ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Fildaus NYIRAHABIMANA

ANALYSIS OF PARAOXONASE-1 GENE IN SMALL FOR GESTATIONAL AGE NEONATES USING MOLECULAR TECHNIQUES

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of thedegree of the Master of Science by the board of jury on 22/02/2018.

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ABSTRACT

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In developed countries, there are a number of studies on the identification, diagnosis and treatment of risk factors responsible for pregnancies resulting in small for gestational age neonates. Although this problem is much more important in developing and underdeveloped countries, there are very few studies in this area and the majority of these studies are insufficient in terms of method and statistical evaluation. In the present work, the PON1 (rs662 and rs705379) gene polymorphisms were examined by broad DNA sequence analysis in small for gestational age neonates and the variation of PON1 gene polymorphisms were determined. The blood samples of SGA babies were taken from Cukurova University Balcali Hospital, Neonatal Intensive Care Unit in first three days of birth. The study analysis was done on 41 SGA neonates by the RT-PCR procedures. As a conclusion, because of small number size of cases used in this study, many studies are required using a large number size of SGA neonates.

Keywords: Small for gestational age, Paraoxonase1 gene, Polymorphisms

ÖZ

YÜKSEK LİSANS TEZİ

GESTASYON HAFTASINA GÖRE DOĞUM AĞIRLIĞI DÜŞÜK OLAN BEBEKLERDE PARAOKSANAZ-1 GENİNİN MOLEKÜLER YÖNTEMLERLE İNCELENMESİ

Fildaus NYIRAHABIMANA

ÇUKUROVA ÜNİVERSİTESİ
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: Doç. Dr. Bertan YILMAZ
: Prof. Dr. Nizami DURAN

Gelişmiş ülkelerde doğum ağırlığı düşük infant doğumu ile sonuçlanan gebeliklerden sorumlu risk faktörlerinin belirlenmesi, teşhis ve tedavisi ile ilgili yapılmış çok sayıda çalışma vardır. Buna karşılık gelişmekte olan ve geri kalmış ülkelerde bu sorunun çok daha önemli olmasına rağmen bu konuda yapılmış çalışmalar çok az sayıda olup bunların da çoğunluğu yöntem ve istatistiksel değerlendirme açısından yetersizdir. Bu çalışmada, gebelik haftasına göre doğum ağırlığı düşük olan yenidoğanlarda paraoksanaz1 geni DNA dizi analizi ile incelenerek PON1 gen polimorfizmlerinin (rs662 ve rs705379) varyasyonları belirlenmiştir. Çukurova Üniversitesi Balcalı Hastanesi Yenidoğan Yoğun Bakım Ünitesinde doğan doğum ağırlığı düşük 41 bebeğin kan örnekleri alınarak RT-PCR yöntemi ile analiz edilmiştir. Bu çalışmadaki hasta sayısının genişletilmesi gereklidir.

Anahtar Kelimeler: Düşük doğum ağırlığı, Paraoksanaz-1 geni, Polimorfizm

EXTENDED ABSTRACT

Biotechnology is a new field that has developed in a wide variety of fields and is now changing gradually in the form of molecular biotechnology, along with its widespread use in molecular techniques. Turkey is a country with a unique geographic position, lying between two continents in the world i.e in northern Asia and partly in southern Europe. Although Turkey is classified as a developed country, there are a few studies that evaluated birth weight for gestational age reference. The gestational age and birth weight are two components of small for gestational age (SGA). The SGA criteria include the combination of birth weight and gestational age together.

The SGA is defined as infants with birth weight (BW) below the 10^{th} percentile for gestational age. Constitutionally small infants are those with a normal BW below 10^{th} percentile due to constitutional factors including maternal height and weight. Accurate gestational dating and measurement of birth weight and length are crucial for identifying children who are born SGA. These neonates are not at increased risk for perinatal mortality or morbidity. The measurement of SGA compares newborn birth weight with a national standard distribution of live births so that weights are relative to neonates of the same gestational age. Because of these variables, birth weight standards obtained in one community may not necessarily apply to another community. Small for gestational age neonates are divided into: neonate weight retardation, growth retardation matched up in weight and height. This sub-classification may help in understanding the mechanisms and implications of being born with SGA. Definition of SGA does not take into account background growth modifying factors such as maternal size, ethnicity, and parity.

Our study was based on small for gestational age, where it faced some problems and evaluation ofSGA neonates. The SGAbabies could be premature (born before 37 weeks of pregnancy), full term (37 to 42 weeks), or post-term (after 42 weeks) of pregnancy. The definition of SGA is not straightforward, it

requires the following points: accurate knowledge of gestational age (based on first-trimester ultrasound exam), accurate measurements at thebirth of weight, length, and head circumference, and cut-off against reference data from a relevant population. This cut-off has been variably set at the 10^{th}percentile, 3rd percentile, or less than 2 standard deviation (SD) from the mean. Most of the studies recommend that SGA should be defined as weight and/or length less than 2SD. This condition affects approximately 3-10% of newborns. The causes of SGA birth include environmental factors, placental factors such as abnormal uteroplacental blood flow, and inherited genetic mutations. The number of genetic variations for any particular gene has been associated with SGA births. Birth weight and gestational age are two very important determinants not only of disability and death among newborns but also of their subsequent health and well being. These children are subjects to follow up for later risk of socially significant diseases in the adult.

Human serum paraoxonase1 (PNO1: 3.1.8.1), is an enzyme that involved in vasodilation and thrombosis and associated with specific high-density lipoprotein (HDL) sub-species that contain apoprotein (apoA1) and clusterin. It is located on long arm of chromosome7 (7q21.3-22.1), PON1 has an enzyme code (EC) of 3.1.8.1 and also implies 43-45 kDa glycoprotein with 6 β bladed propellers with a lid covering the active site passage and 5.1 isoelectric point, it contains 354 amino acids. The purpose of this present study was to investigate the PON1 gene in SGA neonates in order to clarify and find out the variationof PON1 gene polymorphisms with birth weight in SGA neonates using molecular techniques.

According to our knowledge, this study is the first study done in Turkey to report the variation of PON1 gene polymorphisms in small gestational age neonates. Analysis of PON1 gene polymorphisms for SGA neonates was carried out using real time-Polymerase Chain Reaction (RT-PCR). The results of this study can be used to establish and determine the variation of PON1 gene polymorphisms within birth weight group of SGA neonates.

In addition, it can be contributed to the development of new strategies for the diagnosis, complications and treatment of diseases by children healthcare institutions in Turkey and it can be a reference to any research will be done in this area of study all over the world. Because of small number size of cases used in this study, many studies are required using a large number size of SGA neonates.

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Fildaus NYIRAHABIMANA

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LIST OF ABBREVIATIONS

AGA : Appropriate for Gestational Age
BW : Birth Weight
CAD : Coronary Arter Disease
CETP : Cholesteryl Ester Transfer Protein
CHD : Cardiovascular Heart Disease
DNA : Deoxyribonucleic Acid
EC : Enzyme Code
EDTA : EthyleneDiamineTetraAcetic Acid
FFA : Free Fatty Acid
GH : Growth Hormone
HCT : Hematocrit
HDL : High-Density Lipoprotein
HDLC : HDL -Cholesterol
IUGR : Intrauterine Growth Restriction
LBW : Low Birth Weight
LDL : Low-Density Lipoprotein
LDL-R : LDL- Receptor
LGA : Large for Gestational Age
LPL : Lipoprotein Lipase
OP : Organophosphate
OxLDL: Oxidized LDL
PCR : Polymerase Chaine Reaction
PON : Paraoxonase
PUFAs: Polyunsaturated Fatty Acids
ROS : Reactive Oxygen Species
SD : Standard Deviation
SDS : Standard Deviation Score

SGA : Small for Gestational Age

SNP : Single Nucleotide Polymorphism

TC : Thrombocyte count

VLDL : Very- Low-DensityLipoprotein

1. INTRODUCTION

Biotechnology is a new and future-proof field that has developed in a wide variety of fields and is now changing gradually in the form of molecular biotechnology, along with its widespread use in molecular techniques. The increased result of speed up working on the production of products used in the commercial areas is increasing with each passing single day.

In 2010, 32.4 million babies were born SGA in low- and middle-income countries, constituting 27% of all live births. The estimated prevalence of SGA is highest in South Asia and in Sahelian countries of Africa. The prevalence of SGA births is approximately double the prevalence of low-birth weight births globally and in the world's regions(Black, 2015). In North Carolina, the study has found that African American women were twice as likely to deliver a term SGA infant compared with non-Hispanic White women (Schempf et al., 2011). Effective implementation of increasing survival and reducing disability, inhibit and noncommunicable diseasesare avery urgent priority to be resolved in childhood health problem for babies born SGA (Lee et al., 2013).

Recently, in developed countries have a numerous of studies on the identification, diagnosis, and treatment of risk factors for pregnancies resulting in SGA infant birth. Despite the fact that this problem is much more important in developing and underdeveloped countries, where studies on this field are still very low numbered and most of them are found to be inadequate in terms of method and statistical evaluation (McDonald et al., 2010). A large number of risk factors responsible for the birth of the SGA neonates have been reported. Some of them are mentioned in many research projects like low or advanced maternal age, short stature, short interval between pregnancies, smoking, gestational inadequate weight gain, pregnancy vascular disease (Kapadia et al., 2015). Turkey is country with a unique geographic position, lying between two continents on world i.e in northern

Asia and partly in southern Europe. Although Turkey is classified as the developed country, it has a little number of studies of evaluation of birth weight for small gestational agereference. Nevertheless, most studies have been used small sample size and are too limited in their reliability of demonstration (Topcu et al., 2014).

