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Association of Ataxin-2 rs653178 and C10orf107 rs1530440 genes variants with essential hypertension in Arab Iraqis of Babylon province.

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ABSTRACT

Hypertension is the persisted elevation of blood pressure above the normal rates. About 40% of adults in glob suffered from hypertension which Estimated to cause about 12.8% of total annual deaths. This high mortality can be explained because of its major role in the development of cerebrovascular and coronary heart diseases. All hypertensive cases, that do not have an explained etiology are called essential hypertension (EH), this consists about 95% of the total diagnosed hypertension cases . otherwise, it is called secondary hypertension. The recent studies implicate many genes to have an effect on the development and the susceptibility of essential hypertension. genes variants such as; rs653178 of Ataxin-2 (ATX2N) gene and rs1530440 of chromosome 10 open reading frame 107 (C10orf107) gene, represent the top hit variants that found to be significantly associated with essential hypertension in several populations. We aimed by this project, to conduct a case-control association study to evaluate the association of rs653178 and rs1530440 genetic variants with essential hypertension, in one of Iraqi provinces (Babylon) by enrolling individuals from Arab ancestry. The study enrolled 100 unrelated cases of well diagnosed essential hypertensive patients and a 70 unrelated controls of carefully selected normotensive individuals . For genotyping; we designed and optimized a polymerase chain reaction- restriction fragment length polymorphisms (PCR-RFLP) method with the presence of internal splicing sites. Concerning ATX2N rs653178, there was no significant allelic or genotypic association achieved, both patients and control groups did not deviate from Hardy-Weinberg equilibrium distribution , furthermore , the same results were achieved when the samples segregated into male and females. Concerning C10orf107 rs1530440, there was no significant allelic association achieved, but there was a significant difference in the distribution of TT genotype between patient and control groups (p =0.032) , the T allele represent a protective recessive allele which when present as homozygote (TT) it will confer a resistant to the carrier individual, all TT genotype carriers individual (N= 4) were normotensive. Furthermore, There were no significance association recorded when the samples segregated into males and females, and both patients and control groups did not deviated from Hardy-Weinberg equilibrium distribution in all studied groups. We conclude that C10orf107 genes variants represent a genetic factors which can modulate the essential hypertension susceptibility in Arab population of Babylon province .

Keywords: Essential Hypertension, rs653178 , ATX2N , rs1530440 , C10orf107 , PCR-RFLP .

Abbreviations: Chronic kidney disease genetic Consortium: CKDGen consortium, Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium: CHARGE Consortium, disease susceptibility locus: DSL, Essential hypertension :EH, Global Blood Pressure Genetics Consortium: Global BPgen Consortium, linkage disequilibrium: LD , polymerase chain reaction- restriction fragment length polymorphism: PCR-RFLP, polymerase chain reaction: PCR , quantitative trait loci: QTL , single nucleotide polymorphism: SNP.

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INTRODUCTION

Blood pressure : is the pressure of blood flow in arteries. It depends on the pumped blood flow rate and the resistance exerted by vessels against the flow. If the produced blood pressure is higher than normal values ,the heart will be obligated to hard working to maintain the required blood flow through the body organs (Padma and Padma, 2012) .

The Normal adult systolic blood pressure (during the heart beats) is mainly in the average of 120 mm Hg and an average of 80 mm Hg in diastolic blood pressure (when the heart relaxes). If the individual has a systolic blood pressure equal to or exceed 140 mm Hg and/or a diastolic blood pressure equal to or exceed 90 mm Hg the individual considered as hypertensive and the condition is called hypertension (WHO 2010) .

All hypertensive cases that do not have an explained etiology or specific origin are called essential hypertension (EH) , this consists about 95% of the total diagnosed hypertension cases . otherwise , it is called secondary hypertension if the elevated blood pressure has an explained causes such as kidney disease and hormonal disorders (Franceschini and Le, 2014; Zaidan *et al*., 2014).

Hypertension has the highest prevalence among all other diseases , about 40% of adults in glob suffered from hypertension . furthermore , it is recorded among the top five causes of death and disability in the globe and Estimated to cause about 12.8% of total annual deaths . (WHO ,2010 ; Mendis *et al.* 2011). This high mortality , can be explained because of its major role in the development of cerebrovascular and coronary heart diseases (Danaei *et al.*, 2011) .

Antihypertensive therapy and Lifestyle changes can be used to reduce hypertension morbidity and mortality . However, current survey studies indicate that control rates are poor in general . (WHO,2010)

Researchers from several decades till nowadays have been saving no efforts to elucidate the environmental and genetic determinants of hypertension , Because they believe that the best way to fight this huge life threatening disease, is the well understanding of its etiology . any improvement in hypertension etiology understanding, will be positively reflected to an improvement in this disease prevention and treatments . (Arora and Newton ,2010)

From a genetic aspect, There are two well defined forms of hypertension , the monogenic and polygenic forms , the monogenic form appear to be caused by a defect in single gene or locus , while the polygenic form suggested to be caused by collaborative effect of several genes polymorphisms (Wain,2014) .

