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The CRF system, stress, depression and anxiety – insights from human genetic studies

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Abstract

A concatenation of findings from preclinical and clinical studies support a preeminent role for the corticotropin-releasing factor (CRF) system in mediating the physiological response to external stressors and in the pathophysiology of anxiety and depression. Recently, human genetic studies have provided considerable support to several long-standing hypotheses of mood and anxiety disorders, including the CRF hypothesis. These data, reviewed in this report, are congruent with the hypothesis that this system is of paramount importance in mediating stress-related psychopathology. More specifically variants in the gene encoding the CRF_1 receptor interact with adverse environmental factors to predict risk for stress-related psychiatric disorders. In depth characterization of these variants will likely be important in furthering our understanding of the long term consequences of adverse experience.

Keywords

CRF; CRH; receptor; genetic; depression; anxiety

Introduction

Soon after the isolation and chemical characterization of corticotrophin-releasing factor (CRF) from ovine hypothalamus more than 25 years ago, the 41 amino acid containing peptide was unequivocally established as the major physiological regulator of hypothalamic-pituitary-adrenal (HPA) axis activity (1). It's preeminent role in mediating stress-related behaviors in rodents and primates was quickly demonstrated and hypothesized to be important in the pathogenesis of mood and anxiety disorders (2–8). These early findings were followed by a series of clinical studies and additional laboratory animal experiments that support the hypothesis that alterations in central nervous system (CNS) CRF-containing

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Disclosures:
Currently, Dr. Nemeroff serves on the Scientific Advisory Board (SAB) for the American Foundation for Suicide Prevention (AFSP); AstraZeneca; NARSAD, PharmaNeuroboost and CeNeRx. He serves on the Board of Directors of American Foundation for Suicide Prevention (AFSP); George West Mental Health Foundation; NovaDel Pharma, Mt. Cook Pharma, Inc. He owns equity or is stock holder in Corcept; Revaax; NovaDel Pharma; CeNeRx, PharmaNeuroboost, Mt. Cook Pharma. He is inventor on the following patents: Method and devices for transdermal delivery of lithium (US 6,375,990 B1)
Method to estimate serotonin and norepinephrine transporter occupancy after drug treatment using patient or animal serum (provisional filing April, 2001)
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circuits play an important role in the pathophysiology of anxiety and depression (see (9–11) for review).

Because the CRF hypothesis of depression has previously been comprehensively reviewed by our group and others, this monograph will only briefly summarize the CRF system which contains 4 cloned ligands and 2 receptors, as well as major findings derived from animal and human studies that relate this system to stress, anxiety and depression. Our main focus, hitherto not comprehensively reviewed, is on recent developments in the human molecular genetics of the CRF system, how these findings further support the CRF hypothesis of depression and anxiety, and a discussion of future research directions.

The CRF system

Since the initial isolation of ovine CRF in 1981, 3 related ligands and 2 receptors have been identified (Hauger et al 2003 for review (12)).

As noted above, CRF is a 41 amino-acid neuropeptide, expressed both peripherally (blood vessels, skin, lung, testes, ovaries and placenta) as well as in the CNS -with highest expression in the hypothalamus, amygdala, cerebrocortical areas and the septum (13, 14). Urocortin 1, urocortin 2 (stresscopin-related peptide), and urocortin 3 (stresscopin) are CRF-like neuropeptides (15–18). Human urocortin 1 shares approximately 45% homology to human CRF. In the CNS, urocortin 1 is expressed most strongly in the Edinger-Westphal nucleus and the hypothalamus (19, 20), but overall its expression is more widespread peripherally, including in the pituitary, gastrointestinal tract, testes, cardiac myocytes, thymus, spleen and kidney (13, 20, 21). Urocortin 2 and 3 are also expressed both in the CNS and the periphery. Urocortin 2 is expressed in the magnocellular portion of the hypothalamic paraventricular and arcuate nucleus, the locus coeruleus, brainstem and spinal motor neurons; its expression pattern partially overlaps with that of CRF (17). Urocortin 3 is concentrated in the median preoptic area, the rostral perifornical area, the bed nucleus of the stria terminalis and the medial nucleus of the amygdala (16). Its expression pattern shows little overlap with CRF expression. In the periphery, urocortin 2 has been found in the heart, adrenal gland and peripheral blood cells whereas urocortin 3 has been localized in the gastrointestinal tract, muscle, adrenal gland and skin (13, 22).

