



Original article

Bisoprolol responses (PK/PD) in hypertensive patients: A cytochrome P450 (CYP) 2D6 targeted polymorphism study

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ABSTRACT

Background: Bisoprolol is an effective β 1-adrenergic blocker, an inter-individual genetic variability was recorded in its response. This study aimed at investigating the association of CYP2D6*2A (rs1080985) and CYP2D6*10 (rs1065852) single-nucleotide polymorphism (SNP) with Bisoprolol response in cardiac patients attending King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia.

Patients and methods: In the study, 107 patients were enrolled. Five mL of venous blood was collected from each patient and genotyping for CYP2D6*2A and CYP2D6*10 using Vivid® CYP2D6 Green Screening Kit (Life Technologies, USA). Response to Bisoprolol was evaluated through assessment of diastolic and systolic blood pressure and by measuring Bisoprolol plasma level using triple quad mass spectrometer (TQ-MS).

Results: All patients were found to carry homozygous wild type CYP2D6*10 (GG) and none were carrying heterozygous (GA) or mutant homozygous (AA) genotype. CYP2D6*2A allele was detected in the homozygous wild type (GG) in 70 out of 107 patients, the heterozygous (GC) in 19 patients, and the homozygous mutant (CC) in 18 patients with minor allele frequency (MAF) of 25.7%. The plasma concentrations of Bisoprolol in CC carriers were significantly lower than those in GG & CC carriers by 25%, and 51%; respectively. Higher systolic and diastolic blood pressures were also observed in CC carriers than GG and CC carriers.

Conclusion: There is a possible association of CYP2D6*2A genotype with plasma concentration of bisoprolol. This could provide a helpful tool to choose the optimum dose for bisoprolol, depending on the patient's genotyping, in order to increase effectiveness and ameliorate its toxicity.

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1. Introduction

Cardiovascular diseases (CVDs) is a wide term that includes many underlying pathologies in which the heart and vessels are involved resulting in arrhythmias, angina pectoris and hypertension. These disorders share similar causes, mechanisms, therapeutic approaches and linkage to atherosclerosis or arterial pathologies (Kumosani et al., 2011). According to WHO, cardiovascular diseases CVDs are considered as one of the primary causes of death worldwide. The condition is responsible for approximately 31% of the whole death incidences round the world, and some reports pointed out that from all CVD deaths, 85.1% are caused by Ischemic heart disease (IHD) and stroke (WHO, 2017, Quiel

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et al., 2019). In 2018, Saudi Arabia reported 14.2% deaths due to CVDs (MOH, 2018).

Bisoprolol is a selective β_1 -blocker, a class of drugs used primarily in CVDs. β -blockers, are among the most widely prescribed of all drug class and recommended as first line to treat patients with numerous diseases such as hypertension, angina, and heart failure (Dezsi and Szentes, 2017).

Bisoprolol is administered orally, and absorbed from the gastrointestinal tract. It undergoes minimal first pass metabolism in the liver to achieve good oral bioavailability (about 90%). It has long elimination half-life (10–11 h), and suitable for a once daily administration. Finally, bisoprolol is cleared from the body in equal parts by biotransformation in the liver and unchanged by the kidney (Bus-Kwasnik et al., 2017).

Thus, many factors can alter its pharmacokinetics as reduced renal blood flow, hepatic blood flow and mass in addition to the activities of drug metabolizing enzymes (cytochrome P450; CYP2D6 and CYP3A4) that have a major effect on drug metabolism (Taguchi et al., 2005). Previous studies have shown that Bisoprolol had often-variable responses among patients, a phenomenon that might be due to genetic variation (Shin and Johnson, 2007). Therefore, the aim of current study was to investigate the association of CYP 2D6 *2A (rs1080985) and CYP 2D6 *10 (rs1065852) genotypes with Bisoprolol response in patients followed up at King Abdulaziz University hospital (KAUH), Jeddah, Kingdom of Saudi Arabia.