Apart from the rare monogenic form of hypertension , Many researchers suggested that the genes which responsible for hypertension play a role similar to oncogenes , by this similarity these gene are vital to blood pressure regulation in normal individuals, but when these genes fail to fulfill their regulatory role , they involve in the onset of hypertension . Also the recent findings assume the complex multi-factorial genetic architecture of hypertension and its linked risk traits, this complexity may be as a result of pleiotrpy , epistasis and molecular heterosis (Padma and Padma, 2012 ; Wain,2014).

Many genes were found or suggested to directly or indirectly effect the development of EH . According to 'HuGe navigator database' (Yu *et al.*,2008) we can find several hundred genes implicated with hypertension , but the implication of each one of these gene, usually could not be replicated in all studies .

The essential hypertension candidate genes could have an explained role in blood pressure regulation, such as: genes of Renin-angiotensin-aldosterone system (RAAS) (Marcheselli and Micieli ,2008) and ions channel and transporters genes (Li *et al.*,2014) . While other implicated genes did not have a clear role in blood pressure regulation, or their role have not been yet fully understood, most of these gene were discovered during the genome wide scan studies and achieved a significant association with essential hypertension , genes such as the ataxin2 (ATXN2) and chromosome 10 open reading frame 107 (C10orf107) represent a good examples of this category . (Newton-Cheh *et al.,*2009 ; Zhang *et al.,* 2015) .

The human 'chromosome 10 open reading frame 107' (c10orf107) also known as (bA63A2.1) is an open reading frame locating on the cytogenetic location 10q21.2 , and compromising 11 exons , it encode to a

protein called (uncharacterized protein C10orf107) and no further functional information available on its function and regulation (NCBI Gene ID: 219621, updated on 23-Mar-2015).

Ensembl database (Ensembl release 79 - March 2015) show that this gene has 2221 different validated variants, among all of these variants there is the rs1530440 , which was confirmed by several studies to be associated with hypertension or blood pressure , furthermore it was replicated in several different population (Newton-Cheh *et al.*,2009; Jennifer *et al.*,2011; Yang *et al.*2012)

The human ATXN2 gene encode for the ataxin-2 protein which is well known to be the molecular bases of spinocerebellar ataxia-2 (SCA2) disease , (Almaguer-Mederosa *et al.*, 2010). The complete image of Ataxin-2 protein role in the cell still under investigation and several models are proposed to explain the main role of this protein , in general, it is accepted to has a certain role/s in RNA regulation . (Bolduc *et al.*,2008 ; Buchan *et al.*,2008; McCann *et al.*, 2011)

A meta-analysis study conducted by Levy *et al.*, (2009) combined the results of two large genome wide association studies , the CHARGE Consortium (n=29,136) and Global BPgen Consortium (n=34,433) , revealed a three SNPs of ATXN2 gene (rs653178 , rs4766578 and rs10774625) significantly associated with diastolic blood pressure (p values around 10^{-8}) , while the ATXN2 rs653178 only among the other ATXN2 SNPs was associated with systolic blood pressure (p value = $8.5 * 10^{-7}$) . this evidences which implicate the ATX2N gene with hypertension or blood pressure were confirmed and replicated in several populations (Ikram *et al.*,2010; Ho *et al.*,2011; Zhang *et al.*,2015)

This project aimed to study the genetic association of C10orf107 rs1530440 and ATX2N rs653178 variants with the essential hypertension in the Arab population of Babylon province. furthermore we aimed to optimize a PCR-RFLP genotyping method for these variants , which will be suitable for further studies, especially for low and moderate Budget laboratories .

MATERIALS AND METHODS

Sampling :Project methodology was accepted by the Committee of research ethics in Babylon Health Directorate (issue number .1056 , date :26/3/2014) and fulfilled the research ethics requirements of Marjan medical city supervision office at the date 7/4/2014 . Samples were collected during a period of 8 months started in May 2014 , from the consult clinic visitors in Merjan medical city . each participant declare his/her permission to perform the biomedical and genetic tests by signed a written agreement.

The enrolled participant should be a citizen of Babylon province/ Iraq , and had an Arabic ancestry (according to their testimony).

The participant filled a questioner paper for his/her full name ,occupation, sex, age, alcohol consumption, the age of hypertension onset, family history of hypertension , etc. while the participant's weight and height were measured by electronic balance and measuring tape respectively.

By using a disposable syringe (without tourniquet), 5 ml of venous blood was obtained from overnight fasted participant . 2 ml of the blood was mixed gently in EDTA tube and stored at 4C˚ till be used for DNA extraction during the next 5 days . The rest 3ml of blood was drained into gel plain tube for serum preparation, which would be used in biochemical tests during the next 6 hours.

Biochemical tests: A 'cobas c111®' device (Roche® , Germany) was employed to determine the serum urea ,glucose , and creatinine, levels , according to the manufacturer instructions . while a manual method of 'calcium CPC method' kit (Biolabo SA® , France) was employed to determine the serum calcium level .

After the analyzing of biochemical tests results and questioner paper information , the patients group included only who:

- Had a normal values of biochemical tests .
- Had a body mass index less than 30 kg/m^2 .

