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Chapter 5

Calcitonin-Related Polypeptide Alpha Gene Polymorphisms and Related Diseases

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Additional information is available at the end of the chapter

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Abstract

Calcitonin gene-related peptide (CGRP) is a neuropeptide containing 37 amino acids. CGRP is a potent vasodilator neuropeptide, which has protective mechanisms in physiological and pathological conditions. When released, CGRP is a peptide that is active in the cerebral circulation and interacts with the sympathetic nervous system. CGRP is very important in the treatment of cardiovascular diseases. In addition, CGRP, which is also associated with pain processes, has an important role in inflammation. Calcitonin-associated polypeptide alpha (CALCA), one of the isoforms of CGRP, functions through the wide CGRP receptors. Polymorphisms occurring in the CALCA gene are associated with diseases such as ischemic stroke, Parkinson's disease, ovarian cancer, bone mineral density, migraine, schizophrenia, manic depression, and essential hypertension. In this section, the information was given about CALCA gene, which is one of its isoforms of CGRP. In addition, CALCA gene polymorphisms and diseases associated with these gene polymorphisms have also been addressed.

Keywords: CGRP, CALCA, gene polymorphism, PCR, RFLP, diseases

1. Introduction

The CGRP family consists of calcitonin, adrenomedullin, adrenomedullin 2 (intermedin) and amylin. CGRP is a potent vasodilator neuropeptide and it acts through its receptors. CGRP has wide perivascular localization. It is known that sensory fibers to exhibit a wide innervation throughout the body, and CGRP is localized in these sensory fibers. CGRP is also localized in non-neuronal tissues apart from these sensory fibers. CGRP has important protective properties in physiological and pathological conditions. It plays an important role in the treatment of cardiovascular diseases since it has a role as a vascular protective factor. In addition, the

sensory fibers contained in CGRP are associated with pain processes. For this reason, CGRP also plays an important role in migraine pathophysiology. Another condition that is effective of CGRP is inflammation. CGRP has CALCA and calcitonin-related polypeptide beta (CALCB) isoforms [1].

The human CALCA gene is localized on the chromosome 11 (11p15.2-p15.1). This gene codes the calcitonin and CGRP. CALCA gene contains 1 promoter and 6 exons. It is known that the polymorphisms that occur in this gene are related to various diseases. Several polymorphisms have been identified in the CALCA gene. It has been determined that these polymorphisms are related to cerebrovascular, neurodegenerative, psychiatric diseases and hypertension-connected conditions. The most common of the CALCA gene polymorphisms is the CALCA T692C gene polymorphism. Besides this polymorphism, there are also various polymorphisms of CALCA gene such as CALCA-1786T>C, CALCA-624 (T/C), and CALCA (I/D) [2, 3].

Several studies have been conducted to determine whether CALCA gene polymorphisms are genetic risk factors for various diseases. In some of these studies, a significant relationship was found between CALCA gene polymorphisms and disease development risks. However, studies were also found in which this relation is not determined [2].

As a result, in the studies carried out with different populations, different results were found. Findings acquired from these studies that carried out with different ethnic groups will be an important indicator that new treatments for these diseases can be developed.

2. Calcitonin gene-related peptide

CGRP is a neuropeptide produced in consequence of alternative RNA processing of the calcitonin gene and containing 37 amino acids. CGRP gene family is composed of calcitonin, adrenomedullin, adrenomedullin 2 (intermedin) and amylin. CGRP has two important isoforms as CALCA and CALCB (calcitonin-related polypeptide beta). These isoforms of CGRP have similar structures and biological activities. However, separate genes form them. CGRP is also composed of receptor activity modifying protein (RAMP) and calcitonin receptor-like receptor (CLR). RAMP is a protein that changes receptor activity. The CLR receptor is also another receptor bound to the RAMP receptor. CGRP is an extremely powerful vasodilator that has protective mechanisms important for physiological and pathological conditions. Firstly, CGRP released from sensory nerves includes pain pathways. It is a known fact that the sensory fibers contained in CGRP are also related to the pain processes. There are studies showing that CGRP antagonists play an important role in migraine and have the potential to treat migraine. The studies are found that demonstrate that CGRP antagonists alleviate migraine. Apart from this disease, it is also known to have effects on arthritis, skin disorders, diabetes and obesity. Therefore, CGRP is a very important peptide in mammalian biology. CGRP is localized in the sensory fibers, which exhibit an innervation throughout the body, mainly with extensive perivascular localization. These sensory fibers are known to have a dual role in sensory (nociceptive) and efferent (effector) function. The role of CGRP is unclear, but it is also localized in the lesser known neuronal tissues. Sympathetic leakage mediation of CGRP in the brain has been shown. However, when exogenous CGRP was

administered to femtomolar doses to skin of human and animal species, CGRP appeared to have a vasodilatory effect. Vascular protective role of CGRP has been identified via studies in various animal models. Therefore, it has been suggested that CGRP may be an important peptide in the treatment of cardiovascular diseases. CGRP is a very important neuropeptide with various aspects. Firstly, when CGRP is released, it is found as active in the cerebral circulation. In addition to be a powerful vasodilator, it is known that there is a reciprocal interaction with the sympathetic system in the environment. In addition to these, very important role of CGRP is found in inflammation [1].