1.1. Background Of Study

Gestational age is the common word that is used to signify the pregnancy event how is long or short to be born or giving a birth of new-born. Gestational age is determined in weeks or months. The gestational age is described by three main types of its period as well as appropriate for gestational age (AGA) also called normal pregnancy; the small for gestational age (SGA); and large for gestational age (LGA) (Ball et al., 2015).

Our study is based on small for gestational age, where this study faced all problems and evaluation on newborns with SGA. Small for gestational age babies could be born premature (born before 37 weeks of pregnancy), full-term (37 to 42 weeks), or post-term (after 42 weeks) of pregnancy.The small for gestational age distinguishes newborns that have low birth weight and a small number of weeks and days of pregnancy (American College of Obstetricians and Gynecologists, 2013). It also expounds fetuses who fail to attain a specific anthropometric or weight threshold by a specific gestational age (Leng et al., 2016). The SGA invokes the length and/or low weight, body size, widely known gestational age (Boguszewski et al., 2011).

Birth weight (BW) ranging from under 3^{rd} percentile corresponding to two standard deviation (2SD) below the population standard mean for gestational week to below the 10^{th} percentile, this range has been used to determine small for gestational week with describing prenatal growth restriction of newborn (Saenger and Reiter, 2012). This gives a good signification of SGA that is known to be

weight and/or length less than 2SD (Clayton et al., 2007), or the 10th percentile for birth weight according to a population reference (Pay et al., 2015).

Besides, it is widespreaded in many countries and leads to an important public health problem, as it can conduct to a variety of short and long-term consequences as increasing the risk of mortality and morbidity (Cohen et al., 2015). It does not have any compromise; it needs to take care of pregnancies, to get a required knowledge about the gestational week, control birth weight's measurements, head circumference, and the cut-off against reference data from any standard deviation.

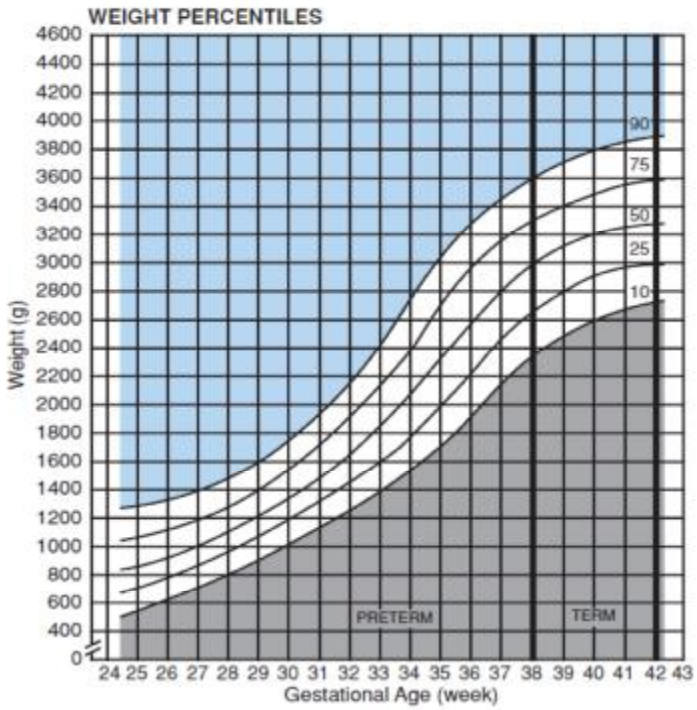


Figure 1.1**.** Lubchenco Growth curve for gestational age (Lubchenco et al., 1963)

The incidence of SGA births in each country is not exactly known because agestational week and birth anthropometric data are rarely recorded in many national databases (Cho and Suh, 2016). Additionally, the highest risk of mortality

and morbidity of infants in many countries posed by SGA that presents and causes important health problems in children's development (Boguszewski et al., 2011).

1.2. Paraoxonase Genes

1.2.1. Paraoxonase genes family and definition

The first study on paraoxonaseenzymes had been done by Mazur in 1946. Previously, many studies have been found that the family of PON is bunched up three enzymes which are Paraoxonase 1 (PON1), Paraoxonase2 (PON2) and Paraoxonase3 (PON3). In humans, all of them are locatedon thelong arm (q) of chromosome 7 (q21.3-22.1) with a closed homology in structure (Liu et al., 2016; Mackness and Mackness, 2015).

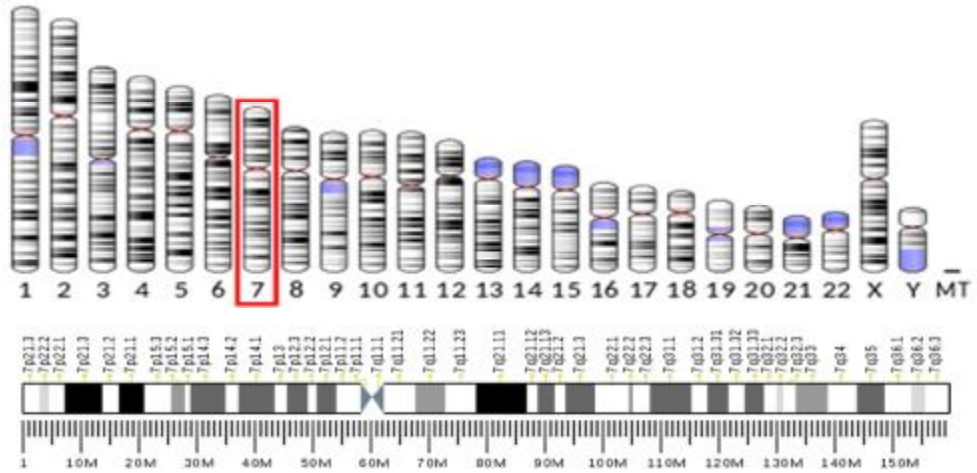


Figure 1.2. PON1 location in Human chromosome 7

1.2.2. Generalstructure of Paraoxonase1 and its activities

Human serum paraoxonase1(EC 3.1.8.1) is an enzyme that involved in vasodilation and thrombosis. PON1 protein starts with an N-terminal hydrophobic sequence associated with specific high-density lipoprotein (HDL) sub-species that contain apoprotein (apoA1) and clusterin. It implies 43-45 kDa glycoprotein with 6

β bladed propeller that covers the active site passage (She et al., 2012) and 5.1 isoelectric point, it contains 355 amino acids, it possesses two calcium binding sites (ca^{+2}) in structure where they are essential for catalytic activity and enzyme stability which has a high affinity, this shows that one side is for the hydrolytic activity another side required to be necessary for antioxidant activity (Mackness, 2015). Serum form of PON1 has three cysteines (Cys) residue at positions 42, 284, and 353. Two of them (Cys42 and Cys353) build a disulfide linkage whereas Cys284 is free. The PON1's activity can be abolished by mutating Cys42 or Cys353 to alanine; either of these variant changes in gene also significantly decreases the secretion of the protein (Sheet al., 2012). In another hand, Cys284 has been shown to be necessary for theability of PON1to protect LDL against copper-induced oxidation modification (Harel et al., 2004). It holds its hydrophobic signal sequence in N-terminal region with the exception of initial of methionine that facilitates PON1 association with HDL (Mackness and Mackness, 2015).

The PON1 is a protein-coding gene, with a size of 27,032 bases and is a glycoprotein composed of 355 amino acids. The structural portion of the paraoxonase protein is encoded by nine exons that form the primary transcript through the use of typical splice donor and acceptor sites. DNA sequences of the regions surrounding all the coding exons have been determined. A polymorphic CA repeat is located in intron 4. The most common allele had 17 repeats in a population of 17 individuals (Clendenning et al., 1996). The two common polymorphisms in coding exon region, one of them is located at 55 position of amino acid (Met/Leu55) where L allele from leucine and M allele from methionine amino acids are mutated. Another mutation encodes Q allele (glutamine) to R allele (arginine) substitution at position 192 (Arg/Gln192) (Rea et al., 2004). Levels of human PON1 activities vary depending on individuals, that may sometimes account for differences in susceptibility to organophosphate poisoning. The purified PON1 from PON1 gene variants 192 Arg/Arg and PON1 55 Leu/Leu individuals has the greatest hydrolytic activity toward paraoxon, whereas one from

PON1 192-Gln/Gln and PON1 55-Met/Met individuals has the least heterozygotes have intermediate levels of activity (Imai et al., 2000; Watson et al., 2001). There is a similarity of another specific substrate of oxons that known as like the methylparaoxon, chlorthion-oxon, andthe amine, which can behaveas PON1 substrates.Nevertheless, the capacity of PON1 to protect LDL against oxidative modification follows the opposite trend to paraoxon hydrolytic activity. The PON1variants from 55-Met/Met/ and 192-Gln/Gln individuals exhibits the greatest protective capacity for LDL oxidization.

