood samples received from study subjects had been used to extract DNA for the study of polymorphism in BNP gene by way of PCR-RFLP technique.

Results: The BNP gene T-381C polymorphism was detecting by using PCR-RFLP. The alleles were designated as TT, TC and CC. There was statistically no significant difference in each the genotyping distribution and allelic frequency between each patient corporations and healthy control group ($P > 0.05$). The current study showed that subjects with TC and CC genotype had the highest level of BNP and NT-proBNP in all study groups than TT genotype, also, all patients ($N=70$) with C allele had significant high level of natriuretic peptides than T allele.

Conclusion: T-381C polymorphism in the BNP gene affects the level of natriuretic peptides where CC genotype and C allele is associated with greater levels of BNP and NT-proBNP in cardiac patients.

Key words: Acute coronary syndrome, heart failure, B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide, polymerase chain reaction, and polymorphism.

Introduction

The human BNP gene, NPPB, is positioned on chromosome 1p36.2 and contains 3 exons and 2 introns. The complete nucleotide sequence of BNP used to be first described at the end of the 1980s¹. Exon 1 of the human BNP gene encoded the 5' untranslated region (UTR) and a part of preproBNP (the 26-amino acids signal peptide and the first 18 amino acids of proBNP); exon 2, the amino acids from 45-129; and exon 3, the 5' terminal amino acids (from 130 to134) and the 3'UTR¹. Single nucleotide polymorphism (SNP) is a DNA sequence variant triggered by means of the changes of bases of a single nucleotide. Human genome consists of about 3 million SNP, and SNP happens for every a thousand bases. Studies show that the polymorphism of NPPB gene is closely correlated with diabetes, hypertension, MI and HF. Therefore, NPPB is now considered one candidate gene for the genetic susceptibility to cardiovascular diseases^{2,3}. BNP concentrations are affected by using not solely age, gender, cardiac load, and clearance, but additionally via genotype^[4]. Our study aims to assess the impact of the common polymorphism (T-381C) in BNP gene on the levels of natriuretic peptides.

Methods

This study was carried out at the laboratories of Biochemistry Department, College of Medicine/ University of Babylon. The collection of samples was conducted during the period from 1st of December 2015 until 30th of April 2016. The present study was conducted on (70) patients, (35) of them with acute coronary syndrome in the age group ranging from 40-75 years (this group comprised of males 48.6 % and females 51.4%) and the other (35) with heart failure in the age group ranging from 48-79 years (this group comprised of males 48.6% and females 51.4%). The study also included (22) apparently healthy individuals were taken as a control group of the age ranging from 40 - 79 years (this group comprised of males 50% and females 50%). The age and sex of this group were matched to age and sex of patient groups, where statistical p. value > 0.05. Each individual who contributed to the study underwent full history and physical examination along with age, gender, dwelling, and smoking, past history of diseases, family history and medications.

DNA extraction

The FavorPrep™ Genomic DNA Extraction Mini Kit was used to extract of DNA from whole blood samples for the study of the extraction of DNA ^{5,6} and polymorphism in BNP gene.

BNP gene amplification for PCR-RFLP analysis

Amplification of BNP gene was done by polymerase chain reaction (PCR). Amplification was performed in a programmable thermal cycler gradient PCR system. The forward primer: 5'-CTG TGA GTC ACC CCG TGC TC-3' and reverse primer: 5'-GGC AGG AAC GCG CTG GAG AC-3' were used⁷.

Table (1): Amplification conditions of BNP gene

Stage	Temp.(C°)	Time(MIN)	Function	Cycles
1	95	5	Initial denaturation	
2	95	1	DNA denaturation	35
	58	1	Primer annealing	
	72	1:30	Template elongation	
3	72	5	Final elongation	
4	8	-	Incubation	Hold

Results:

The presence of DNA extracted detected by using agarose gel electrophoresis technique⁸⁻¹⁰. The extracted DNA was colorless, so a bromophenol blue dye was used with DNA to ease the loading step of the electrophoresis procedure, as shown in fig. (1).