The available evidence suggests that these 4 peptide neurotransmitters bind to 2 identified receptors, CRF_1 and CRF_2 (Hauger et al 2003 for review(12)). CRF and urocortin 1 both bind with high affinity to the CRF_1 receptor, whereas urocortin 1, 2 and 3 but not CRF, bind with high affinity to the CRF_2 receptor. Both CRF receptors are 7-transmembrane G-protein coupled receptors (GPCRs) and share 70% sequence homology. Both receptors are present in the CNS. CRF_1 is expressed in high density in the cerebral cortex, cerebellum, hippocampus, amygdala and pituitary; its peripheral expression is less robust and concentrated to skin, ovaries and testes and the adrenal gland. The CRF_2 receptor is also expressed in the CNS, but largely restricted to subcortical areas, including the hypothalamus, amygdala, bed nucleus of the stria terminalis and raphe nuclei, and in peripheral tissues, such as the pituitary, heart, lungs, ovaries, testes and adrenal gland (14, 23–26).

One unique characteristic of this system is that the biological activity of all four peptides is regulated by a CRF-binding protein (CRFBP), which is highly conserved in mammalian as well as non-mammalian species (27–29). It is present in the circulation and the interstitial spaces as a soluble 37 kDa glycoprotein that binds CRF and all urocortins with high affinity, reduces their bioavailability and prevents their binding to CRF receptors (27). CRFBP is highly expressed in a series of tissues, including brain, heart, intestines, lungs and placenta (13, 30, 31). In the CNS, CRFBP is present in all CRF-related pathways and the pituitary.

The CRFBP is partially colocalized with CRF, as well as CRF receptor expression, thereby permitting its role in modulation of CRF neurotransmission (30).

The CRF system, stress, depression and anxiety

The CRF system and the stress response

The CRF system plays a multitude of physiological roles that are apparently related to orchestrating the stress response at several different anatomical levels (see figure 1). In addition to its well documented role as a hypothalamic hypophysiotropic factor that stimulates pituitary ACTH synthesis and secretion and thereby controls the activity of the HPA axis (1), CRF neurons also innervate the locus coeruleus, thus activating the other major stress response axis, the CNS noradrenergic and sympathetic nervous systems (32). The CRF system also locally regulates adrenal steroidogenesis and catecholamine synthesis and release from the adrenal gland (33), again, dually influencing the HPA axis and norepinephrine and epinephrine secretion. Furthermore, effects of CRF in limbic brain regions have been associated with increased fear, alertness, decreased appetite and libido, all functions relevant in the fight or flight response and dysregulated in depression and anxiety disorders (9). These effects appear to be mediated mainly by the CRF_1 receptor (10, 34, 35). The role of the CRF_2 receptor remains more obscure and likely context and brain region dependent. Anxiolytic effects in a brain region-specific and stress level-dependent manner have been described (35–44). Data from CRF_2 receptor transgenic animals suggest an inhibitory role on adrenocortical function (38, 45). Overall, it appears that the CRF_1 receptor is the principal receptor mediating the stress response, whereas the CRF_2 receptor modulates the effects of CRF_1 signal transduction (10, 11, 46).