This type of PON1 has an ability to frankly hydrolyze the nerve gases soman and sarin compounds (Watson et al., 2001). In the promoter region of PON1 there are 3common polymorphisms among them there is C-108T (rs705379) and it is known to be a predominant promoter polymorphism and gives a greater contribution on enzyme active (phenotypes) (Turgut Cosan et al., 2016). Through the substitution of a cytosine (C) with thymine (T) the polymorphic position C-108T lies within the GGCGGG consensus sequence of the binding site for the transcription factor Sp1(Clendenning et al., 1996).

1.3. Statement Of The Study

Organophosphorus (OP) nerve agents are highly toxic compounds that rapidly inhibit acetylcholinesterase. The genetic factors alone could not explain the increased cases of neurodevelopmental disorders in children, together with their increasing susceptibility to organophosphates (OPs) neurotoxicity but it could be related to gene and environment interaction factors. Epidemiologic studies suggest that children from mothers with the low status of PON1 and who had any contact with OPs during pregnancy tend to give a birth to the smallest head circumference infant and adverse effects in cognitive function during childhood. Infants and children are vulnerable to OPs toxicity. It is better to investigate the placental genes that areinvolved in lipoproteins function during pregnancy as paraoxonase-1 (PON1) gene’s activities with gestational age, especially the SGA because the

changes of lipoprotein concentrations do not play the important key role in the pathology of SGA newborns (Conley and Strully, 2012).

The purpose of the present study is to analyze the variation of the PON1 gene polymorphisms in SGA newborns. The identification and determination of the effects of PON1 (rs662 and rs705379) SPNs in SGA newborns were determined by real time-PCR one of the molecular techniques. Our study is the first study done inTurkey to report the variation of paraoxonase1 gene polymorphisms with small gestational age neonates with their birth weights.

1.4. Significance Of The Study

This study based on analysis of PON1 gene polymorphisms in SGA neonates and the variation of PON1 polymorphisms variants of rs662 and rs705379 determined by molecular techniques. The DNA isolation is a breakdown of cellular structures to form a lysate, separation of the soluble DNA from cell waste and other insoluble material and purification of the DNA of interest from soluble proteins with other nucleic acids.

Analysis of PON1 gene for SGA neonates according to their birth weight was carried out using real-time PCR. The results of this study can be a useful reference for identifying and establishing the specific variation of PON1 gene polymorphisms in SGA newborns or other groups of neonates by their birth weights. In addition, this study can give an immense contribution to the development of new strategies for diagnosis and treatment of diseases appearing in infant and childhood stages by the Children Health Care Institutions in Turkey and all over the world. It may be considered as a reference document on the analysis of PON1 gene polymorphisms in neonates born genetic disorder health problems.

2. LITERATURE REVIEW

2.1. Criteria of Small for Gestational Age

The gestational age and birth weight are two components of SGA; an infant can be preterm but an appropriate weight for that gestational age, and therefore not newborn SGA. The SGA criteria include the combination of lower birth weight for thegestationalweek. Measurement of SGA compares newborn birth weight with a national standard distribution of live births so that weights are relative to infants of the same gestational age. If a baby is not small at birth due to genetic factors, SGA is a measure of intrauterine growth restriction (Oken et al., 2003).

The prevalence of newborns born SGA in the moderate to the late of inappropriateterm group was similar in community and facilities. Overall, the 22.0% were born SGA (Asia 24.4%; Africa 17.0%; Latin America and the Caribbeans 22.7%) (Lee et al., 2013) and in the assessment study done in 16 European countries reported that over 10% of birth neonates are small for gestational age (Zeitlin et al., 2000).In countries with low and middle incomes around 43.3 million newborns means 36% of livebirthsare born either SGA or very soon (preterm). Blencoweet al., (2012) reported that the rate of the health problem of SGA in newborns in Asia is higher than which from some European countries in general.

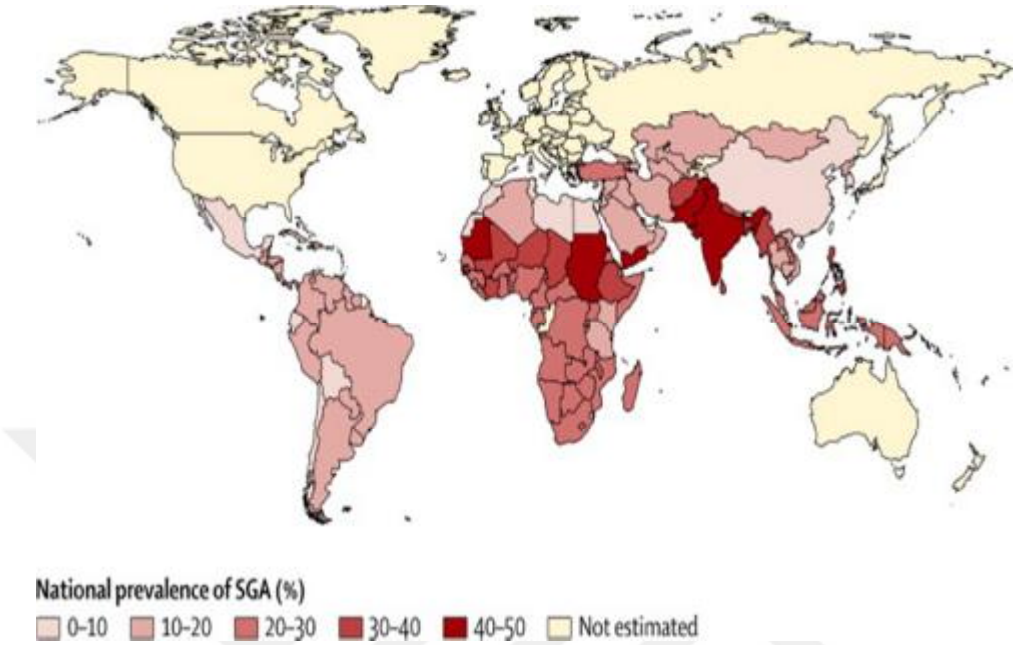


Figure 2.1. Estimation of theprevalence of SGA births in 138 the countries of low income and middleincome (Lee et al., 2013)

2.2. Influencing factors and indications of Small for Gestational Age

There are so many maternal risk factors that might be the cause of new babies born SGA, involving renal and cardiovascular disease, medical history of hypertension, cancer, diabetes, and other chronic diseases. The poor maternal weight gain in pregnancy is one of the maternal risk factors of infants born SGA as well as have been launched by the earlier studies where they found that poor maternal weight gain owing to malnutrition, genital tract infections and fetal distress are also the most cause of SGA (Abdulmoein et al., 2016).

Moreover, placental and environmentalfactors such as abnormal uteroplacental blood flow and/or inherited genetic mutations also are risk factors for babies born SGA. Newborns SGA's mortality i.e death in the 28 days of lifeas forhaving been described by previous studies is posed by the strongest predictors (Saenger and Reiter, 2012). The highest consequences of SGA problems are to increase such kind of high risk of metabolic alterations, prove inhibition of some

hormones and perinatal morbidity (Cho and Suh, 2016). Besides this, there is a risk of cerebral palsy, neurodevelopment, including intelligence in later life growth processes (Cohen et al., 2015).

Association of maternal factors with an increased risk of spontaneous being born small for gestation week, low birth weight and preterm birth have been studied by different researchers. Blencowe et al., (2012) mentioned that poor nutrition, young or advanced maternal age, chronic disease, maternal medical history,infections, potential environmental toxins such as cigarette smoking, organophosphate disposition are primarily SGA risk factors. Additionally, the indicated drugs use, small maternal stature and low prepregnancy weight cause to be born with SGA risks (Leng et al., 2016). Some paternal factors including chronic diseases like diabetes may also contribute tobeing born SGA conditions (Cho and Suh, 2016).

The important indicator of newborns' health with growth development and viability normally is birth weight. Birth weight varies depending on the reflection of both genetic, environmental stressors or determinants (some examples: famine, unemployment rates, air pollution) and the different growth profiles (Conley and Strully, 2012). In most, SGA is associated with increased morbidities and mortalities in neonates and other adverse events including neurodevelopment problems (Xiao et al., 2015). The recent studies have been established the appearance of a positive association between BW, SGA and the current height standard deviation score (SDS) (Cho and Suh, 2016). Early studies have mentioned that birth weight is one of the main signal which has a strong impact on survival of newborns during their first year of life growth, children development and good health (Moreno-Banda et al., 2009). The study made by Zerbeto with his colleagues, (2015) have shown that the lowest gestational age appears as a strong clinical and epidemiological impact and is the greatest risk factors for developmental disorders in childhood.

2.3. Paraoxonase Genes with lipoproteins levels

The main association of high-density lipoprotein (HDL) with PON1 and PON3 are primarily synthesized in the liver, while PON2 is ubiquitously expressed intracellular (Richter et al., 2008; Abelló et al., 2014). The PON2 and PON3 havealso had the capacity to hydrolyze lactones organic molecules similar to PON1, and have also been implicated in the atherosclerosis process. But, their modes of action are not extremely equivalent like that of PON1 produce in metabolisms. Furthermore, it is needed to analyzecarefully and clarify their determinantroles. The anti-atherogenic effect of PON3 could be attributed to the protection against obesity while PON2 were found to play a greater role in theprotectionof mitochondria against oxidative stress (Abelló et al., 2014). Deficiencies of PON2 impair mitochondrial oxidative stress in liver, respiratory complex activities, peritoneal macrophages.