2.1. Structure of CGRP

The structure of CALCA resembles CALCB the other isoform of CGRP. CALCA isoform consists of four domains. The first domain consists of the first seven residues of the NH_2 terminus, and it forms a ring-like structure that is held together with a disulfide bridge. CGRP 8-37 is a CGRP antagonist that occurred from removal of this first domain. Domain 2, composing an alpha helix, occurs from 8 to 18 residues, and these residues constitute deletions that cause 50- to 100-fold decrease in affinity. Residues of 11 and 18 are found in the hydrophilic face of the alpha helix. These residues also play an important role in supporting high-affinity binding. Domain 3 is occurs from 19 to 27 residues, and it is formed from the beta or gamma twist. The fourth domain comprises COOH terminus, and it consists of residues inherit from 28 to 37. It is believed that Domain 4 is required to form a binding epitope, and this domain has two domain rotations. When species differences and structure-activity relationships for CGRP are investigated, various amino acids have been identified. In receptor binding and activation, quite important functions of these amino acids have been found [1] (**Figure 1** and **Table 1**).

2.2. Molecular genetics of CGRP

CALCA gene is localized on the chromosome 11 (11p15.2-p15.1), and it contains six exons. Exon I is an untranslated region. While the exon II encodes signal peptide, exon III encodes N-terminal propeptide. Calcitonin and CGRP are localized on exon IV and V. The untranslated exon VI is the part of the CALCA. All of six exons constitute the primary mRNA transcript and then calcitonin or CGRP mRNA is formed. In consequence of combining the first three exons with exons V and VI, mRNA containing the CGRP is produced. Exon V codes the CGRP. Exon VI encodes the 3′ untranslated region of the CGRP mRNA besides the polyadenylation (polyA) signal. mRNA is translated to produce the pro-CGRP peptide which is cleaved in the conjugated dibasic amino acids and the CGRP is released as 37th amino acid. The structure of the CALCB gene on chromosome 11 is like that of the CALCA gene. However, the exon 4 has lack polyA and thus alternative binding is prevented. In the consequence of this gene transcription, only CGRP is produced [4] (**Figure 2**).

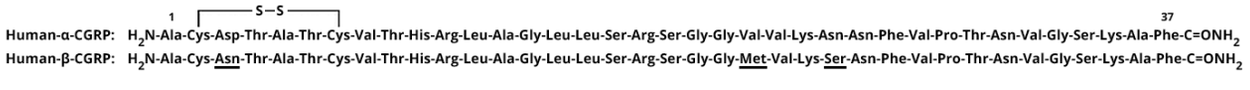
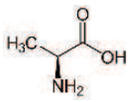
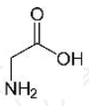
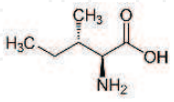
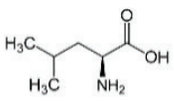
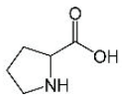
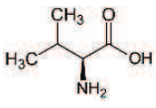
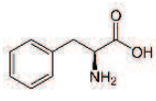
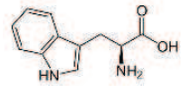
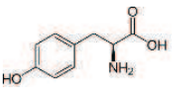
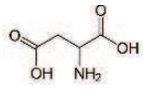
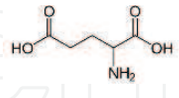
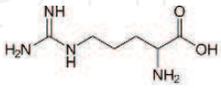
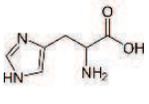
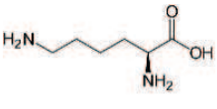
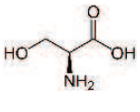


Figure 1. Amino acid residues of human CALCA and CALCB isoforms.

Amino acids	Three-letter abbreviation	One-letter abbreviation	DNA codons	Chemical structure
Alanine	Ala	A	GCT, GCC, GCA, GCG	
Glycine	Gly	G	GGT, GGC, GGA, GGG	
Isoleucine	Ile	I	ATT, ATC, ATA	
Leucine	Leu	L	CTT, CTC, CTA, CTG, TTA, TTG	
Proline	Pro	P	CCT, CCC, CCA, CCG	
Valine	Val	V	GTT, GTC, GTA, GTG	
Phenylalanine	Phe	F	TTT, TTC	
Tryptophan	Trp	W	TGG	
Tyrosine	Tyr	Y	TAT, TAC	
Aspartic acid	Asp	D	GAT, GAC	
Glutamic acid	Glu	E	GAA, GAG	
Arginine	Arg	R	CGT, CGC, CGA, CGG, AGA, AGG	
Histidine	His	H	CAT, CAC	
Lysine	Lys	K	AAA, AAG	
Serine	Ser	S	TCT, TCC, TCA, TCG, AGT, AGC	

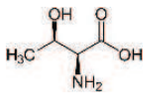
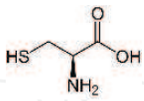
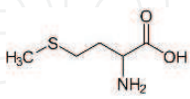
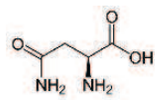
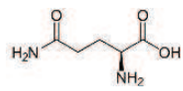
Amino acids	Three-letter abbreviation	One-letter abbreviation	DNA codons	Chemical structure
Threonine	Thr	T	ACT, ACC, ACA, ACG	
Cysteine	Cys	C	TGT, TGC	
Methionine	Met	M	ATG	
Asparagine	Asn	N	AAT, AAC	
Glutamine	Gln	Q	CAA, CAG	

Table 1. The chemical structure and DNA codons of amino acid residues of human CALCA and CALCB isoforms.