The CRF system and depression

Laboratory animal studies in which brain intracerebroventricular (icv) or brain region-specific microinjections of CRF has been employed have revealed that CRF produces behavioral responses reminiscent of major depression in humans, including increased anxiety, reduced slow wave sleep, psychomotor alterations, anhedonia, decreased appetite and libido (47, 48). These studies are complemented by results obtained in transgenic animals either lacking or over-expressing CRF system ligands or receptors, as well as from studies using selective CRF antagonists. This large database indicates that these behaviors are mainly mediated by CRF_1 receptor activation and modulated by CRF_2 receptors (10, 49). Conditional CRF_1 receptor knock-out mice and CRF overexpressing mice restricted to forebrain areas have further demonstrated that these anxiety- and depression-related phenotypes are specific to activation of the CRF_1 receptor in limbic forebrain regions and independent of actions on HPA axis activity (50, 51), though the latter endocrine effects of CRF may indeed contribute to the depressive symptoms. While a negative effect of glucocorticoid receptor activation on CRF expression has been described for the hypothalamus, glucorticoids were shown to increase CRF expression in limbic areas, including the amygdale and the lateral septum (52, 53). In support of the critical role on limbic CRF transmission, recently increased CRF expression in the amygdala induced by use of a lentiviral vector was shown to produce most of the behavioral effects that comprise the depressive syndrome, as well as HPA-axis hyperactivity (54).

In humans, increased CSF CRF concentrations have been repeatedly observed in major depression (8, 55, 56) and suicide victims (57). Moreover, treatment with either electroconvulsive therapy (58) or antidepressants (59–61) reduces CSF CRF concentrations. Notably, persistent elevations of CSF CRF concentration in symptomatically improved depressed patients is associated with early relapse of depression (62).

A series of postmortem studies have provided compelling evidence of increased CRF neurotransmission in major depression and suicide. CRF expression (both mRNA and peptide) is increase in the hypothalamus, cortical areas, pontine nuclei and the locus coeruleus (63–66). This is paralleled by a down regulation of CRF_1 but not CRF_2, receptors in cortical areas of suicide victims, all pointing to an overactive CRF/CRF_1 receptor system in depression (67, 68). In addition, CRFBP has been found to be down-regulated in the amygdala of patients with bipolar disorder (69) and urocortin 1 expression is upregulated in the Edinger-Westphal nuclei of suicide victims (70). The relevance of overactive limbic CRF_1 receptor transmission in depression is underlined by the fact that selective CRF_1 receptor antagonists exert antidepressant effects at doses that do not influence baseline or stimulated HPA axis activation (71, 72). It is important to note that, although not all studies with CRF_1 receptor antagonists are positive in major depression, lack of a CRF_1 receptor positron emission tomography (PET) ligand renders choice of dose problematic (73).

The CRF system and early trauma

Overactivity of the CRF/CRF_1 receptor system also has been demonstrated to be one of the long term neurobiological sequelae of early life trauma, a major risk factor for the development of affective disorders (74, 75). In fact, both rodents and non-human primates exposed to adverse experiences in early life exhibit evidence of hyperactivity of the CRF system as adults. Bonnet macaques reared under stressful conditions exhibit higher CSF CRF concentrations as adults than monkeys reared under non-stressful condition (76). Similar neuroendocrine results have been obtained in Rhesus monkeys (77). In rodents early life stress is associated with persistent alterations in the CRF system, including increased CRF concentrations, increased CRF mRNA expression, and altered CRF receptor expression and binding in the hypothalamus and limbic brain regions (78–80). This overactivity of the CNS CRF system is paralleled by a hyper-reactive HPA axis response to stress in these animals. These findings in laboratory animals are consistent with findings in humans where early life trauma is a strong predictor of CSF CRF concentrations in adults (81–83) and is associated with an enhanced stress response to standardized psychosocial stressors, such as the Trier Social Stress Test (TSST). In addition in endocrine challenge tests, including the CRF stimulation test and the combined dexamethasone suppression/CRF stimulation test (84–87), patients with early life trauma exhibit evidence of marked HPA-axis hyperactivity.

The CRF system and anxiety disorders

Because of its anxiogenic effects in laboratory animals, the CRF system has also been implicated in the pathophysiology of anxiety disorders (10, 88, 89). Increased CSF CRF concentrations have been reported in PTSD (90–92), but studies in adults with panic disorder and generalized anxiety disorder have failed to demonstrate such abnormalities (93–95).