The PON2 has a greater function to protecting against atherogenesis in vivo by modulating lipoprotein oxidation owing to the reduction of intracellular oxidative stress. The study has been concluded by Ozkan et al., (2012) demonstrated that PON2 is an endogenous defense mechanism against oxidative stress of vascular diseases. The PON2 is widely expressed in intracellular proteins with the approximate molecular mass of 44 kDa (Mackness et al., 2000). The messenger RNAs of PON2 arehighly expressed in human tissue such as liver, lung, placenta, testis, and heart just not in the cells of the artery.

The PON2 is able to reduce to the lowest level of the intracellular oxidative stress of cell and prevent oxidation of LDL to the cell-mediated. In another hand, overexpression of PON2 in cells provoke incapability of oxidizing LDL-C and showing considerably less intracellular oxidative stress when they are exposed either to hydrogen peroxide (H_2O_2) or oxidized phospholipids (Mackness et al., 2000). The PON3 is placed between PON1 and PON2, in abunch of Paraoxonase genes, it has approximately 40kDa protein. Like other PON enzymes it is also synthesized by the liver and it has an association with HDL in circulation,

but at a much lowest level compared to the level of PON1 and it has the greatest important in function of bile acid metabolisms. Although, in studies Mackness et al., (2000) have demonstrated that PON3 has very limited activities in arylesterase and no paraoxonase activity, because quickly it hydrolyzes lactone molecules such as statin's products. All PON1, 2 and 3 are very similar at the level of 70% of identical to nucleotide and 60% identical to amino acid levels (Litvinovet al., 2012).

2.4. Paraoxonase1 polymorphisms and plasma lipoprotein levels

The previous study had been shown that PON1 polymorphisms are associated with various plasma lipoproteins such as LDL and HDL-cholesterol. The detection of significant difference was observed in the total mean of cholesterol and LDL cholesterol levels between subjects with the PON1 Leu/Leu-55 and Met/Met-55 genotypes, and in subjects of PON1 Met/Met-55 where they show a better plasma lipoprotein profile (She et al., 2012). The PON1- Gln192 patient cases have been found to have lower plasma oxLDL levels than control cases (Ikeda et al., 2001). The PON1 protein is produced by the liver and accumulates in the plasma membranes and slowly dissociates into the extracellular medium. The PON1 molecule has dissociation that promoted by HDL, very-LDL (Litvinov et al., 2012). Numerous studies have shown that PON1 and HDL have a strong physiological relationship in plasma. The lipoprotein also furnishes hydrophobic environment that could be important for PON1 function. Furthermore, PON1 inhibits LDL oxidation modification, although the extent in vivo to provide this physiological benefit of HDL is less established.

Particularly the relevance of function of HDLi s to be the primary transporters of oxidized lipids in plasma and that oxidation can compromise HDL activities. The paraoxonase and arylesterase activities do not depend on the apolipoproteins content of HDL (Litvinov et al., 2012).

2.5. Role and influencing factors of Paraoxonase1 activity levels

The abilities and activities of PON1 are mostly to prevent and protect the modification of LDL oxidation or to protect the LDL and HDL from the lipid peroxidations (Moreno-Banda et al.,2009). Besides, the PON1 enzyme has the capacity to hydrolyze oxon and other substrates of organophosphate (OP) compounds, and nerve agents (Rea et al., 2004; Andersen et al., 2012). Early studies have shown that PON1 also have lactonase activity which is able to hydrolyze lipophilic lactones and degrade oxidized lipids lipoproteins in cells (Abelló et al., 2014). This enzyme also behaves like one of antioxidants enzymes that have a susceptibility to the reactive oxygen species (ROS) (Mackness et al., 2015). The PON1 activity can vary depending on pathophysiological states, PON1 activity was found to be always decreased in a number of pathological disease conditions.

Evaluation of PON1 functions and activities may be used as an indicator of the capacityof HDL antioxidant during the labor process. Some reports demonstrate how PON1 level of activity values within the association to the mode of giving birth (Vlachos et al., 2006). The decreased level of HDL-C results from hypertriglyceridemia is a major cause reduction of PON1 activity levels during pregnancy and could be one of the high-risk factors of SGA newborns (Vergeer et al., 2010). The PON1 activity levels are influenced by smoking and alcohol consumption and to be with chronic diseases as well as cardiovascular diseases. Additionally, there are interaction effect of genetic and lifestyle determinants on outcomes associated with PON1 gene of interest, and also the increased risk of SGA births isassociated with maternal smoking (Infante, 2010). Through PON1 gene polymorphisms, the lifestyle as nutritional or pharmaceutical modulators of some medicationsmay cause the biggest effect on the activity levels of PON1, which can vary by over 40 folds between individuals (Holland et al., 2015).

2.6. Substrates of Paraoxonase1 Gene

2.6.1. Chemical substrates of Paraoxonase1

Human serum PON1 is known as an A-esterase and it is the most hydrolyzer of many varieties of toxic oxon metabolites of insecticides with different efficiencies. The PON1expresses low affinity towards paraoxon, parathion, diazinon, chlorpyriphos, and similar compounds, but it exhibits much higher catalytic activity to other organophosphates, such as diazoxon and oxon molecules (She et al., 2003). In addition, chemical substrates of PON1 are named like phenylacetate, thiophenylacetate, 2-naphthylacetate, homogentisic acid lactone, c-butyrolactone, and Hcy thiolactone and other aromatic esters (Draganov and La Du, 2004).

2.6.2. Physiological substrates of Paraoxonase1

On the basis of spectrum elucidated by many lines of research, now PONs are proposed to be lactonase enzymes. Oxidized metabolites of polyunsaturated fatty acids (PUFAs) could be physiological substrates of PONs, because of the structure of many of these molecules is similar to that of lactones. Purified PONcan destroy the biologically active lipids in mildly ox-LDL (Khersonsky and Tawfik, 2005). Early, further experiments have been indicated that PON1 has thecapability of mediating the hydrolysis of lipid peroxides at the level of 19% and 90% of the cholesteryl linoleate hydroperoxides in oxidized HDL. The association of PON1 together with HDL are able to substantially hydrolyze H_2O_2; and have the ability to hydrolyzing oxidized metabolites and Hcy-thiolactone allows the PON1 to protect against oxidative stress and inflammatory diseases, including atherosclerosis (She et al., 2012). Assessed based on kinetic parameters of paraoxonases toward different substrates, it is assumed that lactones are the likely physiological substrates of PON1 (Litvinov et al., 2012).

2.7. Mechanisms of Paraoxonase1 Enzyme

The mechanism by which PON1 impedes LDL oxidation is unproven but appears to be involved in the hydrolysis of truncated oxidized fatty acids from the phospholipid, triglyceride hydroperoxides and cholesteryl ester and the result of this process is the production of lysophospholipids, cholesterol, diglyceride and oxidized fatty acids. Low concentration of serum PON1 in human is associated with various hepatic and renal diseases such as renal failure, psoriasis, and macular degeneration, in another hand it is also connected with large inflammatory component diseases including diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus. Thus, individuals with low PON1 levels and activities may be more susceptible to OP exposures and oxidativestress, which occurs when there is an excess of damaging reactive oxygen species compared to the body's antioxidant defense (Holland et al., 2015). PON1 is particularly effective in the decay of the linoleate hydroperoxides. According to the bond between PON1 and HDL, it is better to say that it is one of the strongest epidemiological diseases protectors such as coronary artery and atherosclerosis diseases. Besides, to its physical and chemical properties PON1 works like an antioxidant (Litvinov et al., 2012).

2.8. Paraoxonase1 activities and regulation

The PON1 exhibits a variety of potential atheroprotective properties as the reduction of oxidative stress of macrophage and inhibition of macrophages'ability to oxidize LDL, to decrease cholesterol synthesis and promote cholesterol efflux (Mackness and Mackness, 2015). Moreover, in vitro, the different chemicals try to act through different receptors and/or signaling systems; some examples: statins, glucose and quercetin substrates bind to the PON1promoter receptors and cause PON1 translocation. Recent studies reported that the use of long-term statin treatment is resulting in areduction of PON1 activity.

In newborns, the PON1 activity is measured at a low level and it increases from around 6 to 25 months of age until reaching the PON1 activity levelof adults

(Mackness et al., 2015). The PON1 activity is theoretically determined by the genetic background of the individual, and the PON1 activities are regulated by its polymorphisms (Cole, et al., 2003). Additionally, variation in lipoproteins, cytokines, environmental chemicals, drugs, physio-pathological states, diet, and lifestyle affect expressions ofPON1 and its activities (Mackness et al., 2015).

2.9. Lipids metabolisms

In the study done by Tomas et al., (2004), the building blocks of structures and functions of living cells made up of organic compounds contain hydrocarbons all of them are lipids. De Bie et al., (2010) has been reported that lipid profile and blood pressure are associated with metabolic syndrome in born SGA babies receiving growth hormone treatment. This means that it is important to determine the total concentration of cholesterol, HDL and LDL-C, and especially plasma triglycerides (Hentschke et al., 2013).