- Was not a smoker or alcohol consumer .
- Apparently free from any illness could affect blood pressure .
- Had a diagnosed hypertension for at least one year .
- Had a family history of hypertension .

While the selected control had to be, normotensive , aged more than 50 years , did not have a family history of hypertension and apparently free from any illness could affect blood pressure.

The filtered patient group included 42 males and 58 females with age mean 48.7±9.9. while the filtered control group included 36 males and 34 females with age mean 58.6±7 .

DNA extraction: By following the manufacturer instructions, 'Wizard® Genomic DNA Purification Kit' (Promega. USA) was used for DNA extraction from the venous blood. The extracted DNA quantity and quality was assessed spectrophotometrically by nano-drop device (biodrop , UK), each extracted sample that did not achieve 260/280 ratio equal or more than 1.8 and/or 260/230 ratio equal or more than 2 was re-extracted . then the extracted DNA was subjected to agarose gel electrophoresis (Samboork and Russell ,2001) to determine its molecular weight and integrity.

PCR-RFLP analysis design: The flanked sequence for each SNP was retrieved from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snp) according to human genome assembly GRCH.p2.

The selection of the suitable restriction enzyme was performed by the aid of WatCut® online software (http://watcut.uwaterloo.ca/template) , we selected the AluI and NdeI restriction enzymes to be used in rs653178 and rs1530440 genotyping respectively .

The primers were designed by the aid of NCBI-primer BLAST online software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK _LOC=BlastHome) , at the same time the produced primers was checked for specificity for their target sequences by performing the BLAST against the human genome . The primer ability to form secondary structure was checked by the aid of 'Oligo Calc' online software (Kibbe,2007) (http://www.basic.northwestern.edu/biotools/ oligocalc.html) .

The amplicon of each primers pair was retrieving by the aid of "MFEprimer-2.0" online software (Qu *et al.,*2012) , by performing insilico PCR for the primers pair against the human genome .

Each amplicon sequence retrieved by the former step was subjected to in-silico digestion by the selected restriction enzyme, by the aid of "Sequence Manipulation Suite" online software (http://www.bioinformatics.org/sms2/rest_digest.html) , the designed primers pairs would be rejected if the amplicon showed unaccepted digestion manner .

Table (1) : primers and restriction enzymes used in PCR-RFLP genotyping , the introduced mutation marked by square brackets .

SNP	Primer name	Primer sequence (5'-3')	Amplicon size	Restriction enzyme
Rs653178	Forward primer	AACTCGAGAAGATGGGAATGG	197	AluI
	Reverse primer	ATGATGAAGATGTCCTATGTCA[A]G		
Rs1530440	Forward primer	AGAGTCAGTGCATCCTAAAGG	625	NdeI
	Reverse primer	AGTAGCCCTGGAAATGTCTTC		

Optimization of PCR conditions : a routine optimization protocol was applied for each primers set by employing a gradient annealing temperature (± 10C˚ of the lowest primer TM) , different number of cycles and different reaction ingredient until the achievement of the most specific and sufficient PCR products .

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The optimized PCR thermo-cycling condition for rs653178 were : one cycle of initial denaturation in 94C˚ for 4 min., followed by 40 cycles of (94 C˚ for 40 sec. ,57 C˚ for 35 sec. and 72 C˚ for 30 sec.) then a final elongation step in 72C˚ for 4 min.

The optimized PCR thermo-cycling condition for rs1530440 were : one cycle of initial denaturation in 94C˚ for 4 min., followed by 40 cycles of (94 C˚ for 40 sec. ,65 C˚ for 35 sec. and 72 C˚ for 45 sec.) then a final elongation step in 72C˚ for 4 min.

The optimized PCR reaction mixture for rs653178 genotyping contain, 7.5 µl of 2X go tag green mix® (promiga , USA) , 20Ng of genomic DNA ,0.5 µl of each primer (10 pmol/ µl , bioneer , Korea) and the total volume completed to 15 µl by molecular grade water (promiga , USA) .

The optimized PCR reaction mixture for rs1530440 genotyping contain, 10 µl of 2X go tag green mix® (promiga , USA) , 60 Ng of genomic DNA ,1 µl of each primer (10 pmol/ µl , bioneer , Korea) and the total volume completed to 20 µl by molecular grade water (promiga , USA) .

Restriction digestion of PCR product for rs653178 genotyping: The PCR-product was digested with AluI restriction enzyme , this enzyme cut the product in two sites , the first site represent an internal splicing control which when cut produce a two polynucleotides with a molecular weight of 137 and 60 bp . the second splicing site located on the 60 bp polynucleotides which cut only if the rs653178 C allele presented, into two polynucleotides with a molecular weight of 36 and 24 bp figure (1) .

Restriction digestion of PCR product for rs1530440 genotyping: The PCR-product was digested with NdeI restriction enzyme , this enzyme cut the product in two sites , the first site represent an internal splicing control which when cut produce a two polynucleotides with a molecular weight of 469 and 156 bp . the second splicing site located on the 469 bp polynucleotides which cut only if the rs1530440 T allele presented, the presence of T allele enable the restriction enzyme to cut the 469 bp polynucleotides to two polynucleotide with a molecular weight of 199 and 270 bp , figure (2) .