There is evidence from a series of animal studies that the CRF system is also highly relevant to alcohol dependence (96), particularly during alcohol withdrawal (97), and depression and anxiety disorders exhibit high rates of comorbidity with alcoholism. Indeed several studies have revealed that acute alcohol withdrawal is associated with marked increases in CSF CRF concentrations (98, 99). It is also worth noting that clinically effective benzodiazepine anxiolytics such as alprazolam reduce CRF-ergic activity (100).

In summary, over 3 decades of research have implicated CRF circuits in the pathophysiology of depression and certain anxiety disorders, particularly PTSD, as well as in mediating the long term impact of early trauma. We refer the reader to a series of review articles (10, 11, 46, 101) that comprehensively review the above topics in considerably more detail. This review focuses on the human genetics of the CRF system and how recent

advances in this area inform the field on the critical role of the CRF system in depression and anxiety.

Human genetic studies

Susceptibility to depressive or anxiety disorders is now well established to be due to the combined effect of genes and the environment, with heritability estimates for these disorders ranging from about 30% to 40% (102–106). The CRF system, being highly responsive to the environment, has been posited to serve as a key interface between environmental stressors and the development of depression. In fact, differences in the function of the CRF system due to genetic variation may lead to differences in an individual's response to stressful events and may regulate an individual's vulnerability to stress-related psychiatric disorders.

Table 1 lists the genes encoding all members of the CRF system with their human chromosomal position.

Linkage studies

Before the sequencing of the human genome (107, 108), studies addressing the genetic basis of affective and anxiety disorders mostly consisted of linkage studies, using large pedigrees of families with multiple affected individuals. Most linkage studies have used short or variable tandem repeats or microsatellite markers which often tag larger regions in the chromosome, so that inference of linkage to a certain candidate gene from these studies usually requires extensive follow-up fine-mapping. Nonetheless, if genetic variation in the CRF system is related to increased vulnerability to depressive and anxiety disorders, one might expect to find regions of linkage for these disorders in the neighborhood of the chromosomal loci encoding these genes.

Although none of the recent meta-analyses of linkage scans for bipolar disorder provide strong support for linkage to regions containing CRF system genes (109, 110), some individual scans in major depressive or anxiety disorders (111, 112) have reported suggestive evidence for linkage in some of these regions. Two meta-analyses of linkage scans in bipolar disorder (109, 110) report linkage to the 8q region distal to the CRH gene. The more recent study actually reports genome-wide significance for this locus when bipolar II patients are included in the analysis (109). However, the signal in these studies (8q23) is distal to the CRH gene, which is located in 8q13.1. Testing of a short tandem repeat marker proximal to the CRH gene did not provide any evidence for linkage with bipolar disorder in a smaller sample of 22 pedigrees (113). In contrast, two studies have reported suggestive linkage (LOD < 2.0) for bipolar disorder in the chromosomal regions 8q13.2 and 8q13.3 including the CRH gene (114, 115), and a recent study has also shown linkage of a region containing 8q13.1 to deficit symptoms in a cohort of families affected with schizophrenia, schizoaffective disorder and bipolar disorder (116). Suggestive evidence for linkage to bipolar disorder has also been reported for 3p21, containing UCN2 (117). Interestingly, strong linkage (LOD 3.88) has been found of the combined phenotype of anxiety disorders and early onset major depression with a large locus on chromosome 3 (3p12.3-q12.3) overlapping with the previous locus (118). UCN3 is located centromeric to a linkage peak for obsessive-compulsive disorders on 10p15.3 (119).

Overall, linkage studies provide some evidence for an involvement of the CRF system in the genetics of depression and anxiety disorders, but the data are not compelling. However, such approaches have significant limitations in the study of the genetics of complex disease because they are usually underpowered to detect the smaller combined effects of multiple genes as well as gene x environment interactions expected in these disorders (120). Lack of

evidence from linkage studies can therefore not be extrapolated to an absence of genetic effects of the CRF system in depression and anxiety.