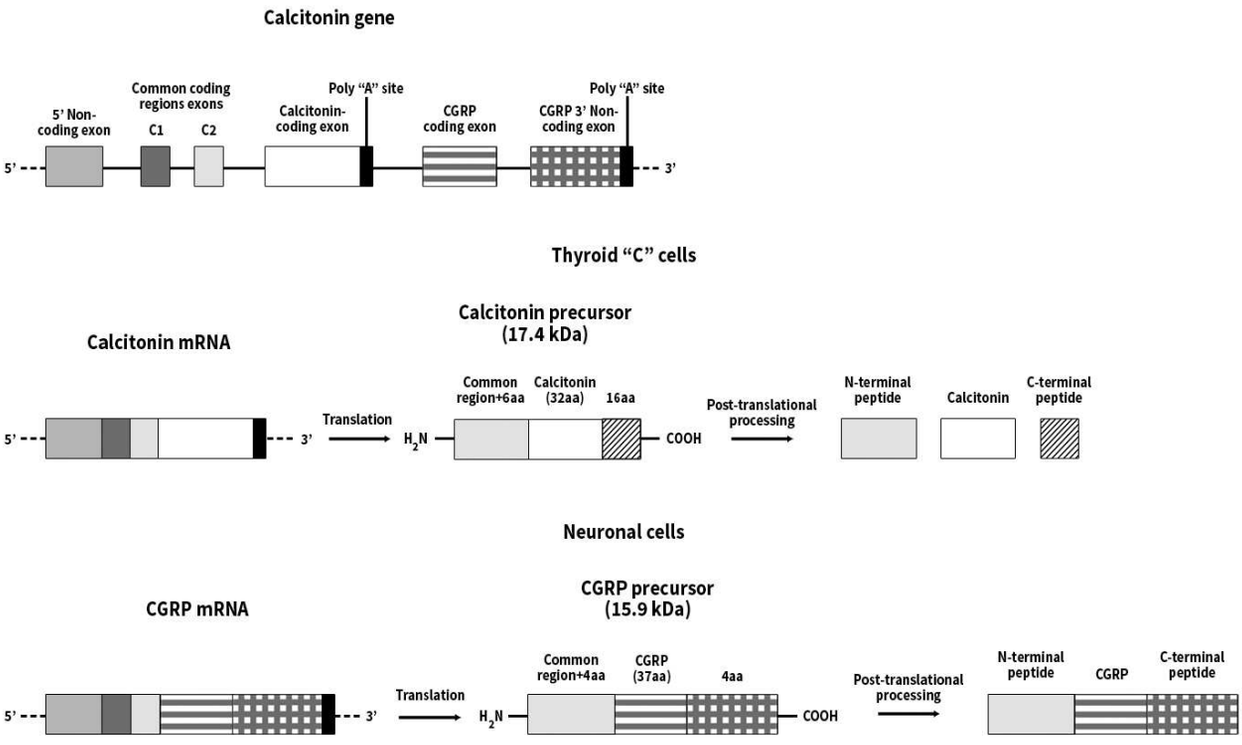


Figure 2. Calcitonin or CGRP production from CALC I gene.

2.3. Isoforms of CGRP

The CALCA and CALCB isoforms of CGRP are called as also CGRP I and CGRP II. These isoforms are synthesized from two different genes on chromosome 11 in different regions. The

CALCB is copied from the CALC II gene, the CALC I gene is to alternative binding to produce calcitonin or CGRP. The CALCA and CALCB sharing are analogous and 90% homologous, but they different from in terms of only three amino acids in humans. Therefore, the biological activities of these isoforms are similar. CALCA is the basic form that found in the central and peripheral nervous system. CALCB is the isoform that found in the enteric nervous system. Calcitonin is produced from the CALC I gene in consequence of expression in the mature protein of exon IV in the gene. Exon V and exon VI are converted to the 121st amino acid pro-hormone in consequence of the expression. CALCA is then cleaved to produce mature 37 amino acid peptides and mRNA is produced. The mechanism determining alternative binding for CALCA, which is predominantly expressed along the central, and peripheral nervous system, is still not fully understood [1].

2.4. Physiological functions of CGRP

2.4.1. CGRP in the cardiovascular system

The distribution of CGRP and its receptors in cardiac tissues such as the sinoatrial node, coronary arteries, atrial and ventricular muscle systems causes an increase in functions such as heart rate, contraction force, coronary heart flow and microvascular permeability. CGRP plays an important role in the regulation of vascular tone and angiogenesis. In consequence of CGRP infusion, perfusion pressure drops in isolated hearts, and vasodilator effect is observed in coronary vasculature. Also, CGRP shows a cardioprotective effect. Thus, capacitance blood vessels are directly affected and environmental vasodilation develops. CGRP receptors, which are found predominantly in the renal blood vessels, have various functions. These functions include increasing renal blood flow, increasing glomerular filtration rate, relieving glomerular afferent arterioles, increasing renin production, and stimulating arterial natriuretic peptide release [4].

2.4.2. CGRP in the central nervous system

CGRP plays a very important role in various functions such as motor, sensory and integrative systems in the central nervous system. Except this, CGRP is a peptide that modulates various senses. CGRP spreads in the central associated with autonomic functions. CGRP also plays an important role in regulating functions such as cardiovascular, respiratory and sleep functions. Apart from these functions, CGRP has a regulatory role. There is a regulatory effect of CGRP in the growth hormone release, hyperthermia, catalepsy, motor activity, and nociceptive responses. In addition, CGRP enhances excitatory actions by increasing the release of excitatory amino acid. CGRP that found in the efferent nerve fibers is found together with neurons containing acetylcholine. Thus, CGRP modulates the acetylcholine release. It also increases the synthesis of acetylcholine receptors and functions as a neurotrophic factor [4].

2.4.3. Other functions of CGRP

Other functions of CGRP are regulation of pituitary hormone secretion, release of pancreatic enzymes, control of gastric acid secretion, thermoregulation, reduction in food intake, insulin

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action, antagonism of insulin, growth factor-like functions. The CGRP effect is induced in the bones through the calcitonin receptors. In consequence of this induction, hypocalcemia, proliferation of osteoclasts, inhibition of both basal and stimulated absorption of the bone occurs. It is also known that CGRP is also distributed in bone tissues. In cases such as pregnancy, menstruation, or oral contraception, plasma CGRP levels increase. Spontaneous contractions occurring in uterus and fallopian tubes are also inhibited by CGRP effect. CGRP, which increases microvascular permeability, is also effective in the formation of inflammatory hyperemia, neutrophil accumulation, and localized edema. CGRP, which has the function of enhancing the migration of endothelial cells, plays an important role in the situations such as ischemia, inflammation, and wound healing [4].