2.9.1. Lipoproteins and Mechanism involved in LipoproteinsMetabolisms

Lipoproteins consist of lipids and proteins known as apolipoproteins (APO). Furthermore, apolipoproteins contribute to the structure and the stability ofthe macromolecules and during the activation/inhibition of enzymes and also interaction with lipoprotein receptors apolipoproteins control the metabolism of lipoproteins. Chylomicrons are triglyceride-rich and that are catabolized by lipoprotein lipase (LPL) within a couple of minutes and produce free fatty acids (FFA), that are taken up by the liver, muscle, and adipose tissues.The HDL plays the mai nrole in reverse cholesterol transport from the peripheral cells to the liver.

The precursor particles of HDL are secreted by liver and intestine in form of disc-shaped structures and they can absorb free cholesterol from cell membranes. Plasma HDL-cholesterol levels are influenced by the complexity of reverse cholesterol transport processes (Batista et al., 2004). Furthermore, the expression of asmall number of LDL receptors may be reduced in mothers with

preeclamptic placentae that bring to the world SGA versus AGA neonates (Hentschke et al., 2013). Low levels of HDL-C are associated with an increased cholesteryl ester transfer protein (CETP) driven theexchange of cholesteryl esters andtriglycerides between pro-atherogenic apolipoprotein containing lipoproteins VLDL, LDL and HDL (Vergeer et al., 2010). Previously, the studies have been using genome scan technologies to prove and find evidence for correlation between birth weights and particular genetic markers although interactions between various polymorphisms may confound these estimates (Infante, 2010; Conley and Strully, 2012).

2.9.2. Effects of smoking on small for gestational age

Prenatal exposure to smoking, such as maternal smoking and passive smoke exposure is one of the most important risk factors that contribute to SGA births of many newborns around the world, and also it depends on how much cigarettes consumed per day. Active maternal smoking has detrimental effects on placental architecture, function, early and late fetal growth, predisposing to the range of adverse offsprings during the third trimester of pregnancy. The most important component of tobacco is nicotine, so it has a major effect onthe concentration level of placenta than in maternal blood,which causes uterine vasoconstriction by inducing maternal catecholamine release (Kabir et al., 2013).

The appearance of smokers and nonsmokers status has been shown that smokers have 14% lower HDL-C levels than nonsmokers, whereas individuals who quit smoking show succeeding increase in HDL-C levels (Titz et al., 2016).

2.10. Birth Weight and Paraoxonase1 activity

High or less level of oxidative stress is pervasive in all three trimesters of pregnancy that are classified according to the duration of gestational age. The early study has been demonstrated that serum PON1 activities are correlated with birth

weights of neonates. Moreover, PON1 activities found to be decreased in a number of diseases during pregnancy (Mogarekar and Rojekar, 2014).

3. MATERIALS AND METHODS

Materials and methods describe the research design and its overall approach, setting, the population and how it was selected, the sample and its characteristics, and the methods of data collection and data analysis.

3.1. Area and period of study

This prospective study's protocol was approved by The Human Subject Ethics Committee of Cukurova University (26/08/2016-E. 110705) and was carried out in Regional Blood Bank Center Laboratory of Cukurova University.The blood samples of SGA babies were taken from Cukurova University Balcali Hospital, Neonatal Intensive Care Unit in first three days of birth while routine blood sampling.The sampling section of the study was conducted during 12 months.

3.2. Data collection and Research design

Data (born SGA babies) were collected through and administered questionnaire by blood sampling.

3.2.1. Population and sampling techniques (Questionnaire)

In our study, the population of thestudy is comprised of the small for gestational ageneonates from themperipheral blood samples were collected.

3.2.2. Sampling techniques and data gathering procedures

We have used random sampling techniques to select the respondents of our study. By this, all mothers who have SGA babies from whom blood samples were collected, they were our interviewer subjects by questionnaire where we used a set of related questions designed to collect all needed information. A total number of 41 women were interviewed by doctors or nurses and then after we filled our data collection protocols in first 3 days of life of SGA newborns.

Particularly, the most asked questions were about cigarette smoking and some genetic diseases appear in afamily of neonates. For this condition of genetic diseases in the family was very hard to find more information about all patients admitted to Neonatal Intensive Care Unit. In our study, we used this technique of data collection because it is easy to administer and save time. Our questionnaire was mainly made up of open-ended questions. This was enabled us to check with exactitude all the answers provided by each respondent.

3.3. Blood sampling

The peripheral blood samples were taken at adifferent time after giving birth but in three days of their lives. The disodium EDTA tubes were used to collect and keep our taken blood samples. The collected samples were brought directly to the cold temperature room (6^{o}C) at Balcali Hospital in Laboratory of Blood Bank Centre. The blood samples were conditioned and waited for DNA isolation and other DNA analysis.

3.3.1. DNA isolation from collected blood samples

The DNA from blood samples were collected and isolated using Roche, High Pure Polymerase Chain Reaction (PCR) Preparation Kit(Roche, Cat. No.11 796 828 001). The followings are adescription of procedures.

3.3.2. Materials

Isopropanol, Absolute Ethanol, Proteinase K, Inhibitor Removal Buffer, Wash Buffer, Elution Buffer and Distilled water.

3.3.3. Standard preparation

- The dilution of Proteinase K into 4.5 mL of distilled water.
- Add 20 mL of absolute ethanol to the inhibitor removal buffer solution.

- The 80 mL of absolute ethanol was added to wash buffer solution.

3.3.4. Sample preparation

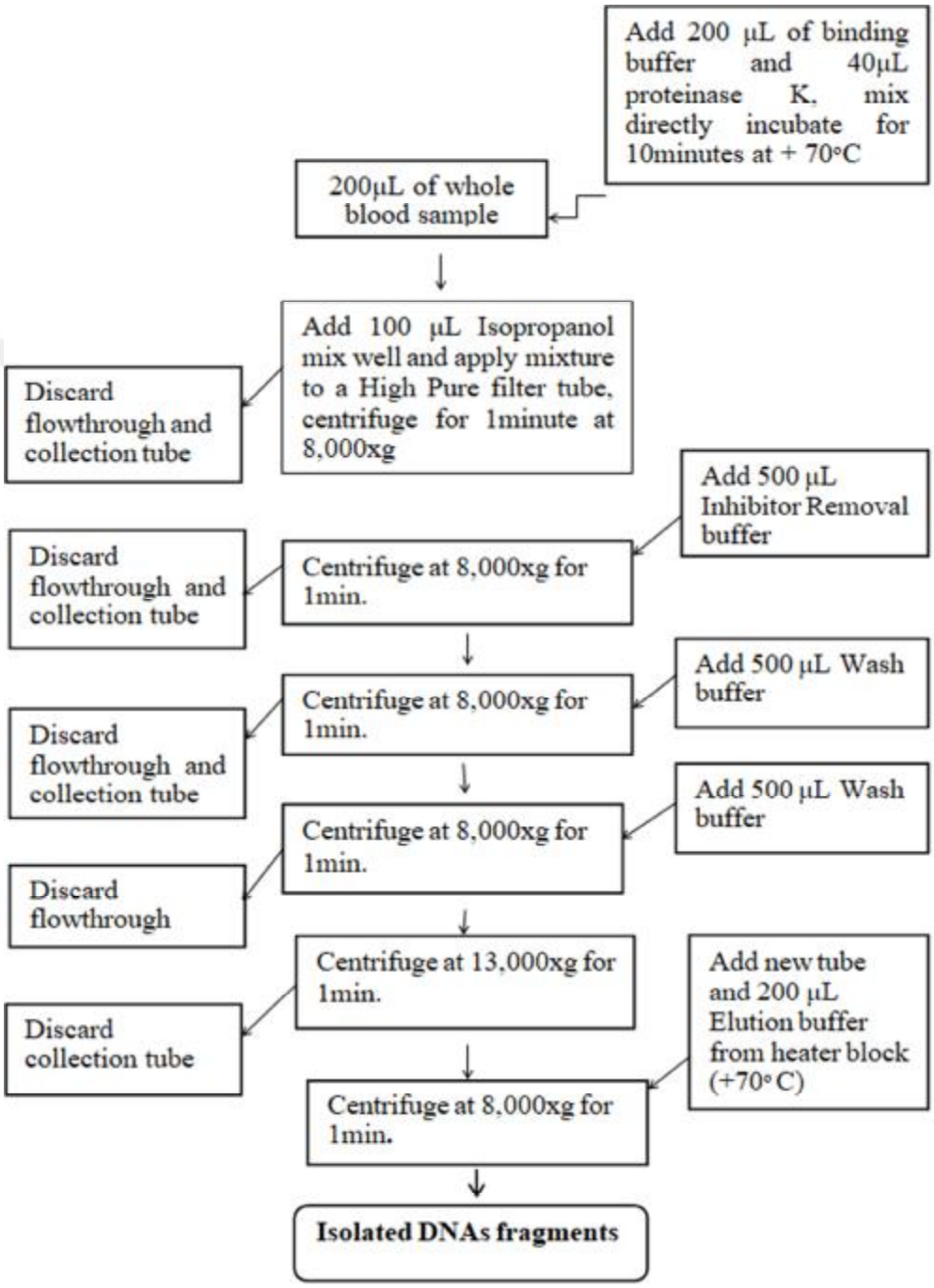
The 2cc of peripheral blood samples were collected in EDTA tubes and were kept in cold room at 6^{o}C. Sera and full blood were separated according totime and number of obtained samples. We took 200µL of whole blood for each sample from 2cc that was used for DNA isolation.

