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RESEARCH

# Molecular analysis of the corticotropin-releasing hormone receptor type 2 gene fragment in anorexia nervosa

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## Summary

**Aim**. CRHR 2 gene can be a candidate gene for anorexia nervosa (AN). The aim of the study was to screen for mutations in exon 1α of the CRHR 2 gene in patients with AN.
**Material and method**. The molecular studies (polymerase chain reaction and direct sequencing) were performed in 20 patients with AN and 10 healthy controls.
**Results**. No genetic variants were found in the analysed region, neither in AN patients nor in the controls.
**Conclusion**. The results do not show mutations within the analysed region of the CRHR 2 gene which may have been involved in the pathogenesis of AN.


**anorexia nervosa / corticotropin releasing hormone receptors**

## INTRODUCTION

Anorexia nervosa (AN) is an eating disorder which is characterized by a progressive loss of body weight in response to a restriction in the intake of food, which can result in concomitant metabolic disorders and abnormalities in the neuroendocrine system.
The aetiology of AN is complex and multifactorial. The results of numerous studies indicate that genetic background plays a significant role in the development of the disease. The increased prevalence of AN is found among the relatives of the proband with the highest prevalence among identical twins [1]. The incidence of AN in monozygotic twins has been estimated at 52 – 56%, and in dizygotic twins at 5–11% [2, 3]. The heritability of susceptibility to AN is estimated at around 50 – 80% [1].
Apart from genetic predispositions, AN may also be triggered by stress factors. Some researchers emphasize that stressful conditions may induce abnormal food-associated behaviour and nutritional disorders especially in individuals characterized by a perfectionistic personality [4]. Sometimes the disease is underlain by a wide range of conflicts, such as a problematic home environment or other dysfunctional relationships (i.e. alcohol abuse by a family member). It has also been noted that having parents who are overprotective, overbearing and who expect excellence and perfection from their children may also contribute to the development of certain kinds of anxiety reactions in those children. Moreover, AN patients have difficulty in maintaining so-

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cial relationships, which can lead to emotional tension, fear and, as a consequence, multisymptomatic reactions to stress [5]. Because the incidence of AN increases during the adolescent period, psychological factors related to sexual development (such as the increased fat panniculus in girls) and a media-promoted thin body image may also play a role in AN.

The word "anorexia" is derived from the Greek (*an* : absence, loss ; *orexis* : appetite) and means lack of appetite or food aversion [6], while "anorexia nervosa" can be translated as a nervous loss of appetite.

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the control of an organism's reaction to stress. Several abnormalities in the action of the HPA axis have been found in AN patients, but it has been suggested that its dysfunction is caused by the hypersecretion of corticoliberin (CRH). Studies show that AN patients have higher concentrations of corticoliberin in their cerebrospinal fluid [7].

Corticoliberin (CRH) is one of several substances which influence the precise and complex nature of the appetite. Central nervous system (CNS) administration of CRH decreases appetite [8], and leads to an acute anorectic effect [9]. The effect of CRH on appetite regulation is associated with the influence of stress factors on the organism [10]. Both, the control of appetite and stress-related behaviours, can be inverted by CRH receptor antagonists [11].

There are two types of CRH receptor which belong to the family of G-protein-coupled receptors, where the signalling is mediated by cAMP [12]. The CRH receptor 1 (CRHR1) has been implicated in mediating ACTH responses to stress, whereas the CRH receptor 2 (CRHR2) affects the appetite and anxiety-type behaviours. The stimulation of CRHR2 inhibits appetite [11]. Logically, a follow-up study confirmed that CRHR2 antagonist decreases the CRH affect on the appetite, although this affect has not been observed after administration of an antagonist for CRHR1 [12]. In addition, the administration of CRH to the CNS shows a decreased appetite both in mice lacking CRHR1 and in wild-type animals [13].

The CRHR2 has three isoforms : $\alpha$, $\beta$, and $\gamma$, which exhibit different N-terminal extracellular domains [14]. In the hypothalamus—the main centre of appetite control—the $\alpha$ CRHR2 isoform is expressed [11, 15]. The gene for CRHR2 may be a candidate gene in anorexia nervosa. It appears on chromosome 7 (locus p11-p15) [16] and consists of 40 000 bp, and the coding part contains 12 exons (fig. 1). It is the alternative splicing of the 5′ end which results in the $\alpha$, $\beta$ and $\gamma$ isoforms. The mRNA of the $\alpha$ isoform contains a unique exon 103 bp in length. Exon 1 $\alpha$ is followed by a non-coding part 137 bp in length, preceding exon 2 (126 bp), which constitutes the first common fragment for all isoforms [17]. The UTR fragment of isoform $\alpha$ contains around 207 bp [18]. This fragment may be the location of the gene's regulatory sequences, which are responsible for the transcription of a given gene. Exon 1 $\alpha$ CRHR2 codes a unique fragment of the N-terminal part of the peptide, which has the length of 34 amino acids [17]. The 17 first amino acids constitute the signalling fragment of the receptor, while the next 17 encompass the fragment of the first extracellular domain.