3. Calcitonin-associated polypeptide alpha

3.1. Structure of CALCA gene

The human CALCA gene that encodes calcitonin and CGRP is localized on chromosome 11 (11p15.2-p15.1). CALCA gene that consisted of 1 promoter and 6 exons, performs its function through CGRP receptors [2] (**Figure 3**).

3.2. CALCA gene polymorphisms

Many gene polymorphisms are found that occurring in the CALCA gene. In some studies, it has been shown the polymorphisms in the CALCA gene to be associated with cerebrovascular diseases such as ischemic stroke. However, there are studies that show that CALCA gene polymorphisms are not a genetic risk factor for the development of ischemic stroke. Apart from ischemic stroke, it is known that CALCA gene polymorphisms may also be genetic risk factors for various diseases such as Parkinson's disease, ovarian cancer, bone mineral density, migraine, schizophrenia, and essential hypertension [5–8].

The most common polymorphism in the CALCA gene is the CALCA T692C gene polymorphism. Apart from this polymorphism, in the CALCA gene, -1786T>C, -624(T/C), 4218(T/C), -1784 (T/C), -1750 (C/G), -1218 (C/T), -1036 (G/A), rs7948017 (A/C), rs5241 (C/A), rs 2956 (A/T), -855 (G/A), -590 (C/G), CALCA (I/D), 2 bp microdeletion and CALCA A4218T>C gene polymorphisms are determined. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods are used to determine the CALCA gene polymorphisms genotype distributions [5–8].

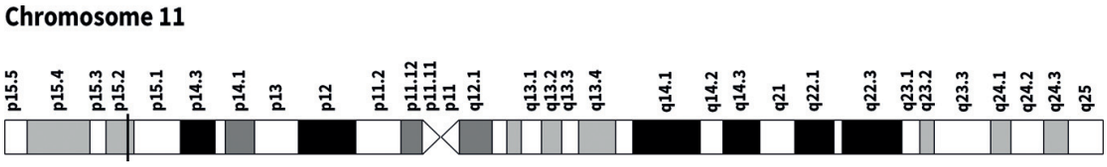

Figure 3. The structure of CALCA gene.

The CALCA T692C gene polymorphism is a single nucleotide polymorphism, and it is characterized by a T/C base transition in position 692 of the CALCA gene. In CALCA T692C gene polymorphism, three genotypes are observed as 692TT homozygote, 692CT heterozygote and 692CC homozygote. The forward primer for the CALCA T692C gene polymorphism is 5′-CGC ATC TGT ACC TTG CAA CT-3′, and the reverse primer is 5′-TCA AAT TCC CGC TCA CTT TA-3′. The PCR conditions for the CALCA T692C gene polymorphism are 5 min for denaturation at 94°C, followed by 38 cycles of denaturation for 50 s at 94°C, annealing for 50 s at 57°C and extension for 1 min at 72°C, followed by 10 min of termination at 72°C. CALCA T692C gene polymorphism is determined using the restriction enzyme PshAI and product length is observed: 636 bp for the TT genotype; 636, 235, 401 bp for the CT genotype; 235, 401 bp for the CC genotype [2].

The CALCA-1786T>C gene polymorphism is another single-nucleotide gene polymorphism that belongs to the CALCA gene. This polymorphism is in the promoter region and arises in consequence of a T/C base exchange in position-1786. TT, CT and CC genotypes are observed in the CALCA-1786T>C gene polymorphism. For the CALCA-1786T>C gene polymorphism, the forward and reverse primers are 5′-CGC TGG GCT GTT TCT CAC AAT AT-3′ and 5′-GTT AGA CAG GAG TTC AAT TAC AGT TGG C-3′. The PCR conditions for the CALCA-1786T>C gene polymorphism are 5 min for denaturation at 94°C, followed by 38 cycles of denaturation for 45 s at 94°C, annealing for 40 s at 62°C and extension for 45 s at 72°C, followed by 10 min of extension at 72°C. The genotype distributions of CALCA-1786T>C gene polymorphism are determined by using BsmAI restriction enzyme and product length is observed: 144 bp for the TT genotype; 144, 115, 29 bp for the CT genotype; 115, 29 bp for the CC genotype [2].

The CALCA 624 (T/C) gene polymorphism occurs because of a T/C base transition in position -624 of the CALCA gene promoter region. TT, CT and CC genotypes are observed in this polymorphism. The forward primer for the CALCA-624 (T/C) gene polymorphism is 5′-GCT GTT TCT CAC AAT ATT CC-3′ and the reverse primer is 5′-CAA TTC CTG GTT GTG TGA TC-3′. For CALCA-624 (T/C) gene polymorphism, the PCR conditions are 10 min for denaturation at 94°C, followed by 35 cycles denaturation for 45 s at 95°C, annealing for 45 s at 60°C, and extension for 45 s at 72°C, followed by 7 min of termination at 72°C. The genotype distributions of CALCA-624 (T/C) gene polymorphisms are determined by using BsmAI restriction enzyme and product length is observed: 109 bp for the TT genotype; 109, 86, 23 bp for the CT genotype; 86, 23 bp for the CC genotype [2].