3.3.5. Procedure

1. The 200 µLof whole blood sample were taken into the eppendorf tube, 200 µL of binding buffer and 40 µL of proteinase K were added to the sample. After mixing of sample and solutions the mixture was incubated at +70^{o}C for 10 minutes. At this time, the preparation of Elution buffer according to the all number of samples X 200 µL and incubate them at + 70^{o}C also.
2. After incubating mixtures we added 100 µL of isopropanol and mixed the solution. After, the all amount of mixture has been pipedto the filter tube and then were labeled.
3. We centrifuged the mixture of ablood sample, binding buffer, proteinase K, isopropanol into filter tube for 1minute at 8000g. And the bottom tubes were thrown and they replaced by cleaned tubes.
4. Addition of 500 µL of inhibitor removal buffer on the filter tubes. Centrifuged at 8000 g for 1 minute. Then we threw away the lower tubes and replaced them with cleaned ones.
5. Again 500 µL of wash buffer solution has been added to the filter tubes with mixtures. And then centrifuged at 8000g for 1 minute. The thrown lower tubes were also replaced by cleaned tubes for next steps.

6. We repeated the5th step, then poured the liquor from the bottom of the tubes and placed them under the filter tube again and centrifuged again at 13000g maximum speed for 1 minute.
7. The mixture containers were taken to the normal 1.5mL eppendorf tubes and we labeled them by writing the number of collected samples on their covers. After that we put the filtered tube in those eppendorf tubes. We added 200 µL heated elution buffer (at + 70^{o}C) and centrifuged them for 1 minute at 8000g of speed.
8. The isolated DNAs were stored at -25° C for waiting along time for final laboratory analysis of PON1 gene mutations.

3.4. Experimental design



3.5. Data classification

The data collected in our study were treated on the computer by the excel software, and hard copies. The interpretation of the results of the survey was primarily centered on the analysis of the distribution of the percentages of answers in the tables and forms of the figures.

The DNAs analysis were analyzed based onbirth weights of SGA neonates, and availability of mothers' smoking habit during their pregnancy period. Participants were stratified into four groups according to thebirth weights of SGA newborns: Group I contains (580-900)gr; Group II (1070-1430)gr; Goup III (1640-1960)gr and Group IV (2000-2340)gr. Group I has 9.756% of SGA newborns, Group II contains 26.829%SGA newborns, Group III has 29.268% SGA newborns and Group IV with 34.146% ofSGA newborns. The total sample size of our study was 41 SGA newborns.

3.6. DNA Genotyping and Analysis

For the amplification of the PON1-rs662 (Q192R) polymorphic fragments the following set of primers were used: 5'-TTTTCTTGACCCCTACTTACA-3'-ASO A (Forward), 5'-TTTTCTTGACCCCTACTTACG-3'-ASO G (Reverse) and 5'-GCATCTAGAACAGCGCCTGGCACATAGTAGGTAGGTACT-3' common with the enzyme kit of NEB Taq Polymerase with Standard Taq Buffer (M0273). The total master mix was 18uL included by:2uLof 10x Std Taq Buffer, 0.4uL of 10Mm dNTPs, 0.4uL of 10Mm rs662 forward primer, 0.4uL of 10Mm rs662 reverse primer 0.1uLof Taq DNA Polymerase and 14.7uL of DEPC water. After the master mixture the 2uL of isolated DNA was added. The reaction was carried out in heat conditions of 95°C for 30sec, and 35cyle in 95°C, 58°C, 68°C for every 30sec and then after 68°C for 5min. The PON1-rs 662 sequencing system was ABI 3130 with sequencing kit of BigDye™ Terminator v3.1 Cycle Sequencing Kit. The cycle sequencing PCR mix was 0.5µL of Big Dye, 2µL of 5X Sequencing buffer, 10µL of DEPC treated water unto, 0.5 µL of primer and 7ng of DNA (rs662).

The amplification was carried out in PCR thermal cycler conditions of initial denaturation (96°C for 1min), the first 25 cycles (96°C for 10sec,50°C for 25sec) and finally 60°C for 4min and then 4°C for ∞.

For the PON1 variant of C-108T SNP number rs705379, primer was 5'-CCGATTGGCCCGCCCCG-3'-ASOG (Forward), 5'CCGATTGGCCCGCCCCA-3'-ASOA (Reverse), the master mix protocol was 2 uLof 10x Std Taq buffer, 0.4 uL of 10Mm dNTPs, 0.4uL of 10Mm rs705379 forward primer, 0.4uL of 10Mm rs705379 reverse primer, 0.1uL of Taq DNA Polymerase and 11.7uL of DEPC water, the total of mixture was 15uL; the 5uL DNA was added to the mixture. The heat conditions were initial 95°C for 30sec, first 35 cycles (95°C for 20sec, 62°C for 30sec, 68°C for 40sec) and then 68°C for 5min. For the sequencing system and enzyme kit was the same as what we used in rs662 SNP number, differ only for the measurements of DNA quantity here we took 12ng of DNA (rs705379). PCR thermal cycle conditions were the same for both of two SPNs in this study. The PCR lengths of SNP regions of PON1 gene, was 608 bp for PON1-rs705379 and 352bp for PON1-rs662.

3.6. PON1 primes and its variantsinformation used in this study

Table 3.1. Primes and Reactive with PCR conditions

Prime	Sequence (5'---3')	⇄	Tm/◦c
rs662 Fwd	CTAGCACGAAGGCTCCATC	→Fwd	56
rs662 Rev	TCCACTACATTTCAGAGAGTT	←Rev	52
rs662common	ATTGCCTTGATTTACATTTTGGTACA	←Rev	56
rs705379 Fwd	GGGTTCCTACTACAGCCCTC	→Fwd	57
rs705379 Rev	GTTAACAGCCTGGACCCAAC	←Rev	56

4. RESULTS

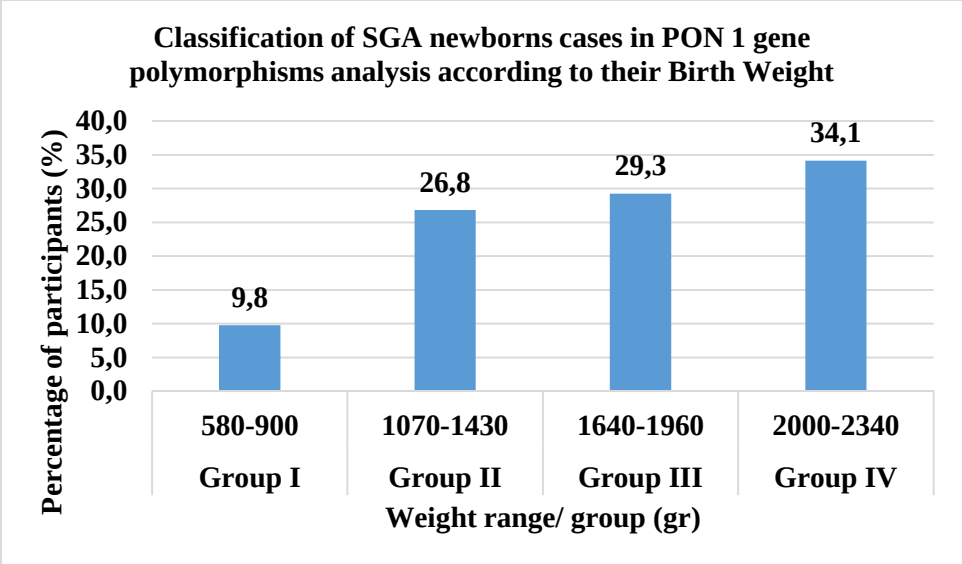


Figure 4.1. Classification of SGA newborns cases in PON1 gene polymorphisms analysis according to their birth weights