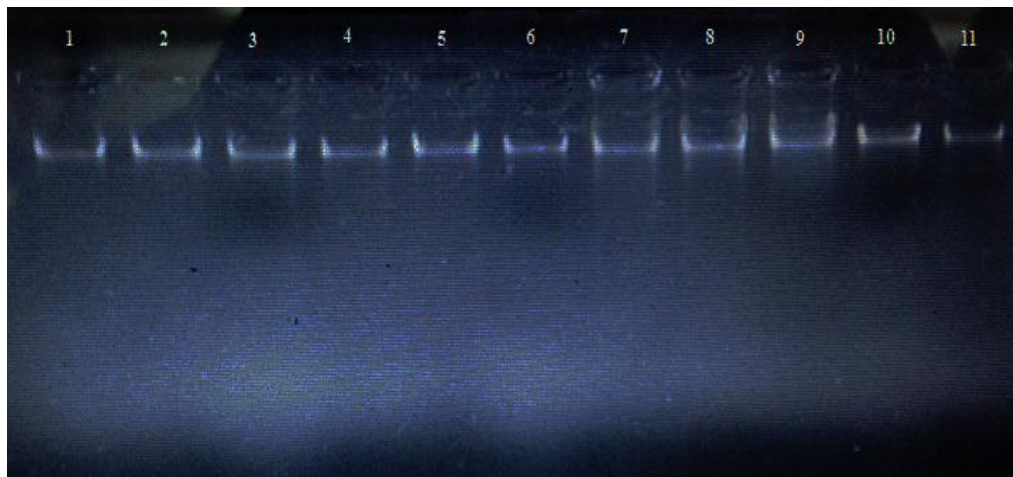


Figure (1): Genomic DNA extracted from whole blood. Lane (1-5): DNA extracted from controls, and lane (6-11): DNA extracted from patients, 1% agarose.

Then the amplification products were separated by electrophoresis through 2% agarose gel stained with SimplySafe™ blue stain. The PCR product length was 186 bp¹ as shown in fig. (2). The PCR product was digested with the restriction enzyme depending on the methods that described by MspI (Moraxella species)⁷

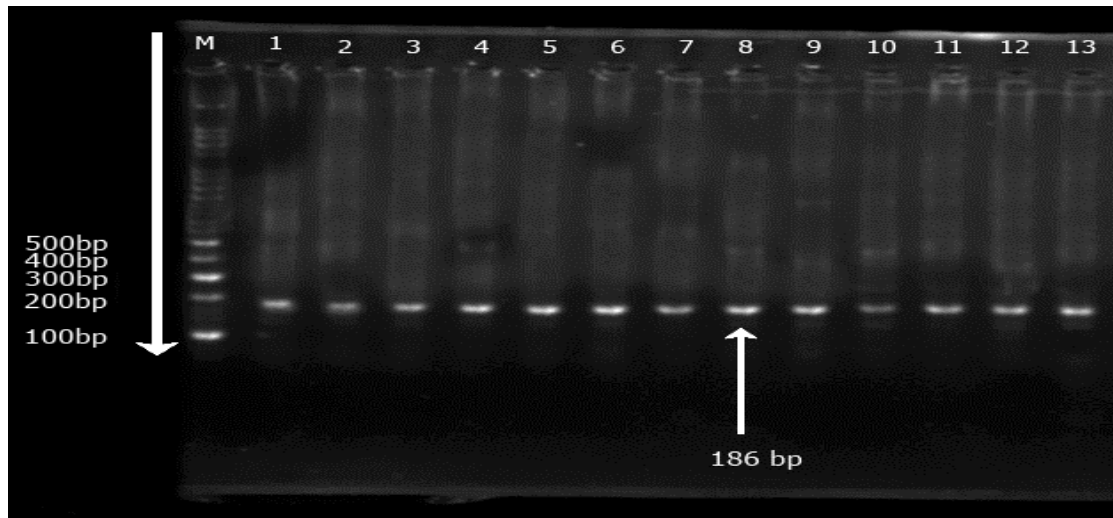


Figure (2): Amplification and PCR product (186 bp) picture of BNP gene on 2% agarose. Lane M, DNA ladder 100 bp and lanes (1-13), PCR products (186 bp).