The forward primer for the CGRP 4218 (T/C) gene polymorphism is 5′-GGA AGA AGC AAA GAC CAG GA-3′ and the reverse primer is 5′-CTG CAA GAA CAA TTC CCA CA-3′. The genotype distributions of CGRP 4218 (T/C) gene polymorphisms are determined by using AluI restriction enzyme and product length is observed: 202, 169, 106 bp for the TT genotype; 371, 202, 169, 106 bp for the CT genotype; 371, 106 bp for the CC genotype [9].

TT, CT and CC genotypes are observed in CALCA A4218T>C gene polymorphism. Primer sequences are used to determine this gene polymorphism; forward primer: 5′-AGC CTG CAC TGA GTT TGC TTC CC-3′ and reverse primer: 5′-ATC CAC CTT CCT GTG TAT TGC TG CG-3. For CALCA A4218T>C gene polymorphism, the PCR conditions are 10 min for denaturation at 95°C, followed by 35 cycles denaturation for 45 s at 95°C, annealing for 45 s at 60°C, and

extension for 45 s at 72°C, followed by 7 min of termination at 72°C. The genotype distributions of CGRP 4218 (T/C) gene polymorphisms are determined by using AluI restriction enzyme and product length is observed: 140, 96 bp for the TT genotype; 236, 140, 96 bp for the CT genotype; 236, 96 bp for the CC genotype [10].

II, ID and DD genotypes are observed in the CALCA (I/D) gene polymorphism. Primer sequences are used to determine this gene polymorphism; forward primer: 5′-TTG GGG AGA AGG GTA GGA CT-3′ and reverse primer: 5′-GAA CTT TTG GAA GCC CAT GA-3. For CALCA (I/D) gene polymorphism, the PCR conditions are 10 min for denaturation at 95°C, followed by 30 cycles denaturation for 45 s at 95°C, annealing for 45 s at 60°C, and extension for 45 s at 72°C, followed by 4 min of termination at 72°C. Product lengths in the CALCA (I/D) gene polymorphism are 303 bp (wildtype-I) and 287 bp (deletion-D) [6].

CC, CG and GG genotypes are observed in the CALCA-1750 (C/G) gene polymorphism. Primer sequences are used to determine CALCA-1750 (C/G) gene polymorphism; forward primer: 5′-TAG CTG GTA TTT CCC ACA GAG-3′ and reverse primer: 5′-CCC ATT TCA AAG ATG AGT ACC CTG-3. The genotype distributions of CALCA-1750 (C/G) gene polymorphisms are determined by using Bsu36I restriction enzyme and product length is observed: 167 bp for the GG genotype; 167, 142, 25 bp for the CG genotype; 142, 25 bp for the CC genotype [3].

Primer sequences are used to determine CALCA 2 bp microdeletion gene polymorphism; forward primer: 5′-CCC AGA AGA GGA GGA CAG CTC TGG GT-3′ and reverse primer: 5′-AGA GCT GGA GGA GCG ATC CTA GAG GGA-3. For CALCA (I/D) gene polymorphism, the PCR conditions are 3 min for denaturation at 96°C, followed by 60 cycles denaturation for 25 s at 98°C, annealing for 30 s at 63°C, and extension for 30 s at 72°C, followed by 10 min of termination at 72°C. Product lengths in the CALCA 2 bp microdeletion gene polymorphism are 184 and 182 bp [11].

TT, CT, and CC genotypes are observed in the CALCA-1218 (C/T) gene polymorphism. Primer sequences are used to determine this gene polymorphism; forward primer: 5′-CAG GTT CTG GAA GCA TGA GGG TGA CGC′ and reverse primer: 5′-CGA CTG CTC TTA TTC CCG CCG CTG T-3. For CALCA-1218 (C/T) gene polymorphism, the PCR conditions are 3 min for denaturation at 96°C, followed by 60 cycles denaturation for 25 s at 98°C, annealing for 30 s at 63°C, and extension for 30 s at 72°C, followed by 10 min of termination at 72°C [11].

GG, GA and AA genotypes are observed in the CALCA-855 (G/A) gene polymorphism. The wild-type sequence used to determine this gene polymorphism is: 5′-GGC TTC CGC ATC TGTA-3′ and mutation sequence: 5′-GGC TTC C**A**C ATC TGTA-3′. For CALCA-855 (G/A) gene polymorphism, the PCR conditions are 35 cycles denaturation for 40 s at 94°C, annealing for 45 s at 56°C, and extension for 1 min at 72°C. The genotype distributions of CALCA-855 (G/A) gene polymorphisms are determined by using AciI restriction enzyme [10].

CC, CG and GG genotypes are observed in the CALCA-590 (C/G) gene polymorphism. The wild type and mutation sequences are used to determine the polymorphism of this gene respectively as 5′-ACA CTG AGC CTC TGT-3′ and 5′-ACA CTC AG**G** CTC TGT-3′. For CALCA-590 (C/G) gene polymorphism, the PCR conditions are 35 cycles denaturation for

40 s at 94°C, annealing for 45 s at 56°C, and extension for 1 min at 72°C. The genotype distributions of CALCA-590 (C/G) gene polymorphisms are determined by using PshAI restriction enzyme [10].

3.3. Migraine and CALCA gene polymorphisms

Migraine, a common disease, is characterized by unilateral throbbing headache with autonomic symptoms such as nausea, vomiting, and photophobia. Although the pathogenesis of migraine is still unclear, it is known that genetic and environmental factors play a role in the pathophysiology of this disease [12].

At the onset of migraine attack, trigeminovascular system is activated. Vasodilatation occurs in the cranial blood vessels in consequence of the release of substance P, neurokinin A and CGRP at sensory nerve endings. As a result, neurogenic inflammation occurs in these veins. Pain signals are induced, and they transmitted to the thalamus. These signals are perceived as headache by the cerebral cortex [12].