2. Patients and methods

2.1. Patients

The study protocol was approved in advance by the Biomedical Ethics Research Committee at King Abdulaziz University (Reference No 63–14). Informed consent letter for participation in the study was obtained from each patient contributing to this study. One hundred and seven in and outpatients were recruited from KAUH from October 2014 to October 2015. Inclusion criteria included all patients with cardiac diseases and Bisoprolol therapy, aged eighteen year or above and agreed to participate and sign the informed consent, while any patients taking drugs known to interact with Bisoprolol especially inducers and inhibitors of CYP 2D6 and CYP 3A4 were excluded. Therapeutic doses and duration of Bisoprolol therapy were determined by the treating physician, depending on the disease severity and patient condition. In addition, follow-up survey based on the medical records of KAUH was carried out.

2.2. Methods

Briefly, 2–4 h after administration of Bisoprolol 5 mg (Concor 5 tablet, Merck KGaA, Germany), two venous blood samples of 5 mL each was collected from patients. Genomic DNA was extracted from leukocytes of first blood sample using QIAamp DNA mini kit (Qiagen, Alameda, CA, USA). Thereafter, the isolated genomic DNA concentration was determined by using Nanodrop-2000 spectrophotometer (Thermo Scientific, USA), based on measuring the optical density at wavelength 260 nm. For genotyping for CYP2D6*2A and CYP2D6*10, the isolated DNA was amplified via PCR, followed by purification from unattached primers, free nucleotides and other ready reaction mix dyes. The purified PCR products were next used as template DNA to generate single stranded sequences with Big-Dye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Life Technologies, and USA) either with forward or reverse primers in separate reactions. These products were run on ABI 3500 genetic analyzer (Life Technologies, USA) using Vivid® CYP2D6 Green Screening Kit (Life Technologies, USA).

In addition, pharmacodynamic response to bisoprolol was evaluated through assessment of diastolic and systolic blood pressure and bisoprolol plasma level was measured using triple quad mass spectrometer (TQ-MS) (Delahaut et al., 2003). The HPLC system consisted of an Agilent 1200 system, solvent delivery module, quaternary pump, autosampler, and column compartment (Agilent Technologies, Germany). The column effluent was connected to an Agilent 6460 triple quad mass spectrometer (TQ-MS) controlled by MassHunter software. The screw-capped (PTFE/silicon) total recovery 1-mL autosampler vial, 12x32 mm was used. Injection volume was 5 μ L. The TQ-MS conditions utilized were a gas temperature of 330 °C, gas flow of 10 L/min, nebulizer pressure of 36 psi, capillary voltage of 4000 V, and cell accelerator voltage of 7 V. The dwell, fragmentor voltage, and collision energy (CE) were optimized for each compound and a positive multiple reaction-monitoring (MRM) mode was applied. The separation was performed on an Agilent Eclipse Plus C18, 3.0 \times 150 mm, 5 μ m HPLC column (Agilent Technologies, USA) maintained at 25 \pm 2 °C. Mobile system was composed of 28% acetonitrile and 72% Ammonium acetate buffer, pH 4.7, pumped at flow rate of 0.5 mL/min. propranolol was used as an internal standard.

2.3. Statistical analysis

Various parameters including numbers, percentages of participants with demographic characteristics of age, gender, and nationality in correlation with each genotype was determined. Difference in the mean of the analyzed variables including clinical and demographic characteristics were verified using descriptive statistics. Results were expressed as the means \pm standard deviation or number (%) depending on the type of tested variables. All statistical analyses were done by using Excel (Microsoft Excel 2010, USA).