Polyacrylamide gel electrophoresis: the digested PCR product subjected to non-denaturing polyacrylamide gel electrophoresis according to (Sambrook and Russell ,2001), then the gel was imaged by gel imaging device (ATTA, Japan) and analyzed by CS® analyzer software (ATTA, Japan).

Statistical analysis: the genetic association parameters were carried out by the aid of SNPStats® online software (Sole *et al.*,2006) (http://bioinfo.iconcologia.net/en/SNPStats_web) and by 'case-control studies tool / institute of human genetic /Helmholtz center / Munich' (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl) . except that genotypes association which was carried out by Fisher's exact test according to (Agresti,1992).

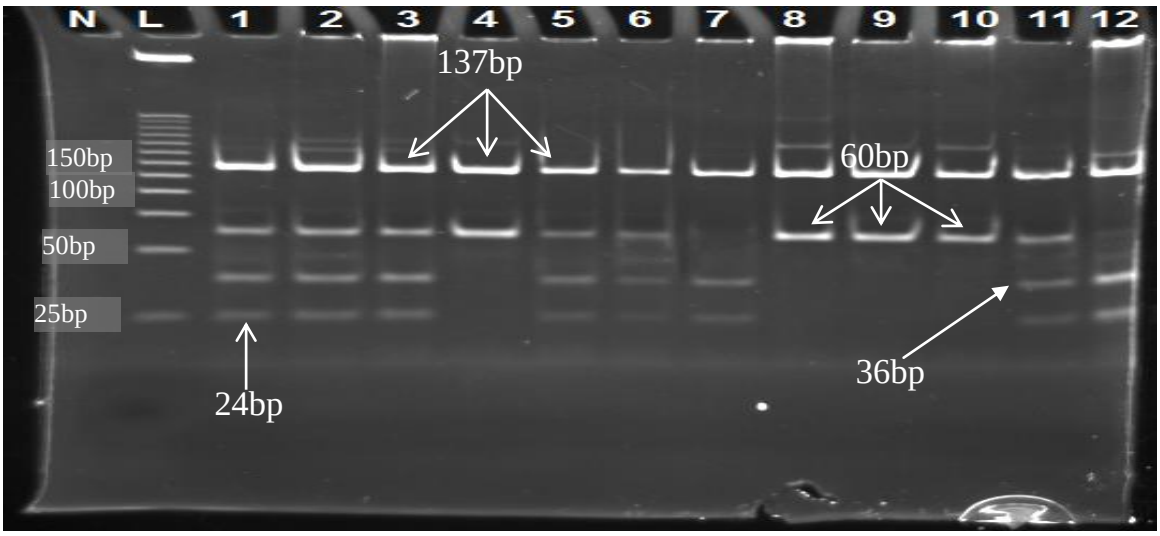

Figure (1): Genotyping of rs653178 by the designed PCR-RFLP method . (lanes : N = internal negative control ; L=25 bp step ladder ; 1,2,3,5,6 and 11= CT genotype ; 4,8,9 and 10 = TT genotype ; 7 and 12= CC genotype .

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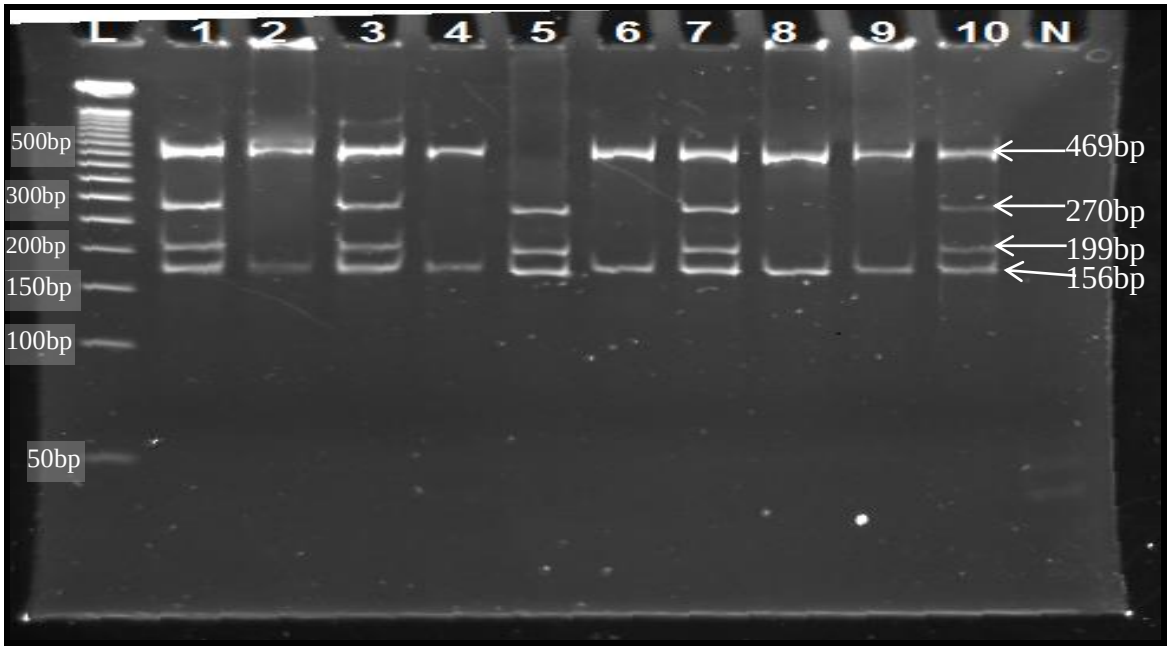