Increased levels of CGRP obtained from jugular vein are related to the development of migraine attacks. These CGRP levels return to normal following headache interruption. Intravenous infusion of CGRP is effective in the formation of a like migraine headache. When properly administered, CGRP antagonists can prevent migraine attacks. Nitric oxide is another substance that plays an important role in the pathogenesis of migraine, and nitric oxide effect is seen in consequence of CGRP release in the trigeminal nerve terminals [12].

CGRP is very important in migraine pathophysiology. CGRP is a peptide, which is responsible for neurological inflammation and vasodilatation in head trauma. It plays an important role in the regulation of vascular tone and angiogenesis by causing vasodilatation in blood vessels. It is known to be a neurotrophic factor modulating pain sensation in the nervous system. CGRP, an important peptide, must be synthesized correctly for biological activities to be regular. The molecular structure, function and reaction can change in consequence of the polymorphisms occurring in the CALCA gene [12].

Many studies have been conducted to investigate the relationship between migraine development risk and CALCA gene polymorphisms. In a study conducted in the Thracian population, CALCA T692C gene polymorphism genotype and allele, distributions in female migraine patients were not determined different from healthy controls. This polymorphism was found not to be associated with severity and frequency of migraine attacks. The significant difference was not found in terms of CALCA T692C gene polymorphism in comparison carried out between migraine types with and without aura [12].

In a study conducted by Menon et al., in the Australian population, the significant difference was not found in terms of CALCA (I/D) gene polymorphism between migraine patients (migraine with aura-migraine without aura) and controls. In a study performed by Lemos et al., the significant difference was not determined in terms of CALCA-1750 (C/G) gene polymorphism between migraine patients (with and without aura) and controls. In this study, it was also found that the coexistence of the CG genotype of CALCA-1750 (C/G) gene

polymorphism the AT genotype of the brain natriuretic factor gene polymorphism of increased the risk of the resulting migraine [12].

In a study conducted in the Han-Chinese population, no significant relationship was found between CALCA rs 3781719 and rs 145837941 gene polymorphisms and the risk of developing migraine. However, in this study, CALCA rs 3781719 gene polymorphism was an important risk factor for the development of migraine with aura, but significant result was not found statistically. In a study conducted in the Japanese population by Masakazu et al., it was determined that CALCA rs 3781719 and rs 145837941 gene polymorphisms were not genetic risk factors for migraine complications due to excessive drug use in migraine patients. In another study conducted by Sutherland et al. in the Australian Caucasian population, there was no relationship between CALCA rs 3781719 and rs 145837941 gene polymorphisms and the risk of developing migraine. In the study carried out by Lemos et al., in a European population, CALCA rs 1553005 gene polymorphism was not found as a genetic risk factor for the development of migraine [12].

3.4. Essential hypertension and CALCA gene polymorphisms

Essential hypertension, which affects about 20–25% of the world's population, is a very important health problem. It is known that hypertension increases the risk of coronary heart disease, ischemic stroke, and congestive heart failure. Essential hypertension is a multifactorial disease, and it is quite complex. Environmental and genetic factors play a role in the development of essential hypertension. Through changes in CGRP synthesis and release, CGRP plays an important role in the onset, progression of essential hypertension and its maintenance of essential hypertension [7].

Several single nucleotide gene polymorphisms have been found as effective in the development of essential hypertension. It has been determined that some polymorphisms that occur in the genes are important in influencing the expressions of enzymes and proteins associated with essential hypertension such as angiotensinogen, endothelial nitric oxide synthase. CGRP is a neuropeptide that plays an important role in the pathophysiology of essential hypertension. CALCA and CALCB isoforms of CGRP are associated with increasing of blood pressure. Differences in CGRP plasma concentrations were not fully determined between healthy subjects and hypertensive patients. However, significantly lower plasma CGRP concentrations were found in hypertensive patients and preeclamptic pregnant women than normotensive controls. In some studies performed in patients with hypertension, a significant relationship was found between elevated plasma CGRP levels and systolic and diastolic blood pressures [7].

In consequence of the polymorphisms occurring in the CALCA gene, heart diseases and renal damage due to hypertension are also increasing. In some studies performed with experimental animals, it was observed that systolic blood pressure increases in consequence of CALCA gene polymorphisms. In a study conducted in Japan, a 2-bp microdeletion polymorphism has been shown in the intron 1 of the CALCA gene. This gene polymorphism is associated with the risk of developing essential hypertension. In another study conducted with the Chinese population,

it was determined that CALCA T692C gene polymorphism is a genetic risk factor in the development of essential hypertension [7].

3.5. Ischemic stroke and CALCA gene polymorphisms

Cerebrovascular diseases occur in consequence of sudden emergence of local or global neurological symptoms. Ischemic stroke is one of these diseases, and it occurs in consequence of blocking of blood flow to any region of the brain. Ischemic stroke is classified into five subgroups. The emergence of large or small vessel diseases is a major cause of cerebral ischemia. Large artery disease arises due to atherothrombosis of the carotid, vertebral and proximal cerebral arteries. Lipohyalinosis of the vessel wall in the distal penetrant branches of the veins results in small artery disease. Another ischemic stroke subtype arising from endometrium diseased cardiac valves is cardioembolism. In consequence of these pathogenic mechanisms, a decline is observed in cerebral blood flow. In consequence of decrease the levels of oxygen and glucose required to feed of the brain reduce cell damage occurs. Another type of stroke that causing hypercoagulability is cryptogenic stroke. The other one is also an unclassified ischemic stroke subtype [2].