3. Results

3.1. Patients' demographic data

The patients' characteristics data are summarized in (Fig. 1). A total of 107 patients were enrolled in the study. All of them were bisoprolol users (dose 5 mg). Twenty-seven of them were Saudi (24.8%), while 82 were non-Saudi (75.2%). Males were 74 patients (67.9%) and females were 35 patients (32.1%). Average age for male was (56.9 \pm 11.4 year), average height for male was (166.6 \pm 8.5 cm), average weight was for male (81.6 \pm 18.4 kg), average age for female was (56.3 \pm 15.6 year), average height for female was (162.8 \pm 8.2 cm), and average weight for female was (71.9 \pm 17 kg).

3.2. Frequency of studied population affected by various diseases

Table 1 shows that among all study population who had cardiovascular diseases, most of them 44.2% had diabetes. But the other diseases were in low percentage such as gastrointestinal diseases by 13.3%, thyroid diseases by 9.3%, nephrological diseases by 6.2%, hepatobiliary diseases by 5.2%, respiratory diseases by 5.1%, neurological diseases by 3.1%, and dermatological diseases by 2.1%.

3.3. Genotype CYP2D6*2A and CYP2D6*10 analysis

After collecting 5 mL blood sample, measuring the blood pressure, and collecting data from the patient, the custom TaqMan assay genotyping data was done for all individuals including wild type and mutant genotypes for rs1080985 (CYP2D6*2A) alleles and for rs1065852 (CYP2D6*10) are summarized in Table 2. For the gene CYP 2D6* 10 allele it was found all the patients were carrying homozygous (GG) and none of them are heterozygous (GA)

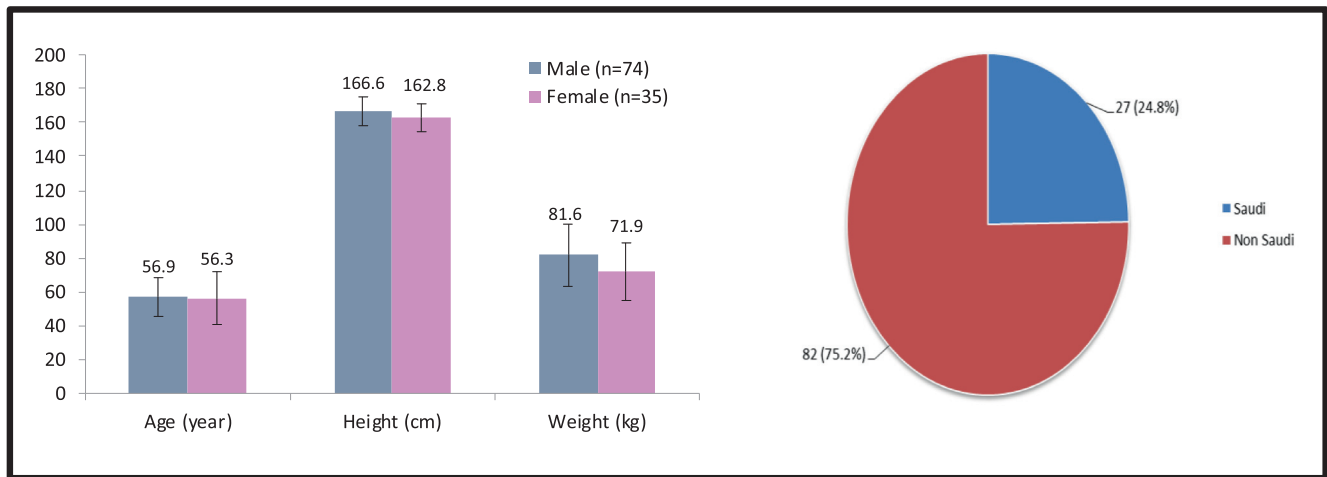


Fig. 1. Demographic characteristics of enrolled population (n = 107).

Table 1

Table of frequency of the studied population affected by various diseases.