The goal of this study was to identify the mutations within the 1 $\alpha$ exon of the CRHR2 gene in AN, which may contribute to the pathogenesis of the disease.

## MATERIAL AND METHODS

### Patients

The studies included 20 female patients aged from 13 to 23 years (the mean age was 18.4 years) with anorexia nervosa recognized according to the diagnostic criteria of ICD-10 [19] and DSM-IV [20] classification, hospitalised in the Department of Pediatric Endocrinology and Diabetes, as well as in the Department of Child and Adolescent Psychiatry in Poznań, in the period 2004 – 2005 (18 female patients had the restrictive type of AN, and 2 female patients had the bulimic type).

The control group consisted of 10 healthy individuals aged from 26 to 36 years (with a mean age of 30.4 years), with proper body weight above 85%, and with a negative result in a clinical examination and family history with regards to nutritional disorders and mental

diseases. The difference in age between these two groups permitted us to limit inclusion in the control group individuals with an increased genetic risk of AN development. The study was approved by the institutional ethics committee and all patients gave their informed consent.

### Gene analysis

The molecular studies performed included isolating DNA from the peripheral blood leukocytes, DNA amplification using polymerase chain reaction (PCR), agarose gel electrophoresis, and sequencing of the PCR products. The studies aimed to amplify the CRHR2 gene fragment within exon 1$\alpha$ (103 bp). During the amplification, the following primer sequences were used:
CRHR2 for : 5'GAGACTGAGCCCCTCCGAGA 3'
CRHR2 rev : 5' GGTGTAGAGCAGGCAGCGAG 3'
The primers were constructed so as to amplify the greater fragment of the gene, which extended the area of the analysis. The PCR product obtained had a size of 699 bp.

## RESULTS

The sequencing of the 699 bp PCR reaction product yielded the nucleotide sequence of the fragment under investigation of the CRHR2 gene (Performed at the Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University in Poznań). As a result of this one sequencing procedure, the 310 bp sequence of 5' PCR product (the 91 bp–401 bp fragment) encompassing exon 1$\alpha$ (103 bp) and the non-translation region (207 bp) was obtained. The analysis of the obtained sequences of CRHR2 gene did not reveal genetic variants in the study group or in the control group.

## DISCUSSION

In recent years, significant technological progress has been observed in the field of molecular genetics. It is believed that the results of genetic studies allow the identification of genes associated with the pathogenesis of this disease.
Although the genetic background of AN has already been convincingly substantiated, the use of molecular investigation of specific genes is still rare. To date, only a few genetic studies have addressed genes affecting appetite regulation which may be candidate genes in AN. The recognized genetic variants within certain genes were then analysed using associative studies. The studies performed concerned the genes in the serotoninergic [21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32], dopaminergic [33, 34, 35, 36] and melanocortin [37, 38] systems, estrogen receptor genes [39, 40], neuropeptyde Y [41], $\beta$ 3-adrenergic receptor [42], as well as genes for leptin [43], AgRP (Agouti Related Protein) [44], uncoupling proteins [45], the brain-derived neurotropic factor [46] and ghrelin [47]. To our knowledge, however, there are no molecular studies dealing with the incidence of genetic variants within the CRH system in AN. Therefore the study presented here on a group of AN patients is one of the first studies dealing with the possible mutation affect of CRHR2 on AN pathogenesis.
Although the results obtained do not show that the investigated fragment of CRHR2 has any correlation to AN development, the lack of genetic variants within this gene may result from the relatively small number of individuals examined. These results also suggest an outline for further research, which should include investigations of the remaining part of the CRHR2$\alpha$ gene, the whole CRHR2 gene, mutations within UTR segments which may influence protein translation and associative studies in single nucleotide polymorphisms. Since there are obvious differences between the concentrations of CRH in patients with AN and the control group, other genetic studies should be designed to assess other components of the CRH system in order to gain information concerning the expression of this gene and the function of ligand-receptor complexes in the CNS.

## CONCLUSIONS

1. There are no mutations within exon 1$\alpha$ of the CRHR2 gene in the AN patients.

2. Studies show that one cannot find that the CRHR2 gene, which includes exon 1$\alpha$, contributes to AN development.
3. The lack of change within the investigated sequences of the CRHR2 gene, increases the need for further research to examine possible mutations in other portions of this gene in AN patients.

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