Figure (2): Genotyping of rs1530440 by the designed PCR-RFLP method . (lanes : N = internal negative control ; L=50 bp step ladder ; 1,3,7and 10= CT genotype ; 5 = TT genotype ; 2,4,6,8 and 9 = CC genotype .

RESULTS AND DISCUSSION

The association of rs653178 with EH:

The results showed that there was no significant allele frequency differences between patients and control group . furthermore the same results was achieved when the samples segregated into males and females , table (2) .

Table (2): Alleles percentage ,alleles risk association and alleles odd ratio of rs653178

Samples	Allele	Patients	Controls	Odd ratio (95% C.I.)	P- value[a]
All	T	66%	65%	1.069 (0.678-1.684)	0.77
	C	34%	35%	0.936 (0.594-1.474)	
Males	T	69%	63%	0.765 (0.408-1.432)	0.40
	C	31%	37%	1.308 (0.699-2.448)	
Females	T	65%	69%	0.831 (0.405-1.707)	0.61
	C	35%	31%	1.203 (0.586-2.469)	

a:Pearson's goodness-of-fit chi-square (degree of freedom = 1)

Both control and patients groups and even after their segregation in to males and females , did not record any significant deviation from the distribution of hardy-Weinberg equilibrium , table (3) .

Table (3): rs653178 genotypes distribution in patients and control groups and segregated into males and females , the deviation from hardy-Weinberg equilibrium represented by the exact test P-value .

Samples	Groups	Genotype			HW. P-value
		TT	CT	CC	
All	patients	40%	53%	7%	0.075
	control	39%	52%	9%	0.29
Males	patients	45%	47.6%	7.4%	0.72
	control	36.9%	52.1%	11%	0.54
Females	Patients	36.2%	56.9%	6.9%	0.088
	control	41.7%	54.2%	4.1%	0.36

The association of each genotypes with EH was further tested and under different models of inheritance , the result showed that there was no significant association of any genotype with EH, under any tested inheritance model , and the same results was achieved when the samples segregated into males and females ,tables (4) .

Table (4): association of rs653178 genotypes with essential hypertension under different models of inheritance .

Model	Samples	Genotype	Patients	control	OR (95% CI)	P-value[a]
Codominant	All	T/T	40%	38.5%	1.00	0.94
		C/T	53%	52.9%	1.03 (0.54-1.97)	
		C/C	7%	8.6%	1.27 (0.38-4.19)	
	Males	T/T	45.2%	37%	1.00	0.77
		C/T	47.6%	52.2%	1.34 (0.55-3.24)	
		C/C	7.1%	10.9%	1.86 (0.39-8.99)	
	Females	T/T	36.2%	41.7%	1.00	0.92
		C/T	56.9%	54.2%	0.83 (0.31-2.23)	
		C/C	6.9%	4.2%	0.52 (0.05-5.33)	
Dominant	All	T/T	40%	38.6%	1.00	0.87
		C/T-C/C	60%	61.4%	1.06 (0.57-1.99)	
	Males	T/T	45.2%	37%	1.00	0.52
		C/T-C/C	54.8%	63%	1.41 (0.60-3.31)	
	Females	T/T	36.2%	41.7%	1.00	0.80
		C/T-C/C	63.8%	58.3%	0.79 (0.30-2.10)	
Recessive	All	T/T-C/T	93%	91.4%	1.00	0.77
		C/C	7%	8.6%	1.25 (0.40-3.88)	
	Males	T/T-C/T	92.9%	89.1%	1.00	0.72
		C/C	7.1%	10.9%	1.59 (0.35-7.08)	
	Females	T/T-C/T	93.1%	95.8%	1.00	1.00
		C/C	6.9%	4.2%	0.59 (0.06-5.54)	
Overdominant	All	T/T-C/C	47%	47.1%	1.00	1.00
		C/T	53%	52.9%	0.99 (0.54-1.83)	
	Males	T/T-C/C	52.4%	47.8%	1.00	0.83
		C/T	47.6%	52.2%	1.20 (0.52-2.77)	
	Females	T/T-C/C	43.1%	45.8%	1.00	1.00
		C/T	56.9%	54.2%	0.90 (0.34-2.33)	

a: two tailed p-value of Fisher's Exact Test

The rs653178 is a di-allelic (C or T) single nucleotide polymorphism located at the position **111569952** of the chromosome 12 forward strand ,this variant represent an intronic variant of Ataxin-2 gene (ATXN2) (Ensembl release 80 - May 2015) . The complete image of Ataxin-2 protein role in the cell still under investigation and several models are proposed to explain the main role of this protein , in general it is accepted to has a certain role/s in RNA regulation (Bolduc *et al.,*2008 ; Buchan *et al.,*2008) .