Results from whole-genome association studies

For whole genome association studies, the markers of choice have been single nucleotide polymorphisms (SNPs) because they are common genetic variations (single base exchanges) and their genotyping is amenable to high-throughput methods (121, 122). Over 6.5 million of these variants have been validated in humans so far (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi). In past years, data from the HapMap project, an international consortium to catalogue human genetic variation (HapMap) (123) and other publicly available resources have become available online (http://www.hapmap.org/, http://genome.perlegen.com/, and others) to facilitate the selection of the most informative SNPs to adequately cover the genetic variation in different populations. Most commercially available SNP assays and whole-genome SNP arrays rely on these resources for SNP selection.

Several whole-genome studies have been published for bipolar disorder (124–128) and two for unipolar depression (129, 130) and none show reproducible association of SNPs within CRF system loci in the samples for which association data is readily available online. In fact, data from the GWAS for unipolar depression of the GAIN-study (130) show that within 200 kb 5’ and 3’ of the CRF-system genes, no association with a p-value smaller than 0.01 is observed, except for one SNP about 40 kb proximal of UCN3 (p-value between 0.01 and 0.001). Results from whole-genome studies for anxiety disorders or gene x environment interactions have not been published yet. It is however noteworthy that the arrays used in these studies contained especially few SNPs within the CRH, UCN and UCN2 loci. The Illumina hap550 v3 array for example with over 550,000 SNPs across the whole genome has only 2 SNPs within 20kb surrounding the CRH gene (http://genome.ucsc.edu/). Overall the genetic coverage of the used SNP arrays is not complete and especially weak for rarer variants. Negative results in these studies may thus also be confounded by a lack of coverage of the genetic variation in some of these loci.

Candidate gene approaches

In contrast to the genome wide approaches described above, candidate gene approaches have the advantage of directly testing association of genetic variations within a gene or locus of interest. This focused approach allows to answer specific hypotheses, including gene x gene as well as gene x environment interaction analyses and to home in on the actual functional variant. Candidate gene approaches have investigated a number of different genetic polymorphisms, including putatively functional variable tandem repeats and insertion-deletion. In the absence of a know functional variant, researchers often rely on a selection of marker variants to tag a genetic site making use of information on the linkage disequilibrium structure of the investigated chromosomal region (131).

In the following section we will describe the genetic structure of the genes encoding the various components of the CRF system, as well as evidence for functional polymorphisms within the genes of interest and results from candidate gene association studies.

The CRH locus

While the official name of the peptide is corticotropin releasing factor (CRF), is it also often referred to as corticotrophin releasing hormone (CRH) and *CRH* is also the official gene symbol for the locus encoding CRF.

Genetic architecture—*CRH* is a short gene, spanning about 2 kb with 2 exons located on the long arm of chromosome 8. It encodes the 196 amino acid long CRF precursor which is then processed to the 41 amino acid long CRF.

In the HapMap project, 12 SNPs have been genotyped within the *CRH* locus (+− 1.5 kb), of which most are either non-polymorphic or rare. Shimmin et al (2007) (132) have comprehensively re-sequenced this locus in over 200 individuals of Mexican-, European- and African American descent and have detected 37 SNPs, one insertion-deletion polymorphism and a microsatellite marker in the same region. At the time of publication, only 23% of these variants were listed in dbSNP, the most comprehensive database cataloguing these polymorphisms. This would indicate that current SNP databases and thus commercially available genotyping assays and arrays, vastly under-represent the genetic variation in this locus. Negative association results may therefore also be related to the fact that none of the potentially relevant markers have been genotyped in previous association studies.