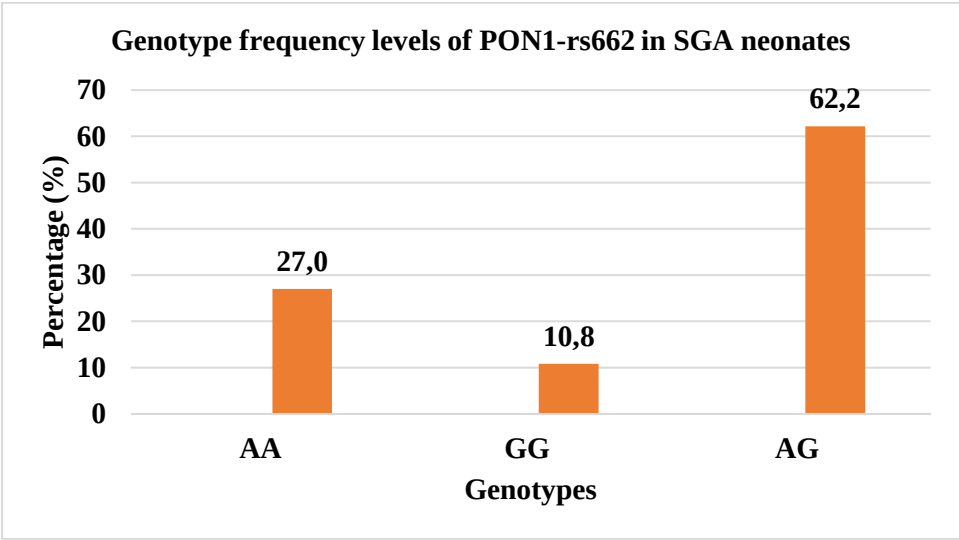


Figure 4.2. Genotype frequency levels of PON1-rs662 in SGA neonates

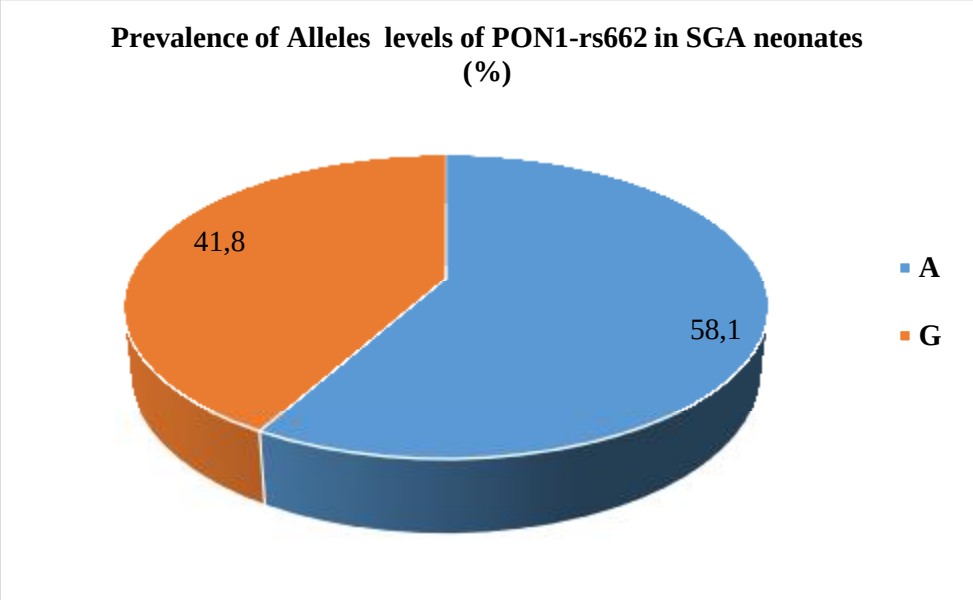


Figure 4.3. Prevalence of alleles levels of PON1-rs662 in SGA neonates (%)

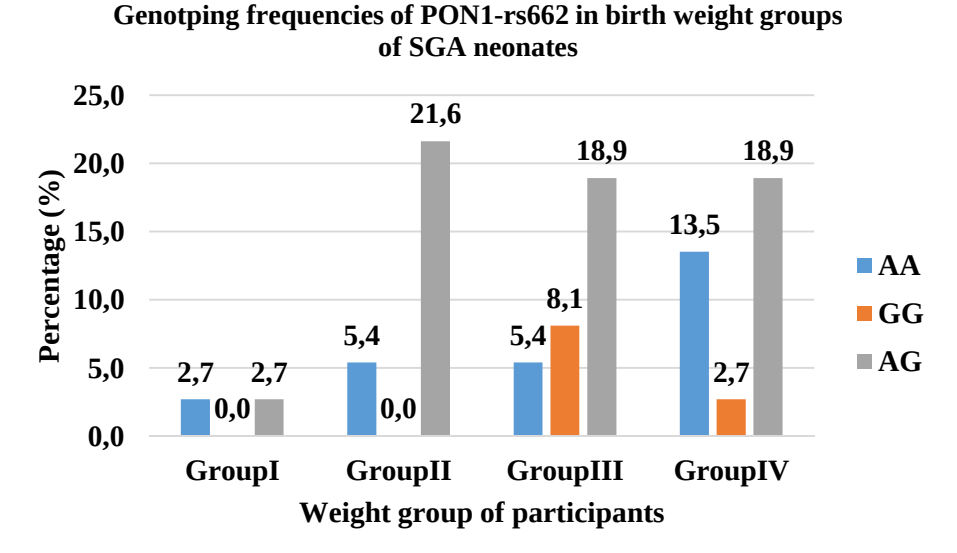


Figure 4.4. Genotyping frequencies of PON1-rs662 in birth weight groups of SGA neonates

Abbreviation: (AA: Normal genotype, GG: Carrier genotype, AG: Heterozygous)

Our results, Figure3 shows the characteristics of SGA newborns in term of birth weights groups. We grouped SGA newborns according to their low birth weight by the study design. Some of their mothers' lifestyle like cigarette smoking and alcohol consumption during pregnancy was mentioned in the study analysis evaluations. Figure4 demonstrates the frequency genotypes of PON1-rs662 polymorphism in SGA neonates in all their birth weight's group classification. In group I, heterozygous 2.7% was identified and homozygous found in this group was 2.7%. The heterozygous were 21.6% and also there was 5.4% of the homozygous present in group II. Group III has heterozygousand homozygous: heterozygous was 18.9% whereas homozygous is represented by 5.4%. Our results show that in group IV, heterozygous genotype was 18.9% and homozygous was found in 13.5%.

The results demonstrate that heterozygous low birth weighed newborns of group II have a high level of SGA risk and concerning to their gestation week these babies are in the range of ≤ 22 to ≥ 37 weeks. The total number of heterozygous found in thisstudy is 62.2% and homozygous was found at 27.0%. Group III and IV of SGA cases were obtained to be on the same level to show the chance to have the high level of heterozygous where their total percentages are 37.8%. Group I contains the lowest birth weighted SGA babies the heterozygous level is 2.7%.

In addition, in our results we found the 27.0% of homozygous that relates to the range of ≤ 34 and ≥38 gestation weeks of pregnancy but in another handthe birth weights were varying between 1700 to 2300gr. On another side, according to the gestational age of newborns we had in our study, the results showed that the 62.2% of heterozygous genotypes varying in therange of ≤31 weeks and ≥ 42 weeks but birth weights are appearing in different groups as it is mentioned in Figure3. Another side of our results we have the SPN rs705379 of PON1 gene polymorphism.