The remarkable notice of rs653178 is the significant allele frequency differences among different populations , it is relatively had a high minor allele frequency in European (Newton-chen *et al.*,2009) while a very rare minor allele frequency in chines and other Asian populations (Nie *et al.*,2010) .

Our results showed that the studied population has allele frequency similar to that found in European and Americans populations , but the frequency is significantly differ from other studied Asian and African populations as depicted in figure (3) .

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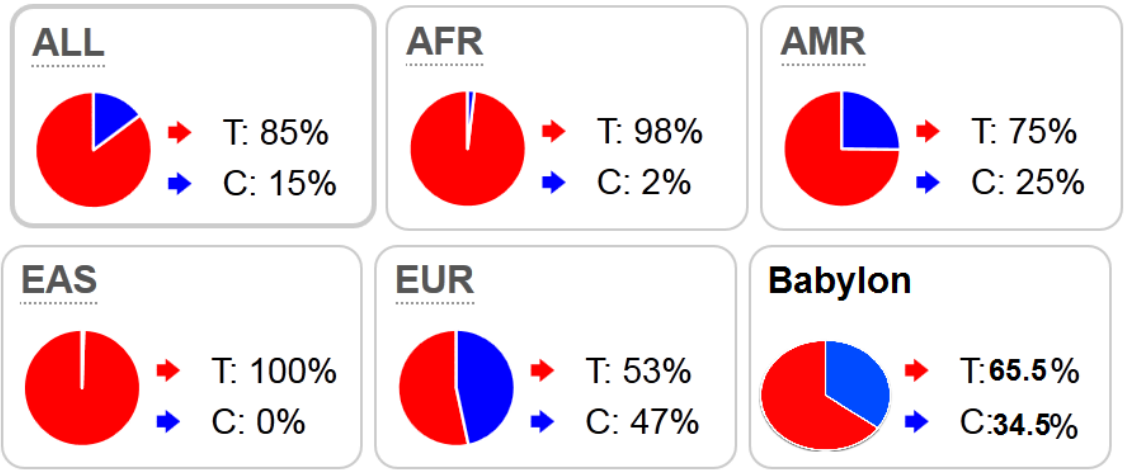

Figure (3): rs653178 allele percentages in Babylon province population compared to several populations . the data and figures other than studied population (Babylon) are adapted from 1000 Genomes Project Phase3 .(ALL= all phase3 individuals ; AFR= Africans ; AMR= Americans ; EAS= east Asians ; EUR= Europeans).

Our results disagreed with many other studies that showed the significant association of rs653178 with EH or blood pressure , such as the meta-analysis study of Levy *et al.* (2009) (see introduction) .

another meta study performed by CKDGen consortium included several hundred thousands of samples as attempt to explain the relationship between the genetic variants that implicated in kidney traits and cardiovascular phenotypes , the study found that only rs653178 had a direct association with diastolic , systolic blood pressure, coronary artery diseases and with a high level of retinal venular caliber (Olden *et al.*,2013) .

Another evidence for the implication of rs653178 with cardiovascular disease come from a large genome wide association study , Kullo *et al.*(2014) as attempt to find the genetic bases of peripheral arterial diseases (PAD) , they performed a genome wide association study for 537,872 SNPs , enrolled 1641 cases and 1604 controls , followed by replication for the top 48 SNPs in 740 cases and 1051 controls , the project's results showed that only rs653178 was significantly associated with BAD achieved P-value equal to $5.5 * 10^{-5}$ and $8.9* 10^{-4}$ in discovery and replication cohorts respectively .

The direct implication of ATX2 gene in blood pressure regulation and other cardiovascular diseases was suggested by several researchers but they did not demonstrate the clear explanation for this implication (Ikram *et al.,*2010;Zhang *et al.*, 2015) .

While the indirect association of this gene with blood pressure was suggested and explained , the association could be due to the occurrence of this gene in the region of 'SH2B adaptor protein 3' gene (SH2B3) . SH2B3 gene encodes for one of SH2B protein family which have a divers physiological roles such as hematopoiesis , immune response and signaling (Takaki *et al.,*2002 *;* Devalliere *et al.,*2012) , this gene was reported to be associated with several phenotypes such as Celiac disease (Hunt *et al.,*2008) and diabetes mellitus (Barrett *et al.,*2009) .

Furthermore the SH2B3 rs3184504 variant was reported to be significantly associated with blood pressure (Ehret *et al.,*2011) . Kullo *et al.*(2014) performed a dynamic analysis to explain the effect of this variant , they demonstrate that rs318450 can be the causal locus for several phenotypes by altering the lipids binding ,protein-protein interaction and by the introduction of a new phosphorylation site that may effect on the signaling pathway of SH2B3 .