Functional polymorphisms—A set of 4 polymorphisms (SNPs) in the 5' regulatory region of the *CRH* locus up to 4000 bps upstream of the transcription initiation site have been described and appear to be associated with differential transcription of the gene product using reporter gene assays (133–135). One of these polymorphisms, rs5030876 appears to alter binding of activating transcription factor 6 to the putative promoter region (135, 136), thus providing a potential molecular mechanism for differences in gene transcription. Rosmond et al (2001) tested one of these putatively functional promoter variants (rs503875) for its effects on baseline and dexamethasone-suppressed saliva cortisol measures. This T/G SNP is rare in European populations (8–10% carrying the G allele), but more frequent in Africans (close to 40% carrying at least one G allele) (Hapmap and (133)). In a Swedish sample of 284 men, no effects of the SNP on diurnal saliva cortisol variation or dexamethasone suppression (0.5 mg) were observed. However, its rare allele showed a significant interaction with a functional SNP (ThtIII1) in the glucocorticoid receptor (GR). Only the combination of the rarer CRH promoter variant and the allele of the GR polymorphism previously associated with higher diurnal cortisol secretion was also associated with higher salivary cortisol levels in this study (137). The sample size (N = 12) in the combined genotype group that carried the effect was, however, very small. With the exception of another small study that linked the CRH locus to a congenital isolated ACTH deficiency in one family with two affected and two non-affected siblings (138), we are not aware of any other studies relating genetic variation in the CRH locus to endocrine measures. Recently, a functional variant in the promoter of the CRH gene of Rhesus monkeys has been reported that disrupts a glucocorticoid response element (GRE). This functional variant was associated with lower CSF CRH concentrations as well as more exploratory and bold behavior as infants and adolescents (139).

Association studies—A handful of studies have investigated the association of the CRH locus to anxiety-related phenotypes. Smoller et al (2003, 2005) have investigated the phenotype of behavioral inhibition in children of parents with anxiety disorders and found association with a repeat polymorphisms about 20kb 3' of the gene (140) and 3 SNPs located just 3' as well as 5' of CRH (141, 142). Two of these SNPs were also tested for association with panic disorder in a case-control study in adults, but no significant associations were observed (143). Finally, the synonymous coding SNP rs6159 (amino acid position 96 Gly/Gly), associated with behavioral inhibition (142) as well as two SNPs 5' of the CRH genes were neither associated with major depression nor with response to antidepressant treatment in a German sample (144). Wasserman et al (2008) also reported negative results for an association of 2 CRH SNPs with suicide attempt (145).

Even though some interesting functional SNPs regulating CRH expression have been identified, so far no definitive conclusions can be reached on the involvement of genetic variation within the CRH locus in anxiety- and depression-related phenotypes due to the limited sample sizes in these studies and the small number of studies overall. However, putatively important gene x environment interactions have not yet been investigated for this gene.

Urocortins and CRHBP

Genetic architecture and functional variants—The genes encoding the preproproteins for the three urocortins are located on different chromosomes and each has 2 exons. The *CRHBP* locus, encoding CRFBP, is located on chromosome 5 and contains 7 exons. Public databases also indicate two additional exons 3' of the gene and an alternate isoform skipping exon 7 and containing these 2 3' exons. We are currently not aware of any validated functional variants in these genes, but putative functional variants such as non-synonymous coding SNPs and other variants potentially influencing transcription or splicing regulation are predicted *in silico*.

Association studies—To our knowledge, except for negative associations with response to citalopram treatment in major depression (146) no psychiatric genetic association studies have specifically investigated the urocortin gene variants. An initial positive study on the association of CRHBP SNPs with recurrent unipolar depression (147) could not be verified in an enlarged sample of the same cohort and did not replicate in a second independent sample (148). The same group has also reported a lack of association of these variants with bipolar disorder (149). Enoch et al., 2008 report the association of variants within a CRHBP haplotype block with anxiety disorders in Plains Indians and alcohol use disorders in Caucasians. The same haplotypes were also associated with alpha power as measured by resting EEG in these individuals (150). These results may suggest that CRHBP variants could play a role in anxiety and addiction by moderating arousal states. A recent study from our group showed an ethnicity-specific association of a CRHBP SNP with response to citalopram treatment in the STAR*D cohort (146). This study investigated the association of polymorphisms within all CRF system genes discussed in this article and response to antidepressant treatment. The only association withstanding correction for multiple testing was with a SNP located 3' of the CRHBP gene, rs10473984. In the African American and Hispanic subset of STAR*D patients, the T allele of rs10473984 was associated with poorer treatment outcome. This allele was also associated overall higher plasma ACTH levels, suggesting functional effects of this variant on CRHBP levels and thereby CRF signalling at the pituitary level. Finally, the association with poorer treatment outcome was most pronounced in individuals with anxious depression, supporting the role of this system in anxiety-related behaviors (146).