Diseases	Frequency		Percent (%)		Total
	Yes	No	Yes	No	
Cardiovascular	98	0	100	0	98
Diabetes	42	56	44.2	55.8	98
Gastrointestinal	11	87	13.3	86.7	98
Thyroid	9	89	9.3	90.7	98
Nephrological	6	92	6.2	93.8	98
Hepatobiliary	5	93	5.2	94.8	98
Respiratory	5	93	5.1	94.9	98
Neurological	3	95	3.1	96.9	98
Dermatological	2	96	2.1	97.9	98

Table 2

Allele Genotype Frequency of SNP CYP 2D6*2A AND SNP 2D6*10.

SNP	Genotype	Number	Percentage	MAF
	Homozygous (CC)	18	16.5%	
	Heterozygous (GC)	19	17.4%	
SNP CYP2D6*10 (rs1065852)	Homozygous (GG)	99	100%	0.00
	Homozygous (AA)	0	0	
	Heterozygous (GA)	0	0	

MAF is minor allele frequency

or homozygous for minor allele (AA). However, for CYP 2D6 * 2A, allele 70 (65.4%) of patients were homozygous (GG), 19 (17.7%) of patients were heterozygous (GC), and 18 (16.8%) of patients were homozygous (CC) (Table 2).

3.4. The effect of CYP2D6*2A genotype on blood pressure, heart rate, and bisoprolol concentration

As shown in Table 3, the plasma level of bisoprolol in homozygous GG carriers was 14.2 ± 5.6 ng/ml while systolic BP and

diastolic BP were 129.7 ± 29.7 mmHg, 73.8 ± 16.9 mmHg, respectively and heart rate was 72.6 ± 12.2 bpm. In homozygous CC carriers, the plasma level was 11.9 ± 4.4 ng/ml and decreased by 16% compared to GG carriers and accordingly systolic BP increased by 7% (138.6 ± 30.1 mmHg) and Diastolic BP increased by 9% (80.1 ± 16.7 mmHg). On the other hand plasma level of bisoprolol in heterozygous GC carriers increased by 51% (21.5 ± 8.1 ng/ml) compared to GG and this caused a decrease in systolic BP by 6% (122.3 ± 19.8 mmHg) and no changes in the diastolic BP (73.3 ± 15.9 mmHg). Similarly, the plasma level of bisoprolol

Table 3

The Effect of CYP2D6*2A Genotype on blood pressure, heart rate, and bisoprolol concentration.

Genotype staut SNP CYP2D6*2A	Systolic BP (mmHg)	Systolic BP (% change)	Diastolic BP (mmHg)	Diastolic BP (% change)	Heart rate (bpm)	Blood Bisoprolol concentration (ng/ml)	% Change of plasma level
Homozygous (GG)	129.7 ± 29.7	-7% ²	73.8 ± 16.9	-8% ²	72.6 ± 12.2	14.2 ± 5.6	+19.3% ²
Homozygous (CC)	138.6 ± 30.1	+7% ¹	80.1 ± 16.7	+9% ¹	74.8 ± 15.0	11.9 ± 4.4	-16% ¹
Heterozygous (GC)	122.3 ± 19.8	-6% ¹ -16% ²	73.3 ± 15.9	-9% ²	81.4 ± 13.7	21.5 ± 8.1	+51% ¹ +81% ²

Results are averages mean \pm SD, Total no of patients = 107.

- 1 compared to homozygous (GG).
- 2 compared to homozygous (CC).

in heterozygous GC carriers increased by 81% (21.5 ± 8.1 ng/ml) compared to homozygous CC carriers and this caused a decrease in systolic BP by 16% (122.3 ± 19.8 mmHg) and in the diastolic BP by 9% (73.3 ± 15.9 mmHg).

3.5. The effect of CYP2D6*10 genotype on blood pressure, heart rate, and bisoprolol concentration

As shown in Table 4 for CYP2D6*10 genotype no carriers of heterozygous GA and homozygous AA, and all patients homozygous (GG) and the average plasma level was 14.7 ± 13.4 ng/ml, average systolic BP was 129.9 ± 28.5 mmHg, average diastolic BP was 74.8 ± 16.7 mmHg and heart rate average was 74.6 ± 13.4 bpm.