CGRP is an important member of the calcitonin peptide family, and it plays an important role in the dilation of the cerebral arteries in the human. It is a neuropeptide that especially associated with central and peripheral nervous system disorders. It is known that the polymorphisms occurring in the CALCA gene is associated with ischemic stroke. However, in some studies, it has been determined that CALCA gene polymorphisms were not to be genetic risk factors for the development of ischemic stroke. In a study conducted in the Thracian population, it was determined that CALCA T692C, -1786T>C and -624 (T/C) gene polymorphisms were not genetic risk factors for the development of ischemic stroke. In addition, the significant difference was not determined in terms of the CALCA gene polymorphisms in the patients' subtypes with ischemic stroke in the same study. A limited number of studies have found aimed to investigate the relationship between the risk of ischemic stroke development and CALCA gene polymorphisms [2].

3.6. Ovarian cancer and CALCA gene polymorphisms

Calcitonin, which is synthesized by parathyroid cells of the thyroid, is a peptide hormone that plays an important role in suppressing blood calcium levels. It is known that reducing extracellular calcium levels is associated with the regulation of calcitonin. The risk of ovarian cancer can be reduced by an antiproliferative mechanism. In the ovulation period, the ovarian surface epithelium is subjected to repeated injuries and healing. Repeated proliferative stimulation plays an important role in the malignant transformation of ovarian epithelial cells. Extracellular calcium is elevated via receptors that sensing the calcium. Unconverted ovarian surface epithelial cells multiply in response to this condition [13].

The differentiation and proliferation of small intestine and chest epithelial cells are also modulated by extracellular calcium. There is a very significant relationship between this differentiation,

proliferation and ovarian cancer. Therefore, impaired calcium regulation is believed to be an important risk factor for the development of ovarian cancer. Calcitonin, which plays an important role in the prevention of bone resorption, causes a decrease in serum calcium and is known as an important regulator of calcium metabolism by this property. Calcium inhibits the production of proteins necessary for the regulation of hypocalcemia. Increased calcium reduces proliferation and thus regulates wound healing of the ovarian surface epithelium [13].

Hypercalcemia is also associated with ovarian epithelial tumors. Proteins that are effective in the regulation of hypocalcemia stimulate protein kinase C and phospholipase C signaling pathways. Thus, mitosis is triggered and cancer-related apoptosis reduces. It can also alter the cancer susceptibility by interacting with insulin-like growth factor (IGF) and binding protein, hormones and other growth factors [13].

In studies conducted in the Japanese population, it was found that the C allele of CALCA-624 (T/C) gene polymorphism associated significantly with ovarian cancer. In women with TT genotypes, ovarian cancer is much more common than in women with CC genotype. It has been determined that the C allele is a genetic risk factor in women who consume less calcium. In addition, because of reduced calcium intake, serum calcium levels decrease and the concentration of required calcitonin decreases [13].

3.7. Parkinson, schizophrenia, manic depression and CALCA gene polymorphisms

Parkinson's disease occurs because of damage of gray matter nuclei in the lower part of the brain. Because of this damage, degeneration occurs in cells that secrete dopamine [14]. Schizophrenia, a chronic brain disease, is also known as a developmental disorder of the brain. It is known that subcortical dopamine pathways in the brain are highly active in this disease. In consequence of this over activity, excessive dose of dopamine is released from the nerve endings. As a result, dopamine receptors are stimulated largely [15]. The dopaminergic hypothesis plays an important role in the pathophysiology of manic depression, a common neuropsychiatric disorder. There are homeostatic mechanisms that develop in response to hyperdopaminergic agents in the manic phase of the disease [16]. Because of these mechanisms, decrease occurs in dopaminergic function together with a hypodopaminergic situation and depression can developed [10].

Calcitonin plays an important role in bone calcium metabolism and is produced by C cells known as thyroid parafollicular cells. Polymorphisms that occur in calcitonin receptors are associated with changes in bone density in postmenopausal women. It is believed that the disorders that occur in calcitonin function are related to osteoporosis. Concentrations of procalcitonin are increased in bacterial infections and in cases such as bacterial sepsis. This increase is performed as independent of thyroid C cells. Catacalsine (PND-21) is another product of procalcitonin and is important in the development of medullary thyroid carcinoma [10].

CALCA, which produces a strong vasodilator effect in the brain and peripheral organs, is released from nerve fibers that bind blood vessels to the nervous system. CALCA, released from fibrils that come from the ventral tegmental area, amygdala, and ventral striatum plays

an important role as moderator of dopaminergic transmission in these areas. The dopaminergic system consists of mesolimbic and mesostral axis. Both axes are influenced by CALCA. There was a relationship between cerebrospinal fluid and elevated CALCA levels in patients with major depression. Polymorphisms of CALCA gene have been associated with disorders such as Parkinson, Schizophrenia, and manic depression. It is believed that gene polymorphisms of CALCA 855 (G/A), CALCA-624 (T/C), CALCA-590 (C/G) and CALCA (I/D) may be associated with psychiatric or neurological diseases in dopaminergic transmission [10].

3.8. Aseptic loosened total hip arthroplasty and CALCA gene polymorphisms

One of the most important causes of long-term failure of arthroplasty is aseptic loosening of total joint replacement. There are various studies to understand the aseptic relaxation mechanism. Aseptic relaxation is known as a multifaceted process resulting from events such as foreign body reaction, cell-cell interaction, allergic reactions, hydrostatic pressure, body weight, and implantation. Protein-surrounding local osteolysis is initiated by an aseptic inflammatory response following the addition of abrasion particles by the macrophages. Fibroblasts are stimulated by macrophages in consequence of proliferation and differentiation of precursor osteoclasts to mature osteoclasts. This stimulation performs via cytokines. An important process in the regulation of homeostasis in tissues is apoptosis. Some important proteins regulate the most important process and the proliferation of normal tissues.