BNP gene polymorphism analysis

The results of amplification and digestion by restriction enzyme of BNP gene by PCR-RFLP assay were of two alleles (T and C) and three genotypes were digested by restriction enzyme (MspI): TT has two bands 48 and 138 bp, and CC has three bands 48, 67, and 71 bp, and TC has four bands 48, 67, 71, and 138 as shown in fig. (3).

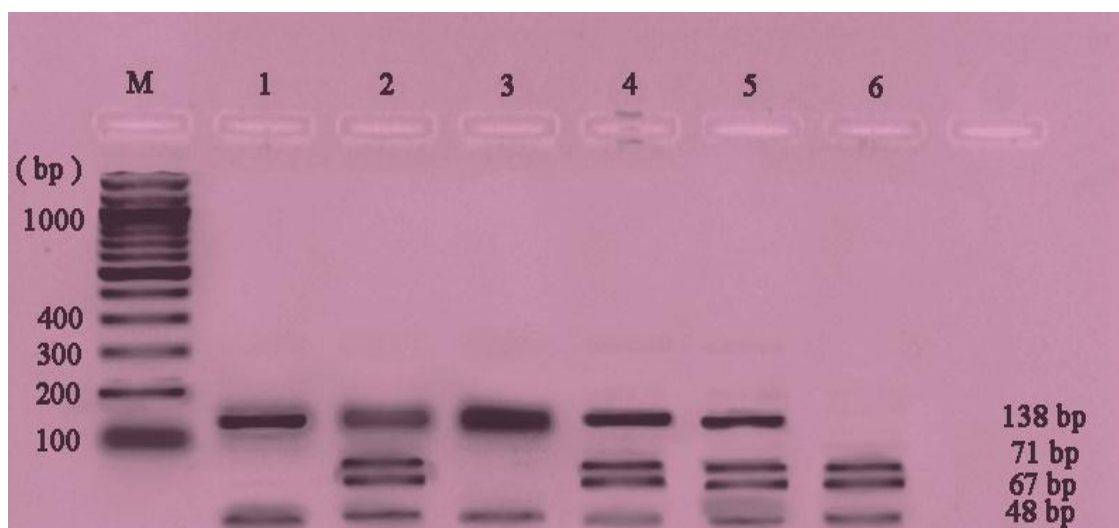


Figure (3): Electrophoretic picture represents the BNP genotyping, (5% agarose), where lane M is 100 bp DNA ladder, lane (1, 3) has two bands at (48bp+138bp) representing the homozygous of (TT), lane (6) has three band at (48bp+67bp+71bp) representing homozygous (CC) allele ,and lane (2, 4, 5) has four bands at (48bp+67bp+71bp+138bp) representing the heterozygous (TC).

Frequency distribution of the genotyping and each allele reported among the studied group is shown in table (3), where no statistically significant differences had been found among the three groups regarding the frequencies of genotypes or separated allele, ($P > 0.05$)

Table (3): Distribution of genotyping among the studied groups

Genotype	ACS		HF		Control	
	No.	%	No.	%	No.	%
T allele	41	41.0	34	34.0	25	25.0
C allele	29	34.5	36	42.9	19	22.6
TC	15	42.9%	16	45.7%	11	50.0%
TT	13	37.1%	9	25.7%	7	31.8%
CC	7	20.0%	10	28.6%	4	18.2%
$\chi^2 = 1.78$, $P = 0.79$ (not significant) $df = 1$						

Heart failure group had the higher mean BNP and NT-proBNP levels across all three genotypes, from other point of view, patients with CC genotype had the higher mean BNP and NT-pro BNP level in all three groups; HF, ACS and controls, in all comparison ($P < 0.001$ significant), furthermore, the comparison mean BNP and NT-proBNP of all the 70 cardiac patients across the genotypes revealed almost similar results i.e. patients with CC genotype had the higher levels of these two parameters than other two genotypes, TT and TC, ($P < 0.001$ significant), and the TC group had higher levels of BNP and NT-proBNP than TT groups and the patients with C allele had the higher levels of these two parameters than those with T allele, table (4) and figs. (4 and 5).