3.6. The association of genotype CYP2D6*2A and bisoprolol side effects

Table 5 shows that among the 7.2% of patients who had tiredness 6 of them were homozygous GG carriers and 1 patient was heterozygous GC carriers. Similarly, among the 6.2% who had chest pain, 4 of them were homozygous GG carrier and 2 were heterozygous GC carriers. In troubled breathing patients (6.2%), 5 of them were homozygous GG carrier and 1 patient was heterozygous GC carrier. In dizziness (4.6%), cough (4.1%) and fever (3.1%) all patients were homozygous GG carriers. For weakness, 3 of the patients (4.1%) were homozygous GG carriers and 1 patient was heterozygous GC carriers. For headache, 2 patients (3.1%) were homozygous GG carriers and 1 patient was heterozygous GC carriers. For blurred vision (2.1%), one patient was homozygous GG carrier and one patient was heterozygous GC carrier, and in nightmares (2.1%) and anxiety (1%) all the patients were homozygous GG.

4. Discussion

Cardiac and respiratory disorders are highly reported in Saudi population. An estimate in Saudi Arabia showed that 42% of all deaths are from CVDs (Alshaikh et al., 2016). Also, diabetes and ischemic heart disease are main reasons for hospitalization. The changing lifestyles in oil rich Middle Eastern countries lead to more CVDs risk. These include eating high fat food especially red meat, smoking, increase blood sugar and cholesterol, and other risk factors. In Saudi Arabia, most of the young generation has multiple risk factors (Mahmood et al., 2015). Aldiabi et al., showed in a cohort group in Saudi Arabia population that hypertension and pre-hypertension are most common conditions. The prevalence of pre-hypertension in male was 66.1%, in female was 48.1%, and in all subjects was 54.9%. It was also observed that the highest risk of hypertension was associated obesity and overweight (Aldiabi et al., 2018).

Various phenomena have been identified in causing inter-individual variability in response to Bisoprolol despite the tailored dose for achieving the goal of blood pressure and heart rate. One of these phenomena is pharmacogenomics which causes of the inter-individual variability in response to Bisoprolol. Therefore, the aim of present study was to investigate the association of CYP 2D6

*2A (rs1080985) and CYP 2D6 *10 (rs1065852) genotypes with the Bisoprolol response in patients at KAUH, Jeddah, Kingdom of Saudi Arabia.

Current results showed that all patients carry homozygous wild type CYP2D6*10 (GG), but none were carrying heterozygous (GA) or mutant homozygous (AA) genotype. CYP2D6*2A allele was detected in the homozygous wild type (GG) in 70 out of 107 patients, the heterozygous (GC) in 19 patients, and the homozygous mutant (CC) in 18 patients with minor allele frequency (MAF) of 25.7%. The plasma concentrations of Bisoprolol in CC carriers were significantly lower than GG and CC carriers by 25%, and 51% respectively. Higher systolic and diastolic blood pressure was also observed in CC carriers than GG and CC carriers so there is a window to increase the dose for these patients. The average systolic blood pressure in CC carriers was 139.8 mmHg compared to 128.8 mmHg in GG carriers and 121.5 mmHg in GC carries. Similarly, the average diastolic blood pressure in CC carriers was 79.9 mmHg compared to 73.6 mmHg in GG carriers and 73.7 mmHg in CC carriers.