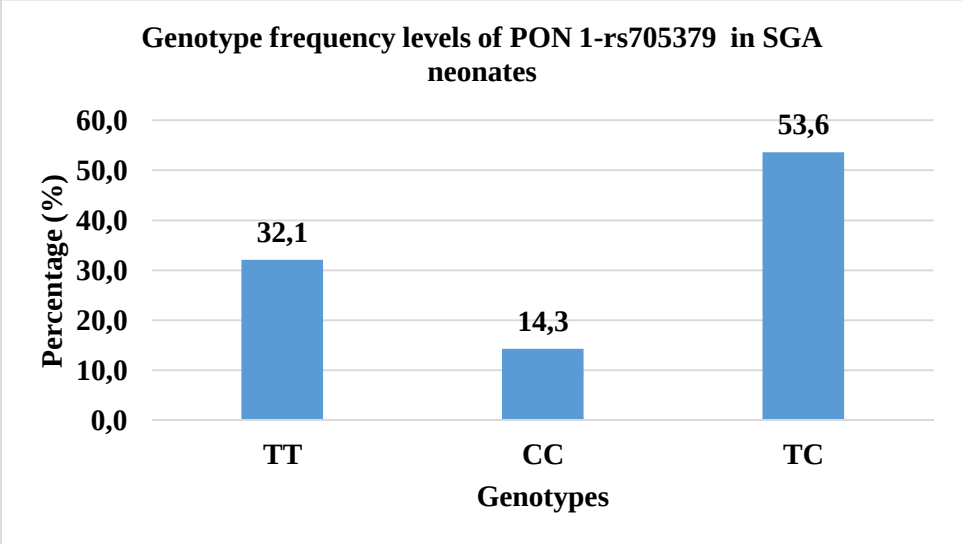


Figure 4.5. Genotype frequency levels of PON1-rs705379 in SGA neonates

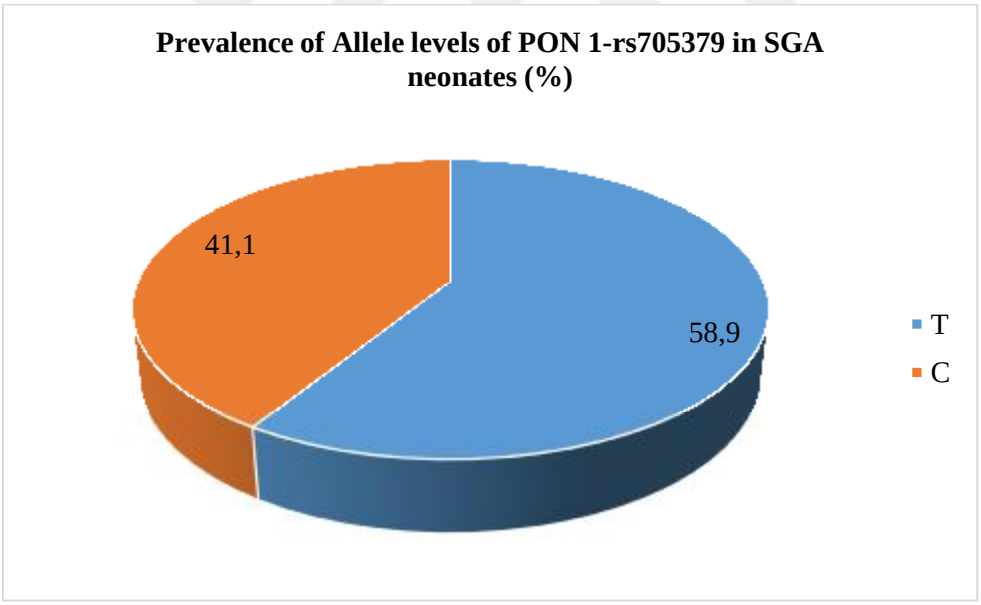


Figure 4.6. Prevalence of Allele levels of PON1-rs705379 in SGA neonates

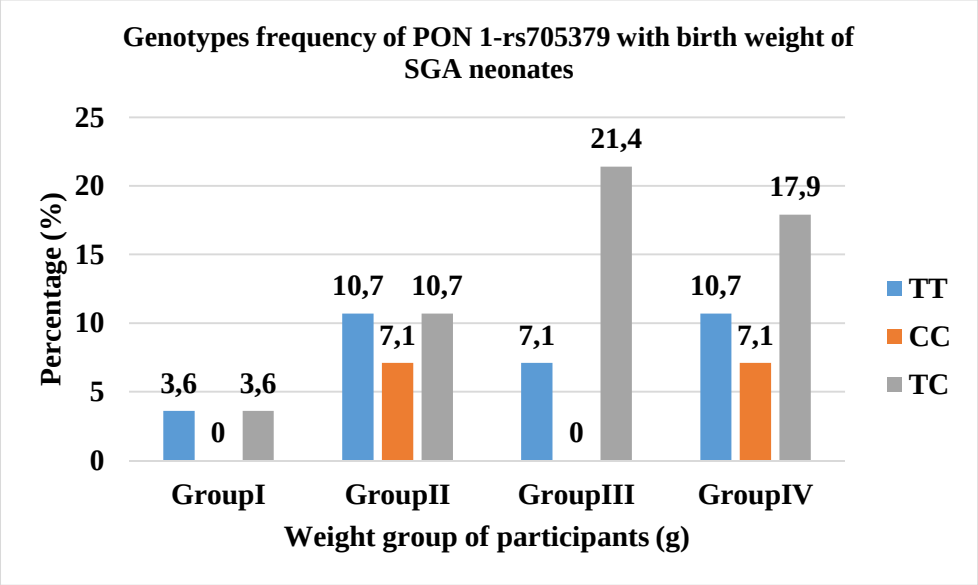


Figure 4.7. Genotypes frequency of PON 1-rs705379 with abirth weight of SGA neonates

Abbreviation: (TT: Normal genotype, CC: Carrier genotype,TC: Heterozygous genotype)

In other hand of analysis of PON1 polymorphisms in SGA neonates, in Table 6 of our results shows that PON1-rs705379 homozygous and heterozygous was on same percentages of 3.6% in SGA neonates group I. In group II the presence of homozygous and heterozygous is on the same level of 10.7% while group III has 7.1% of homozygous and 21.4% of heterozygous. Homozygous in group IV is presented by 10.7% whereas heterozygous is 17.9% (Figure 9).Our study was done on 41 SGA neonates, and with many different circumstances of the quality and quantity of isolated DNA fragments were very low and also because low quality of blood samples from SGA neonates,the analysis of PON1-rs662 with Real Time-PCR resulted in 37 on the rate of 90.2% SGA neonates blood samples and in PON1-rs705379 results was found in 28 related to the rate of 68.3% SGA neonates samples.

5. DISCUSSION AND CONCLUSION

5.1. Discussion

In our study, the frequencies of genotypes of PON1 rs662 (Q192R)) and PON1 rs705379 (C-108T) variants in Turkish SGA newborns were evaluated. According to the best of our knowledge there is no previous study has assessed the correlation of PON1 gene polymorphisms with small for gestational age neonates using molecular techniques. The small for gestation age is recognized as the most important high-risk factor for mortality and morbidity of newborns all over the world (Lubchenco et al., 1963). It is hard to clarify the incidence ofSGA neonates birthsin every single country; it hasreported that birth anthropometric data and determination of real gestational week are not recorded for some places'databases or are based to use national SD (Cho and Suh, 2016).

The assessment study done across European countries reported that the prevalences of SGA vary dependingon country-specific or common European references; Italy, Portugal and France contain 39.9% of lower term birth weights according to common references whereas, Sweden, Denmark and the Netherlands have 28.9% of higher term birth weights. Besides, there was no observation with specific country references (Zeitlin et al.,2017). Genetic changes of PON1 gene polymorphisms are considered as the risk factor of having a large number of SGA newborns.

The PON1 gene polymorphisms can change depending on numerous genetic and environmental factors occur during pregnancy period for any woman. The PON1 is highly associated with HDL, which can control the metabolisms of many molecules in human especially for oxidative modification ofLDL (Infante, 2010). The PON1-rs662 polymorphismpromulgates differential catalytic activity toward some OP substrates, while PON1-rs705379 the polymorphism is the major contributor to differences in the levels of PON1 expression.

Both contribute to determining the status of PON1 levelin an individual (Costaet al., 2013). In our study the PON1 occurrence in small for gestational age neonates was the PON1-rs662's incidence of 27.0% of homozygous and 62.2% of heterozygous whereas PON1-rs705379 was 32.1% homozygous and 53.6% of heterozygous. Many studies have been discussed that PON1 activityis correlated with birth weights of neonates (Mogarekar and Rojekar, 2014). This can also support that prevalence of PON1 activity can be reduced by cigarette smoking that can affect maternal offsprings birth weight and gestational age which result in delivering SGA newborns. As well as environments factors, our study based on cigarette smoking variation for daily smoking and nondaily smoking mothers of neonates admitted to Neonatal Intensive Care Unit during blood samples collection. The cigarette smoking during pregnancy is the most important modifiable risk factor associated with adverse pregnancy outcomes.Of the 41 women attended in our study, 17.1% of the mothers were daily smokers, while the 82.9% of the women were non-daily smokers. For instance, mothers who smoked during pregnancy have an increased risk of having too lower birth weighed neonates compared to non-smoking mothers (Kabir et al., 2013).

Our results demonstrated that 17.1% of neonates whose mothers were smokers during pregnancy their birth weights were low compared to those born to non-smokers mothers. The study done on British population has shown that cigarette smoking during pregnancy increased the neonatal mortality rate by 28% and reduced birth weight by 170gr (Butler et al.,1972). Cigarette smoking has a serious effect on appetite in general this case can limit the weight gain during pregnancy even before pregnancy. According to the results ofour study the small for gestational age (both gestational age and birth weight) of offsprings of mothers who smoked during all pregnancy trimesters were seemed to be lower than those of the non-smokingmothers.

Although there is a convincing evidence for the association between SGA and lifestyle status, it is not known to what extent explanatory factors contribute to this association.

5.2. Conclusion

Maternal smoking is responsible for increased incidences of small for gestational age newborns therefore, smoking cessationshould be advised to pregnant women to reduce morbidities in their neonates. Further studies are needed to clarify the variation and correlation of small for gestational age neonates with passive smoking and exposure to environmental cigarette smoke and to parental smokers in the family.

Many studies using a large sample size are required to elaborate the correlation of Paraoxonase1 gene polymorphisms with small for gestational age neonates. And find out many genetic and environmental factors that influence the SGA neonates.

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BIOGRAPHY

Am Rwandese, was born on March 3, 1989, in theMutete sector, Gicumbi district. I am second born raised ina family of two siblings and my mother. I attended Secondary School at Groupe Scolaire Notre Dame du Bon Conseil Byumba graduating from Biochimie option in 2008, and went on to earn an undergraduate at University of Rwanda- College of Science and Technology former Kigali Institute of Science and Technology (KIST) in Faculty of Science, Applied Chemistry Department, Biochemistry Option.I successfully got my Bachelor's degree in September, 2013. Following my graduation from KIST, I won Turkish Scholarships in September 2014. Now, I am completing my Master's Degree in Biotechnology Department at Cukurova University.

APPENDICES

Appendix I: Tables

Table 1:Classification of Participants in Paraoxonase 1 (PON1) Gene analysisaccording to birth weight

Group Number	**Weight range (gr)**	**Number**	**%**
Group I	580-900	4	9.756
Group II	1070-1430	11	26.829
Group III	1640-1960	12	29.268
Group IV	2000-2340	14	34.146

Table 2:Genotype frequency of PON1-rs 662 in SGA neonates classification groups

PON1 (rs662) Group	**AA (%)**	**GG (%)**	**AG (%)**
Group I	2.7	0	2.7
Group II	5.4	0	21.6
Group III	5.4	8.1	18.9
Group IV	13.5	2.7	18.9

Table 3:Genotyping and allelic frequencies of PON1-rs662 polymorphism in SGA newborns

PON1-rs662 (Q192R) A>G	**Cases**	**%**
Genotypes		
A/A	10	27.02
A/G	23	62.16
G/G	4	10.81
Alleles		
A	43	58.10
G	31	41.89

Table4: Genotypefrequency of PON1-rs705379 in SGA neonates classification groups

PON1-rs705379 / Groups	TT (%)	CC (%)	TC (%)
Group I	3.6	0	3.6
Group II	10.7	7.1	10.7
Group III	7.1	0	21.4
Group IV	10.7	7.1	17.9

Table5: Genotyping and allelic frequencies of PON1-rs705379 polymorphism in SGA neonates

PON1-rs705379 (C-108T) T>C	Cases	%
Genotypes		
T/T	9	32.1
T/C	15	53.6
C/C	4	14.3
Alleles		
T	33	58.9
C	23	41.1