Table (4): Comparison of mean BNP and NT-proBNP among the studied groups according to the genotype

Parameter	Genotype	Group			P.value*
		HF (n=35)	ACS (n=35)	Control (n=22)	
mean BNP	TT	386.4 ± 75.8	183.4 ± 21.4	35.1 ± 4.7	< 0.001
	TC	675.0 ± 106.8	211.9 ± 26.2	33.4 ± 2.7	< 0.001
	CC	957.2 ± 70.9	215.2 ± 27.7	45.7 ± 6.0	< 0.001
	P.value*	< 0.001	0.007	< 0.001	
mean NT-proBNP	TT	912.6 ± 177.8	303.8 ± 72.9	47.7 ± 6.1	< 0.001
	TC	1538.4 ± 237.3	499.6 ± 83.0	60.1 ± 7.9	< 0.001
	CC	2171.1 ± 231.9	690.3 ± 124.5	68.6 ± 4.5	< 0.001
	P.value	< 0.001	< 0.001	< 0.001	
<i>P.value* ANOVA test used in comparison between and within groups</i>					

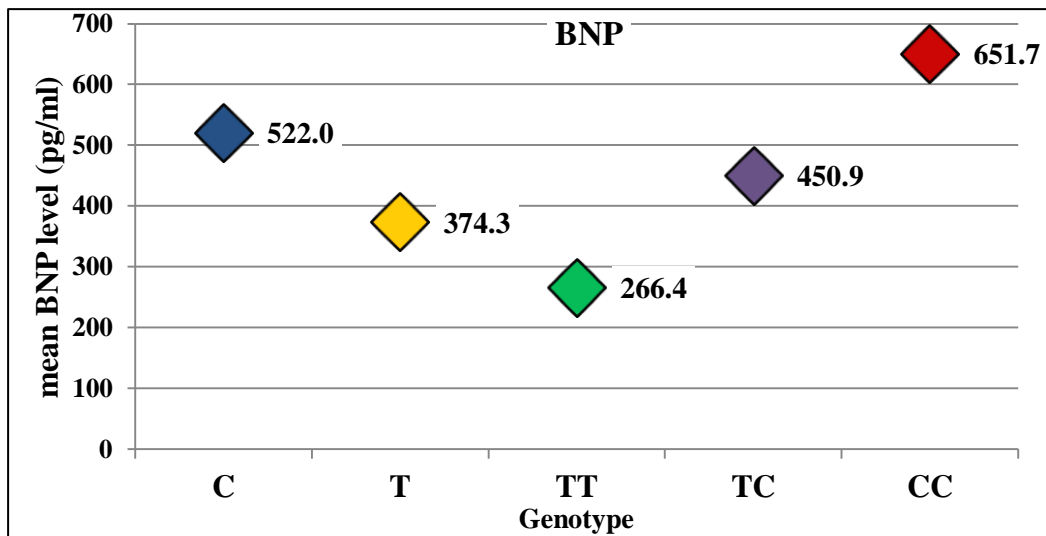


Figure (4): Comparison of mean BNP level according to genotype in cardiac patients (N = 70).

