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Impact of fractalkine polymorphisms (rs 614230, rs 170364) on the occurrence of inappropriate body mass

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ABSTRACT

Introduction: Fractalkine chemokine (CX3CL1) is a protein cytokine which is associated with the pathophysiology of lifestyle-related diseases such as obesity, diabetes and atherosclerosis. This study aimed to assess the influence of fractalkine gene variants (rs 614230, rs 170364) on body mass.

Material and methods: Study group consisted of 295 patients (152 women and 133 men). The blood samples were collected and stored at –70˚C. Afterwards the DNA material was isolated and prepared (quality and quantity were checked by spectrophotometry). Allelic discrimination of fractalkine gene (rs 614230, rs 170364) was performed using Roche Lightcycler96 thermocycler. Real-Time PCR was conducted using fluorescence-labeled TaqMan Pre-designed SNP Genotyping Assay probes.

Results: The CC genotype of fractalkine (rs 614230) had a positive association with BMI compared to CT and TT genotypes. The GG genotype of fractalkine (rs 170364) had a negative association with body size, body mass index and body fat content compared to GT. The TT genotype of fractalkine (rs 170364) shows a negative association with BMI compared to GT.

Conclusions: Fractalkine polymorphism rs 614230 may be associated with high body mass index (BMI), but not with waist size or body fat content. Single nucleotide polymorphisms (SNP) of fractalkine rs 170364 may be associated with high body mass. Further studies in a larger group of patients are necessary to completely assess this association.

Keywords: fractalkine; obesity; single nucleotide polymorphisms

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Introduction

Fractalkine (Fkn), chemokine CX3CL1, is a protein cytokine coded by gene located on chromosome 16q13. Unlike most chemokines, CX3CL1 is not synthesized in leukocytes but in the vascular endothelium, parenchymal organ cells and nerve cells [1]. The expression of its membrane form is stimulated by pro-inflammatory cytokines such as IL-1 or TNF-α. Receptors for CX3CL1 are also found on T lymphocytes, NK cells, macrophages, neutrophils and platelets. In recent years, an increasing number of publications have been published linking Fkn with various processes taking place in the body. Studies have proven its relationship with chronic inflammatory reaction and the relationship between fractalkine serum concentration and inflammation and conversion of brown to yellow adipose tissue. It has been shown that high Fkn expression in patients with colorectal cancer is associated with a better prognosis [2].

Recent studies have demonstrated the relationship of CX3CL1 cytokine to pathophysiology of lifestyle-related diseases such as obesity, diabetes and atherosclerosis [3–6]. Schinzari et al. published research proving the relationship between high circulating fractalkine and obesity and type 2 diabetes. According to this research, activation of fractalkine as well as other chemokines is involved in atherogenesis and contributes to premature vascular damage in diabetes and obesity. The study showed that in both metabolically healthy and unhealthy

obese subjects vasodilator responses to drugs were impaired and plasma fractalkine levels were increased. Moreover, oral antidiabetic drugs in diabetic patients reduced circulating levels of fractalkine with no impact on vascular responses. The finding indicates that antidiabetic treatment may provide cardiovascular benefits by lowering the level of circulating fractalkine [7].

New research also indicates a relationship between high serum concentration of fractalkine and polycystic ovary syndrome (PCOS). İsmail Demi R et al. investigated fractalkine levels in two groups of patients: with metabolic and reproductive system disorders and healthy controls, both groups without other diseases. They showed positive correlation between the Fkn levels and PCOS diagnosis. Furthermore CX3L1 was also elevated in patients with insulin resistance, BMI > 25 kg/m^2 and high CRP levels [8].

As suggested by many scientific reports, fractalkine seems to be important in the development of many diseases associated with chronic inflammation and insulin resistance. Detailed understanding of its mechanisms of action could help to identify new therapeutic targets.

The aim of our study was to investigate the association between two (rs 614230, rs 170364) single nucleotide polymorphisms (SNP) and increased body mass as determined by body mass index (BMI), waist size and body fat content in a group of Polish patients.

Material and methods

The blood samples were collected from patients who visited a primary care clinic in Southern Poland. Informed consent was granted by all patients. The research included 285 adults participants(152 women and 133 men). Their average age was 53.