Blood sample was collected from the patients 2–4 hrs after bisoprolol (5 mg) dosing. Studies have shown that peak plasma concentration of Bisoprolol occurs within 2–4 hrs of dosing (Tjandrawinata et al. 2012). The effect of CYP2D6*2A genotype on bisoprolol plasma level showed that the range of bisoprolol plasma levels in patients was almost comparable to that reported by Nozawa et al. (Nozawa et al., 2005). In the present study, homozygous CC carriers decreased by 16%, compared to homozygous GG carriers. However, plasma levels in heterozygous GC carriers increased by 51% compared to homozygous GG carriers, and by 81% compared to CC homozygous carriers. Therefore, it could be inferred that homozygous CC carriers are extensive metabolizers, homozygous GG carriers are intermediate metabolizers and heterozygous GC carriers are poor metabolizers.

These changes in bisoprolol plasma levels were reflected on responses of systolic and diastolic blood pressure. Heterozygous GC carriers showed 6% decrease in systolic blood pressure compared to homozygous GG carriers, 16% and 9% decrease in both systolic and diastolic blood pressure respectively when compared to CC carriers. Similarly, CYP2D6*2A homozygous GG carriers showed 7% decrease in systolic blood pressure and 8% decrease in diastolic blood pressure compared to homozygous CC carriers. So, it might be suggested that patients who are carriers of the G allele might have a better response to bisoprolol, and carrier of the allele C might have a reduced response to bisoprolol. On the other hand, patients who are carriers of the C allele might have a reduced response to bisoprolol and might require increased dose of the drug. It has been observed that patients who were homozygous GG carriers and heterozygous GC carriers had response rate in systolic blood pressure (41% and 44%) respectively which was much higher than homozygous CC carriers (19%). In addition, patients who were homozygous GG carriers and heterozygous GC carriers had response rate in diastolic blood pressure (59% and 50%) which was much higher than homozygous CC carriers (31%). However, the effect of CYP2D6*2A genotype on response in heart rate did not show any statistically significant change between the various genotype groups. Other studies have shown that analysis of

Table 4
The Effect of CYP2D6*10 Genotype on blood pressure, heart rate, and bisoprolol blood concentrations.

GenotypeSNP CYP2D6*10	Blood pressure systolic (mmHg)	Blood pressure diastolic (mmHg)	Heart rate(bpm)	Bisoprolol concentration in the Blood (ng/ml)
Homozygous (GG)(100%)	129.9 ± 28.5	74.8 ± 16.7	74.6 ± 13.3	14.7 ± 13.4
Homozygous AA(0%)	NA	NA	NA	NA
Heterozygous GA(0%)	NA	NA	NA	NA

Total number of patients = 107, Results are means ± SD. NA: Not applicable.

Table 5
Association of genotype CYP2D6*2A and observed side effects in study population on bisoprolol.

Side effects	No of patients (Frequency)		Percentage		PatientGENO TYPE		Total
	Yes	No	Yes	NO	GG	GC	
Tiredness	7	90	7.2%	92.8%	6	1	97
Troubled breathing	6	91	6.2%	93.8%	5	1	97
Chest pain	6	91	6.2%	93.8%	4	2	97
Dizziness	5	92	4.6%	84.4%	5	0	97
Cough	4	93	4.1%	95.9%	4	0	97
Weakness	4	93	4.1%	95.9%	3	1	97
Fever	3	94	3.1%	96.9%	3	0	97
Headache	3	94	3.1%	96.9%	2	1	97
Blurred vision	2	95	2.1%	97.9%	1	1	97
Nightmares	2	95	2.1%	97.9%	2	0	97

CYP2D6*2A (rs1080985) genotype might be a useful predictor for poor response with other drugs for example with Donepezil treatment in patients with Alzheimer's disease (Albani et al., 2012). This was supported also by Xiao and co-workers in a meta-analysis study including 1266 patients with Alzheimer's disease treated with donepezil (Xiao et al., 2016).