The CRF receptor genes

In the following, we will present data for the two CRF receptor genes, and begin with the gene encoding the CRF_2 receptor (CRHR2), for which fewer studies are available, followed by the more intensely studied CRF_1 receptor gene (CRHR1).

The *CRHR2* locus

Genetic architecture—The CRHR2 locus contains 15 exons with three different promoter sites and 3 different first exons (151) encoding the CRF_{2alpha}, $CRF_{2\ beta}$ and CRF_{2gamma} isoforms (152–154). These three isoforms differ in the initial part of the N-terminal ligand binding domain, but otherwise the receptor structure is the same, starting with the common second exon and they do not appear to differ in their ligand binding profile

(152, 153). The CRF_{2alpha} is the predominant isoform in human brain and its expression is responsive to glucocorticoids (155).

Association studies—For variants within the CRHR2 locus, positive associations have been reported. Tochigi et al (156) reported an association of an intronic SNP in CRHR2 with the personality trait openness on the NEO personality inventory in Japanese individuals. DeLuca et al (157) identified a three microsatellite marker haplotype that was associated with the severity of suicidal behavior, but not suicide attempts *per se* in families with bipolar disorder. Another intronic SNP (not in the same haplotype block as the one reported by Tochigi et al (158) was also associated with response to citalopram treatment in a Spanish cohort. Negative associations of CRHR2 variants with unipolar, bipolar and panic disorder as well as antidepressant treatment response have also been published (143, 146, 159, 160), painting a controversial picture of the association of variants in this gene with depression and anxiety-related phenotypes.

The *CRHR1* locus

Genetic architecture—The *CRHR1* gene spans 51 kb on the long arm of chromosome 17 (see figure 2). Its 13 exons encode the 415 amino acid CRF_1 receptor for which 8 splice variants have been reported (161–163). CRF_{1alpha} is the main isoform, containing all 13 exons. CRF_{1beta} has 29 amino acids inserted into the first intracellular loop (encoded by an additional exon without any homologous sequence in mouse). CRF_{1c} has 40 amino acids deleted from the N-terminus (lacking exon 3) and CRF_{1d} results from a skipping of exon 12 and thus a 14 amino acid deletion from the 7^{th} transmembrane domain. CRF_{1e} lacks exons 3 and 4 - coding for the N-terminus, CRF_{1f} lacks exon 11 and CRF_{1g} lacks exon 10 and part of exons 9 and 11. CRF_{1h} has a cryptic exon inserted between exons 4 and 5. All receptor variants result in either altered/impaired binding affinity or signal transduction. In addition, CRF_{1h}, in which the cryptic exon generates a translation terminator in exon 4, only has a functional CRF binding domain but no transmembrane domains. CRF_{1e} and CRF_{1h} are soluble isoforms and might serve in an analogous function to the CRFBP. In fact in *in vitro* systems, the presence of CRF_{1e} decreases and CRF_{1h} increases CRF_{1alpha} activation (162). CRF receptors isoform CRF_{1alpha} is expressed in both the central nervous system and the periphery. CRF_{1e} through CRF_{1h} have been discovered in the skin and $CRF_{1beta-1h}$ in the myometrium (161, 164, 165). The lack of isoform-specific antibodies has made it difficult to test in which tissues the receptor isoform proteins are actually expressed. So far, no report has described how genetic variation might influence this alternative splicing, but polymorphisms altering the ratio of the different isoforms are likely to have a strong impact on CRF_1 signaling.