Inappropriately high body mass was determined by 3 indicators:

— body mass index (underweight < 18.5, normal weight 18.5–24.9, overweight 25–29.9, obesity ≥ 30);
— waist size (waist circumference > 80 cm for women, > 94 cm for men);
— body fat content (> 32% for women, > 25% for men).

The DNA isolation was performed with the use of column method and the samples were stored at –70°C. Then, according to the dilution protocol, the DNA was mixed with water to obtain a concentration of 15 ng/μL. This was verified by using the spectrophotometer denoviX.

Afterward, we performed allelic discrimination using fluorescently labeled probes, according to the manufacturer's protocol. PCR was performed in Roche LightCycler 96. Alleles were marked as C in VIC and T in FAM for rs 614230 and G in VIC and T in FAM for rs 170364.

Eventually, statistical analysis of the data distribution was performed by the Shapiro-Wilk test. Subsequently, we used the Kruskal-Wallis ANOVA rank test with multiple comparisons to determine statistically significant differences between distributions of genotypes, gender and indicators associated with inappropriate body mass. Finally, we compared significant data between the two groups by the Mann-Whitney U test.

Results

We found a significant difference between fractalkine genotypes of rs 614230 and body mass index. This correlation was not found when comparing waist size and body fat content with rs 614230 polymorphisms (Table 1). We determined that only the CC genotype, as opposed to CT and TT genotypes of fractalkine, had positive association with BMI (Table 2).

Single nucleotide polymorphisms (SNP) of fractalkine rs 170364 were associated with all 3 indicators of inappropriate body mass (Table 3). GG genotype had a negative association with body size, BMI and body fat content compared to GT (Table 4, Table 5, Tabel 6). In this SNP, TT genotype showed a negative association with BMI (Table 5).

Discussion

Many publications have focused on fractalkine and its impact on health [7–11]. As opposed to the cytokine, genetic variants of fractalkine have not been widely researched. The potential implications of polymorphisms rs 614230 and rs 170364 are yet to be discovered. No prior studies focused on the correlation between body mass and these gene variants.

However, there have been studies that researched the association between previously mentioned single nucleotide polymorphisms and proinflammatory risk factors that play a role in the development of coronary artery disease (CAD) and common carotid artery intima-media thickness. Study conducted by Zang X et al. showed a correlation between fractalkine polymorphism of rs170364 and the prevalence of coronary artery disease. Research demonstrated that patient carrying fractalkine rs170364 allele T had a 1.25 times increased risk of developing CAD compared to non-carriers [12].

Table 1. Comparison of fractalkine genotype (rs 614230) distributions in patients with and without indicators associated with inappropriate body mass

Genotype fractalkine rs 614230							
	CC		**CT**		**TT**		
Gender	n	%	n	%	n	%	
Women	27		63		62		
Men	19		60		54		
Waist size (waist circumference > 80 cm for women, > 94 cm for men)	n	%	n	%	n	%	p = 0.6079
Yes	36	78.26%	87	70.73%	83	71.55%	
No	10	21.74%	36	29.27%	33	28.45%	
Body mass index	n	%	n	%	n	%	p = 0.0315
Underweight < 18.5					1	0.86%	
Normal weight 18.5–24.9	9	19.57%	45	36.59%	42	36.21%	
Overweight 25–29.9	25	54.35%	56	45.53%	59	50.86%	
Obesity ≥ 30	12	26.09%	22	17.89%	14	12.07%	
Body fat content (> 32% for women, > 25% for men)	n	%	n	%	n	%	p = 0.1585
Yes	39	84.78%	87	70.73%	83	71.55%	
No	7	15.22%	36	29.27%	33	28.45%	

Table 2. The result of Mann-Whitney U test between genotypes of fractalkine (rs 614230) and Body Mass Index, showing statistical difference p < 0.05

Compared group	**P**	**Z**
CC-CT	0.037757	2.077489
CC-TT	0.015763	2.414361

Another study conducted by Jin SG et al. showed similar results. Based on the results obtained in 229 subjects, it was concluded that being a carrier of TT genotype of rs170364 in CX3CL1 may decrease the risk of developing coronary artery disease. However, no statistically significant correlation has been found between the single T allele of rs170364 and the risk of CAD. Moreover, CC genotype and C allele of fractalkine rs614230 were found to significantly decrease the risk of CAD [13]. The protective nature of allele C of CX3CL1 rs614230 was confirmed in another study, where its presence was associated with the absence of atherosclerotic plaque in HIV-infected individuals [14].