The inclusion of rs653178 and rs3184504 within the same haplotype block (HapMap project results) , may represent the clue that the rs653178 was not the causal locus but it appeared to associate with the hypertension due to the high degree of linkage disequilibrium between this variant and the suggested causal variant (rs318450) .

On the other hand , our results partially agreed with two studies performed on east Asian populations (Niu *et al.,*2010; Lin *et al.*,2011) , both studies found that rs653178 did not achieve the significance associate with hypertension , they demonstrated that this variant had a very low minor allele frequency (0.0036 and 0.018) , this finding made them excluded this variant from their further genetic analysis and concluded that ATX2 gene may has a different genetic contexts in different populations , furthermore they concluded that the rs653178 might not represent a hypertension susceptibility locus in chines .

The conflicted results of the implication of certain genetic variant with hypertension was recognized early by the researchers and lead them to suggest that, the solution can be in the construction of hypertension-variants database for each ethic group (Izawa *et al.*,2003) .

The association of Rs1530440 with EH :

The results showed that there was no significant allele frequency differences between patients and control group . the same results was achieved when the samples segregated into males and females, table (5) .

Table (5) Alleles percentages ,alleles risk association and alleles odd ratio of rs1530440

Samples	Allele	Patients	Controls	Odd ratio (95% C.I.)	P- value[a]
All	C	85%	79%	1.474 (0.831-2.615)	0.18
	T	15%	21%	0.678 (0.382-1.203)	
Males	C	82%	75%	1.524 (0.694-3.347)	0.29
	T	18%	25%	0.656 (0.299-1.441)	
Females	C	87%	84%	1.51 (0.85-2.71)	0.55
	T	13%	16%	0.772(0.328-1.816)	

a:Pearson's goodness-of-fit chi-square (degree of freedom = 1)

The result also revealed that both control and patients groups genotype frequency flow the Hardy-Weinberg equilibrium, and did not record any significant deviation , the same results were achieved when the samples segregated into males and females, table (6) .

Table (6) : rs1530440 genotypes distribution in patients and control groups and segregated into males and females , the deviation from hardy-Weinberg equilibrium represented by the exact test P-value

Samples	Groups	Genotype			HW. P-value
		CC	CT	TT	
All	patients	69.9%	30.1%	0.0%	0.21
	control	64.3%	30%	5.7%	0.47
Males	patients	64.1%	35.9%	0.0%	0.5
	control	55.6%	38.9%	5.6%	0.32
Females	Patients	74.1%	25.9%	0.0%	0.58
	control	73.5%	20.6%	5.9%	0.18

The association of each genotype with EH was further tested and under different models of inheritance , the result showed that there was a significant difference in the distribution of TT genotype between patients and control groups (P = 0.032) , the T allele represent a protective recessive allele which when present as homozygote (TT) it will confer a resistant to the carrier individual , all homozygote TT individuals (No.=4) are normotensive . on the other hand , There was no significance difference recorded when the samples segregated into males and females, tables (7) .

The rs1530440 located at the cytogenetic region 10q21 . it is an intronic SNP has a di-allelic C or T at the position **10:61764833** forward strand (GRCH38p2) . this SNP occur within the 'chromosome 10 open reading frame 107' gene (C10orf107) , there is a little and confused information concerning the physiological and pathological function of C10orf107 gene . the rs1530440 region include two important genes that have a candidate effect on blood pressure , the first is the 'AT rich interactive domain 5B' (ARID5B) and the second gene is 'Rho BTB GTPase' (RHOBTB1) , the ARID5B gene is one of AT rich domain family , considered as

transcriptional factor expressed with high level in cardiovascular tissues and has an important role in the smooth muscles development (Watanabe *et al.,*2002) . while the RHOBTB1 gene encode for the enzyme responsible for the conversion of GTP to GDP , several studies found that such enzymes which modulate the GTP can have a significant effect on blood pressure and hypertension development (Zheng *et al.,*2003 ; Du *et al.,*2008) .

Table (7): association of rs1530440 genotypes with essential hypertension under different models of inheritance