It is worthy noted that diabetes was the most frequent disease associated with our cohort group study by 44.2%. Studies have shown that up to 75% of adults with diabetes also have hypertension (Long and Dagogo-Jack 2011). Thus, hypertension and diabetes are common, intertwined conditions that share a significant overlap in underlying risk factors (including ethnicity, familial, dyslipidemia and life-style determinants) and complications (Ye et al. 2019). All diabetic patients included in the study were of Type 2 diabetes taking Metformin (Glucophage 1500–2000 mg/day). It is unlikely that bisoprolol plasma levels were affected by Metformin as it is not metabolised in the liver nor it is an inducer or inhibitor of hepatic isozymes CYP 2D6 and CYP3A4 responsible for bisoprolol metabolism. Further Nevzorova and co-workers (2007) have reported that a combination of bisoprolol with metformin caused a significant reduction of BMI and improved insulin plasma concentration in patients with arterial hypertension and metabolic syndrome receiving combined therapy. The frequencies of other type of diseases were much lower i.e. gastrointestinal diseases (13.3%), thyroid diseases (9.3%), and nephrological diseases (6.2%) (Ye et al., 2019). So, it was most unlikely that bisoprolol plasma levels and its response on systolic/diastolic blood pressure were affected by these diseases.

Beta blocker therapy was estimated to cause adverse effect in 5% – 43% of patients with heart failure, and 25% of patients are required to stop taking beta blockers due to this intolerance. Several studies suggest that variability may be due to genetic polymorphisms that lead to variable drug response (Myburgh et al., 2012). Notably, Bisoprolol effectiveness and toxicity were found to be related to systemic drug concentration which could vary between individuals due to clinical factors such as age, comorbidities, environmental, period of treatment, dose, drug-drug interaction, drug-food interaction, and genetic factors (Turner et al., 2018).

Limitation of the study includes a) Bisoprolol is metabolized by two CYP isoforms; CYP2D6 and CYP3A4. Polymorphism of CYP3A4 was not determined in this study. b) The relatively limited number of the study population (107 patients) might have some effects on our results.

5. Conclusion

There is a possible association of CYP2D6*2A genotype with plasma concentration of bisoprolol. This could provide a helpful tool to choose the optimum dose for bisoprolol, depending on

the patient's genotyping, in order to increase effectiveness and ameliorate its toxicity. The results of the present study open a new window for studying more SNPs and to define the role of various SNPs in inter-individual variability in Bisoprolol response. The findings can be confirmed by a follow up study of same genes in larger population.