Studies conducted on 2763 subjects showed an association of common carotid artery intima-media thickness with two genetic variants of CX3CL1: rs170364 and rs614230. However, those findings have not been replicated in a follow-up study in a larger study group consisting of 6049 patients [15]. Another study performed by Ma G et al., focused on fractalkine gene polymorphisms showed that allele T carriers of CX3CL1 rs614230 had a higher risk of chronic post-surgical pain [16].

According to the research conducted by Dave Sirois-Gagnon et al., the T280M and V249I polymorphisms of the *CX3CR1* gene encoding the fractalkine (CX3CL1) receptor are associated with obesity [17].

In our work we observed that the SNP of fractalkine (rs 614230, rs 170364) may be associated with the prevalence of obesity as measured by BMI. Also, the gene polymorphism (rs 170364) showed that GG genotype had a negative association with waist size, body mass index and body fat content, compared to GT.

Further studies in a larger group of patients are necessary to completely assess this association.

The main limitations of this study are:

1) serum levels of fractalkine molecule itself were not measured;
2) limited number of TT genotype carriers of **rs 170364** have been found in the study;
3) small study group.

Table 3. Comparison of fractalkine genotype (rs 170364) distributions in patients with and without indicators associated with inappropriate body mass

Genotype fractalkine rs 170364							
	GG		**GT**		**TT**		
Gender	n	%	n	%	n	%	
Women	79	49.69%	62	56.88%	11	64.71%	
Men	80	50.31%	47	43.12%	6	35.29%	
Waist size (waist circumference > 80 cm for women, > 94 cm for men)	n	%	n	%	n	%	p = 0.0383
Yes	105	66.04%	87	79.82%	14	82.35%	
No	54	33.96%	22	20.18%	3	17.64%	
Body mass index	n	%	n	%	n	%	p = 0.0306
Underweight < 18.5	1	0.63%					
Normal weight 18.5–24.9	60	37.75%	29	26.51%	7	41.18%	
Overweight 25–29.9	75	47.17%	55	50.46%	10	58.82%	
Obesity ≥ 30	23	14.47%	25	22.94%			
Body fat content (> 32% for women, > 25% cm for men)	n	%	n	%	n	%	p = 0.0351
Yes	107	67.30%	88	80.73%	14	82.35%	
No	52	32.70%	21	19.27% ^	3	17.65%	

Table 4. The result of Mann-Whitney U test between genotypes of fractalkine (rs 170364) and waist size (waist circumference > 80 cm for women, > 94 cm for men), showing statistical difference p < 0.05

Compared group	**P**	**Z**
GG-GT	0.014181	–2.45267

Table 5. The result of Mann-Whitney U test between genotype of fractalkine (rs 170364) and body mass index, showing statistical difference p < 0.05

Compared group	**P**	**Z**
GG-GT	0.031823	–2.33730
TT-GT	0.042155	–2.03200

Table 6. The result of Mann-Whitney U test between genotype of fractalkine (rs 170364) and body fat content (> 32% for women, > 25% for men), showing statistical difference p < 0.05

Compared group	**P**	**Z**
GG-GT	0.015442	–2.42185

Conclusions

The result suggests that fractalkine genotypes (rs 614230, rs 170364) may be associated with inappropriately high body mass.

The research shows that fractalkine polymorphism rs 614230 may be associated with high body mass index (BMI), but not with waist size or body fat content.

The study found an association between the rs 170364 gene polymorphism and inappropriate body mass (measured by 3 indicators: body mass index (BMI), waist size, body fat content).

Further studies in a larger sample are needed to clarify these findings.

Article information

Data availability statement: *The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.*
Ethics statement: *The study was reviewed and approved by an Ethics Committee and participants*

provided their written informed consent to participate in this study.

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Supplementary material: *All data are in the article.*

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