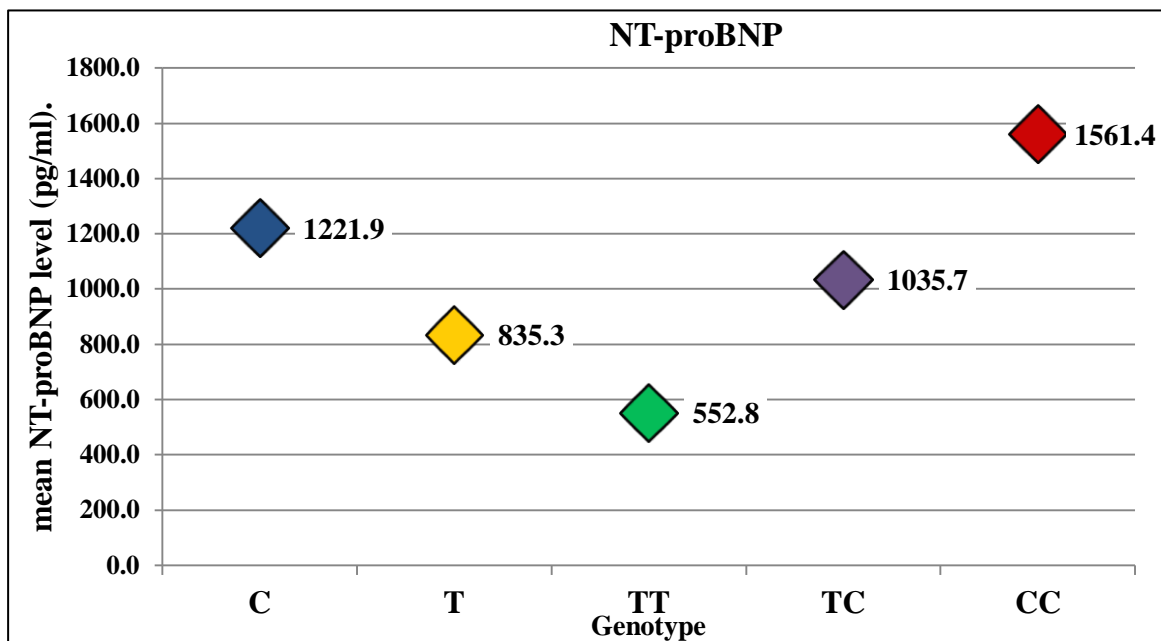


Figure (5): Comparison of mean NT-proBNP level according to genotype in cardiac patients (N = 70).

Discussion

A common SNP in the promoter region of the BNP gene (rs 198389; also recognized as T-381C) was related with 30% BNP levels per C-allele ^[4]. Not only may this genetic BNP elevation confound our interpretation of assay results, it may give BNP's unloading actions, even paradoxically be associated with improved outcomes. Roman P *et al* 2013¹¹ reported that the genotypes of rs198389 (or T-381C polymorphism) associated with BNP levels but there was not significant association between genotypes and HF risk. Takeishi Y *et al* 2007¹² showed that eight SNPs in BNP gene including T-381C variant were significantly associated with BNP concentrations and revealed that individuals with homozygous C-allele had higher BNP level than those individuals with homozygous T-allele and heterozygous, the study evaluated in a large general population of adult Japanese persons. Iuliia P *et al* 2015¹³ reported that the C381C genotype and the C allele of the BNP gene had significant higher levels of BNP in plasma in both healthy men and patients with uncomplicated essential hypertension and LV hypertrophy, residents of Podillia region in Ukraine, age 40-60 years. Berezikova *et al* 2013¹⁴ investigated the BNP gene polymorphism in patients of both sexes with chronic heart failure where it was shown that in healthy individuals of Russian population with the genotype C381C

plasma levels of NT-proBNP was significantly higher than in carriers of the genotype T381T. In patients with established CVD, Karina *et al* 2011¹⁵ showed that the genotypes of NP system polymorphisms including rs198389 were associated with higher levels of BNP and NT-proBNP. Brendan M *et al* 2013¹⁶ proved that there was a relationship between rs198389 polymorphism genotypes and higher levels of NT-proBNP and reduced risk of type 2 diabetes in women. Moreover, the -381C allele was connected with higher BNP concentration and higher BNP promoter activity in reporter gene assays¹⁷. Lisa C *et al* 2011¹⁷ showed that there was nonsignificant difference between genotype frequencies of the BNP gene polymorphism (T-381C) in all study groups including HF and CAD patients ($p=0.98$), but there was association between genotypes and the levels of BNP and NT-proBNP where CC genotypes related with higher levels of BNP and NT-proBNP than other two genotypes TT and TC (CC>TC>TT $p<0.001$ for all assays).

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