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References

- Albani, D., Boneschi, F.M., Biella, G., Giacalone, G., Lupoli, S., Clerici, F., Benussi, L., Ghidoni, R., Galimberti, D., Squitti, R., 2012. Replication study to confirm the role of CYP2D6 polymorphism rs1080985 on donepezil efficacy in Alzheimer's disease patients. *J. Alzheimer's Dis.* 30, 745–749.
- Aldiab, A., Shubair, M.M., Al-Zahrani, J.M., Aldossari, K.K., Al-Ghamdi, S., Househ, M., Razzak, H.A., El-Metwally, A., Jradi, H., 2018. Prevalence of hypertension and prehypertension and its associated cardioembolic risk factors; a population based cross-sectional study in Alkharij. Saudi Arabia. *BMC Public Health* 18, 1327.
- Alshaiikh, M.K., Filippidis, F.T., Baldove, J.P., Majeed, A., Rawaf, S., 2016. Women in Saudi Arabia and the Prevalence of Cardiovascular Risk Factors: A Systematic Review. *Journal of Environmental and Public Health* 2016, 1–15. <https://doi.org/10.1155/2016/7479357>.
- Buś-Kwaśnik, K., Rudzki, P.J., Ksycińska, H., Leś, A., Serafin-Byczak, K., Raszek, J., Bielak, A., Wybraniec, A., Platek, A.E., Szymański, F.M., 2017. Bioequivalence study of 2.5 mg film-coated bisoprolol tablets in healthy volunteers. *Kardiol. Pol. (Polish Hear. Journal)* 75, 48–54.
- Delahaut, P., Levaux, C., Eloy, P., Dubois, M., 2003. Validation of a method for detecting and quantifying tranquilisers and a β -blocker in pig tissues by liquid chromatography–tandem mass spectrometry. *Analytica chimica acta* 483 (1–2), 335–340.
- Dezsi, C.A., Szentes, V., 2017. The real role of β -blockers in daily cardiovascular therapy. *Am. J. Cardiovasc. Drugs* 17, 361–373.
- Kumosani, T.A., Alama, M.N., Iyer, A., 2011. Cardiovascular diseases in Saudi Arabia. *Prime Res Med* 1, 1–6.
- Long, A.N., Dagogo-Jack, S., 2011. Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. *J. Clin Hypertens (Greenwich)* 13, 244–251. <https://doi.org/10.1111/j.1751-7176.2011.00434.x>.
- Mahmood, D., Jahan, K., Habibullah, K., 2015. Primary prevention with statins in cardiovascular diseases: A Saudi Arabian perspective. *J. Saudi Hear. Assoc.* 27, 179–191.
- MOH, 2018. MOH Statistical Yearbook 2018. <https://www.moh.gov.sa/en/Ministry/Statistics/book/Pages/default.aspx> (accessed 20 February 2020)
- Myburgh, R., Hochfeld, W.E., Dodgen, T.M., Ker, J., Pepper, M.S., 2012. Cardiovascular pharmacogenetics. *Pharmacol. Ther.* 133, 280–290.
- Nevzorova, V.A., Zakcharchuk, N.V., Nastradin, O.V., Pomogalova, O.G., 2007. Bisoprolol and metformin in patients with arterial hypertension and metabolic syndrome. *Rational Pharmacotherapy in Cardiology*. 2007;3(5):54–57. <https://doi.org/10.20996/1819-6446-2007-3-5-44-46>
- Nozawa, T., Taguchi, M., Tahara, K., Hashimoto, Y., Igarashi, N., Nonomura, M., Kato, B., Igawa, A., Inoue, H., 2005. Influence of CYP2D6 genotype on metoprolol plasma concentration and β -adrenergic inhibition during long-term treatment: a comparison with bisoprolol. *J. Cardiovasc. Pharmacol.* 46, 713–720.

- Quiel, L., Velásquez, I.M., Gómez, B., Motta, J., Herrera-Ballesteros, V., 2019. Social determinants and cardiovascular disease mortality in Panama, 2012–2016. *BMC Public Health* 19, 199.
- Shin, J., Johnson, J.A., 2007. Pharmacogenetics of β -Blockers. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 27, 874–887.
- Taguchi, M., Nozawa, T., Igawa, A., Inoue, H., Takesono, C., Tahara, K., Hashimoto, Y., 2005. Pharmacokinetic variability of routinely administered bisoprolol in middle-aged and elderly Japanese patients. *Biol. Pharm. Bull.* 28, 876–881.
- Turner, R.M., Fontana, V., Bayliss, M., Whalley, S., Castelazo, A.S., Pirmohamed, M., 2018. Development, validation and application of a novel HPLC-MS/MS method for the quantification of atorvastatin, bisoprolol and clopidogrel in a large cardiovascular patient cohort. *J. Pharm. Biomed. Anal.* 159, 272–281.
- WHO, 2017. Report on cardiovascular diseases, WHO. <https://www.who.int/health-topics/cardiovascular-diseases/> (accessed 20 February 2020)
- Xiao, T., Jiao, B., Zhang, W., Tang, B., Shen, L., 2016. Effect of the CYP2D6 and APOE polymorphisms on the efficacy of donepezil in patients with Alzheimer's disease: a systematic review and meta-analysis. *CNS Drugs* 30, 899–907.
- Ye, X., Kong, W., Zafar, M.I., Chen, L.-L., 2019. Serum triglycerides as a risk factor for cardiovascular diseases in type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *Cardiovasc. Diabetol.* 18, 48.