In a study conducted by Ahmed et al., CALCA immunoreacted nerve fibers were found in the arthroplasty interface membrane. CALCA, produced by central and peripheral nervous systems, is found in almost all tissues and plays an important role in bone remodeling. Large quantities of calcitonin produced by thyroid cells are responsible for calcium homeostasis, leading to the inhibition of osteoclasts. Because of changes in cell cycle, apoptosis, receptor expression, DNA replication, gene expressions and genes, the risk of aseptic loosening following total hip replacement can be increased. In a study conducted in Germany, it was aimed to determine the relationship between aseptic loosening risk and CALCA-1786T>C gene polymorphism following total hip replacement. In consequence of this study, it was determined that CALCA-1786T>C gene polymorphism was not a genetic risk factor for the risk of aseptic loosening [8].

3.9. Analgesic effect and CALCA gene polymorphisms

In most of cancer patients, severe pain occurs in the terminal period. Opioid drugs, known as the most effective drugs in the treatment of cancer pain, can lead to tolerance and hyperalgesia when used for a long time. Fentanyl, one of the opioid drugs, is used as an analgesic in the treatment of terminal cancer pain. Recently, it has been shown that there is a significant relationship between fentanyl and human genes. It is known that CGRP is an important transmitter in the transmission of pain signals. The analgesic mechanism in opioid medications reduces the release of neurotransmitters such as CGRP into the synaptic cleft of nerve fiber ends of the presynaptic membrane [9].

In a study conducted by Cepeda et al., it is found that respiratory depression arising from morphine was found to be more effective in Native American Indians than Caucasians. In another study conducted by Zhou et al. in Caucasians, sedation and respiratory depression were found to be more observed than in Asians. When comparing men and women, it was also determined that the reactions to these drugs were different. In a study by Sear et al., no association was found between plasma concentrations of opioid drugs and clinical effects. The analgesic effect of fentanyl is different. It is believed that this diversity to related to gene motion modes [9].

In some studies, genetic variants that can affect the efficacy of fentanyl have been identified. Because of the polymorphisms that occur in the genes, the analgesic effect and the pain perception mechanism in humans are affected. CGRP is an important neuropeptide in the nervous system. This neuropeptide plays an important role in peripheral nerves via nociceptive information transfer. It is also effective in the production of hyperalgesia in the spinal cord [9].

As a newly discovered polymorphism in exon III of the CGRP gene, CGRP 4218 (T/C) gene polymorphism is believed to be associated with diseases such as Parkinson's and major depression. Whether there is an association between the analgesic effect of fentanyl and the CGRP 4218 (T/C), gene polymorphism is unclear. In a study, CGRP 4218 (T/C) gene polymorphism was identified as a risk factor for the analgesic effect of fentanyl. This study has proven that there is no association between CGRP 4218 (T/C) gene polymorphism and postoperative adverse reactions resulting from fentanyl. In addition to these studies, studies on the relationship between CGRP 4218 (T/C) gene polymorphism and fentanyl pharmacokinetics should be performed [9].

3.10. Psoriasis vulgaris and CALCA gene polymorphisms

The pathogenesis of psoriasis, a chronic disease characterized by reddish, scaly skin, is quite complicated. Intravascular molecules such as intracellular adhesion molecule (ICAM), TNF-alpha, reactive oxygen species (ROS) play an important role in the pathogenesis of this disease [17].

CGRP, widely distributed in central and peripheral nervous systems, is a peptide intermediating to pain. This peptide is also known as a growth factor in cells such as Schwan cells and endothelial cells. Proinflammatory features of CGRP are found in many diseases. In several earlier studies, a marked cutaneous proliferation has been identified in psoriasis. It has also been found that plaques of psoriasis include CGRP. Because of this situation, it has been shown that psoriatic progression is observed. In consequence of various studies, target genes have been identified which can contribute to the development of psoriasis. One of the isoforms of CGRP, which plays an important role in immune regulation and inflammation, is the CALCA gene. It is believed that polymorphisms in this gene may be associated with the development of psoriasis [17].

In a study conducted with the Chinese population, the effect of CGRP mRNA expression and plasma CGRP levels on the development of psoriasis was investigated. It was also aimed to determine the relationship between the T692C gene polymorphism in the CALCA gene and

the risk of development of psoriasis. In this study, morbidity of psoriasis was found to be increased CGRP expression and release. Furthermore, in the CALCA T692C gene polymorphism, TT genotype has been identified as a genetic risk factor in the development of psoriasis in people with alcohol habits. Ethanol that can activate primer sensory neurons can cause neuropeptide release in the skin. In consequence, activation capsaicin receptor of the alcohol results in the release of CGRP from the sensory nerves [17].

It is known that CGRP, a potent vasodilator, plays an important role in skin homeostasis. In consequence of intradermal injection of CGRP, increased blood flow is observed, and microvascular dilation is induced. Consequently, local erythema occurs. There are not many studies aiming to investigate the relationship between CALCA gene polymorphisms and psoriasis [17].

4. Conclusion

Polymorphisms occurring in the CALCA gene are known to be associated with various diseases. Several studies have been carried out to investigate the relationship between CALCA gene polymorphisms and some diseases. In some of these studies, it has been determined that CALCA gene polymorphisms are genetic risk factors in the development of these diseases. However, there are also studies showing that there is no significant relationship between CALCA gene polymorphisms and these diseases. Different results can be obtained in studies; this situation may arise from different selection criteria for patients and control groups. In addition, the studies performed with a small number of cases are another reason for the different results. Different results were found in gene polymorphism studies carried out with different racial and ethnic populations. Findings obtained in consequence of carrying out these studies with more cases and with different populations will be an important indicator for the treatment of diseases.

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Conflict of interest

We declare that there is no conflict of interest with any financial organization regarding the material discussed in the chapter.

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