Model	Samples	Genotype	Patients	control	OR (95% CI)	P-value[a]
Codominant	All	C/C	70%	64.3%	1.00	0.071
		C/T	30%	30%	1.25 (0.63-2.46)	
		T/T	0%	5.7%	12.95 (0.68 - 246.59)	
	Males	C/C	64%	55.6%	1.00	0.38
		C/T	36%	38.9%	1.25 (0.49-3.22)	
		T/T	0.0%	5.6%	6.21(0.28-136)	
	Females	C/C	74%	73.5%	1.00	0.196
		C/T	26%	20.6%	0.80 (0.28-2.25)	
		T/T	0%	5.9%	7.94(0.36-172)	
Dominant	All	C/C	70%	64.3%	1.00	0.50
		C/T-T/T	30 %	35.7%	1.29 (0.67-2.49)	
	Males	C/C	64.1%	55.6%	1.00	0.49
		C/T-T/T	35.9%	44.4%	1.43 (0.57-3.61)	
	Females	C/C	74.1%	73.5%	1.00	1.00
		C/T-T/T	25.9%	26.5%	1.03 (0.39-2.73)	
Recessive	All	C/C-C/T	100%	94.3%	1.00	**0.032**
		T/T	0%	5.7%	12.65(0.66-239)	
	Males	C/C-C/T	100%	94.4%	1.00	0.23
		T/T	0.0%	5.6%	5.72(0.26-123)	
	Females	C/C-C/T	100%	94.1%	1.00	0.15
		T/T	0%	5.9%	8.38(0.39-180)	
Overdominant	All	C/C-T/T	70%	70%	1.00	1.00
		C/T	30%	30%	0.99 (0.51-1.96)	
	Males	C/C-T/T	64.1%	61.1%	1.00	0.82
		C/T	35.9%	38.9%	1.14 (0.45-2.90)	
	Females	C/C-T/T	74.1%	79.4%	1.00	0.62
		C/T	25.9%	20.6%	0.74 (0.26-2.08)	

a: Two tailed p-value of Fisher's Exact Test

Our results showed that, there was a differences in the distribution of alleles frequencies among patients and controls groups but this difference did not reach the statistical significance (P= 0.15) , similar results was achieved when the samples segregated in to males and females , on the other hand the genotypic association showed that the rs1530440 T allele play as a recessive protective allele , the TT genotype showed lesser susceptibility to develop EH comparing to the other genotypes , and all individuals which carry the TT genotype (No.=4) did not develop EH , the statistical significance was lost when the samples segregated in to males and females, this can be due to the small samples number which cannot maintain the statistical significance.

The results were agreed with The consortium of Global Blood Pressure Genetics (Global bpgen) original study, which early indicated the implication of c10orf107 rs1530440 with blood pressure and hypertension, the study enrolled 34,433 European subjects with blood pressure parameters measurements then subjected to direct genotyping and in silico results analyses , the analyses identified eight loci, Among these loci the rs1530440 in c10orf107 which achieves the significant association with both systolic and diastolic blood pressure , also each locus provides substantial evidence for association with hypertension, and they concluded that, these common variants associations with blood pressure and hypertension may elucidate the fine regulation mechanism of blood pressure and can lead to novel targets for prevention from cardiovascular disease (Newton-Cheh *et al.,*2009).

Another study conducted by Jennifer *et al.,*(2011) , enrolled 23,019 north American women to replicate the finding of previous studies , the study confirm the association of several SNPs with blood pressure and the rs1530440 in C10orf107 among those SNPs which confirmed with a statistical significance ($P= 2.3^{-6}$).

Other evidence confirming the association or implication of rs1530440 with hypertension come from a 27 years follow up study which enrolled a cohort of 2357 Finnish individuals , the study designed to investigate the combination effect of 13 candidate SNPs among of which the rs1530440 , the study revealed that the genetic risk score (GRS) were 0.47 and 0.53 mmHg for systolic and diastolic blood pressure respectively ($P < 0.01$) , furthermore they demonstrate that the GRS with diastolic blood pressure began early from the 9 years old , the same study enrolled 1194 individuals to perform a replication study , the replication revealed the same essential results (Oikonen *et al.,*2011).

Furthermore the result also agreed with the finding of Yang *et al.,*(2012) study, which performed on Korean population , to investigate the association of several SNPs with the development of hypertension and arterial stiffness in pre-diabetic and diabetic subjects , the findings demonstrated that only 4 of studied SNPs have statistical significance to be associated with hypertension and arterial stiffness , among of which the rs1530440 ($p = 0.0015$), the study concluded that the diabetic subject who carry this variant may confer an additional risk to develop hypertension and arterial stiffness and also can be effected by antihypertensive medication.

On the other hand Kato *et al.*(2011) conducted a large association study, enrolled 19,608 from East Asian individuals followed by denovo genotyping by two stages of replication involving 20,247 and 10,518 samples, this study failed to establish genome wide association significance among C10orf107 and diastolic or systolic blood pressure , they further investigated this findings and demonstrated that this variant has a different linkage disequilibrium values among the different population , and the Asian ancestry population have a different linkage disequilibrium pattern from the European population.

The previous studies' results pointed to another explanation for the role of rs1530440 in blood pressure regulation , it is likely to say that this variant do not represent a disease susceptibility locus (DSL) or quantitative trait locus (QTL) but it could be in linkage disequilibrium (LD) with another locus or loci that represent the true DSL or QTL . in this case the association of rs1530440 with hypertension or blood pressure would be effected by the degree of LD between this variant and the other QTL or DSL locus , it is well demonstrated that the LD between relatively apart two loci on the human genome usually varied among populations , this argument was well explained in previous studies (Reich *et al.,* 2001 ; Teo and Sim ,2010) . and clearly noticed by HapMap project results.

CONCLUSION

We conclude that, the carrier of TT genotype of rs1530440 have a lesser susceptibility to develop essential hypertension and C10orf107 gene variants represent a genetic factors which can modulate the essential hypertension susceptibility in Arab population of Babylon province .

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