An interesting feature of the chromosomal region containing the *CRHR1* locus on chromosome 17q21.31 is the presence of a 900 kb inversion polymorphism (166). This polymorphism is rare in African and Asian populations, but more frequent in populations of European descent. It is associated with a higher number of off-springs in female carriers and thus under positive selection. Due to an increased potential for misalignment during meiosis in heterozygous carriers of this polymorphism, there is an increased risk for deletion and duplications of this locus. Indeed, this inversion polymorphism has been associated with the occurrence of a microdeletion syndrome of this region encompassing the *CRHR1* and microtubule-associated protein tau (*MAPT*) genes (167–171). The clinical features of this syndrome include a marked hypotonia, dysmorphic features and moderate mental retardation. One report describes friendly and amiable behavior in these patients (167). A duplication of this region has been reported in one female patient (172) in whom hirsutism and sleep disturbances were noted besides mental retardation and dysmorphic features. No

reports about the effects of this polymorphism on endocrine regulation or a more in-depth psychiatric characterization have been published to our knowledge.

Functional polymorphisms—No data on polymorphisms that change *CRHR1* gene function have yet been published. *In silico* analysis of potentially functional SNPs indicates that several SNPs within this locus might have an impact on gene function (http://fastsnp.ibms.sinica.edu.tw). It is noteworthy that for rs12936511, a synonymous coding SNP in exon 2 (Pro/Pro at amino acid position 20), the presence of the rare allele (minor allele frequency less than 10%) results in the loss of an exonic splice enhancer element and could lead to altered splicing regulation. Two missense mutations in exons 3 and 4 (rs41280114 and rs16940655) result in conservative amino acid exchanges (amino acid position 60 - Val/Ala and amino acid position 96 -His/Arg, respectively) and likely do not have a major impact on protein function.

Association studies—Several association studies with *CRHR1* polymorphisms have been published and center around anxiety, depression, response to antidepressant drugs and gene x environment interactions.

Keck et al 2008 (143) have reported an association of CRHR1 polymorphisms with panic disorder. In this study, the authors report nominally significant associations of CRHR1 SNPs in two independent German panic disorder samples. The strongest results were, however, the combined effects of rs878886 in *CRHR1* and rs28632197 in *AVPR1B*, encoding the vasopressin 1B receptor. A two-SNP model showed significant associations with panic disorder in both samples separately and the combined sample of 359 cases and 794 controls. Both SNPs are of potential functional relevance because rs878886 is located in the 3' untranslated region of the *CRHR1* gene and rs28632197 leads to an arginine to histidine amino acid exchange at position 364 of *AVPR1B* which is located in the intracellular C-terminal domain of the receptor, likely involved in G-protein coupling. These genetic data support the large body of evidence demonstrating interactions of the vasopressin and CRF systems in anxiety (48). Another family-based study failed to find association of four polymorphisms in the CRHR1 locus with panic disorder. However, fewer CRHR1 and no AVPR1B polymorphisms were tested in this study (173).

Four studies in ethnically different samples have investigated associations of CRHR1 SNPs with response to antidepressant treatment in major depression (146, 158, 174, 175). Licinio et al (174) initially reported an association of a 3 SNP haplotype within *CRHR1* with response to fluoxetine or desipramine in Mexican-Americans and this has been replicated in a Chinese population (175). Interestingly, both studies reported that the effects of this CRHR1 haplotype were most pronounced in patients with anxious depression. Nominally significant associations with response to citalopram of a SNP within the putative CRHR1 promoter region were also observed in the STAR*D sample (146). Papiol et al (158) found that another intronic SNP (rs110402) was not associated with response to citalopram treatment in 159 Spanish patients, but was correlated with an earlier age at onset for major depression. A significant case-control association of CRHR1 SNPs with major depression was only reported by Liu et al (176).

Another set of studies have examined gene x environment interactions of CRHR1 SNPs and stressful events on different psychiatric phenotypes. Wasserman et al (145) have investigated over 500 family trios of male suicide attempters and found that the rarer T-allele of the intronic CRHR1 SNP rs4792887 is overtransmitted in these families. This overtransmission was restricted to individuals with less stressful life events. Because the vast majority of suicide attempters were also depressed, these data suggest an interaction of CRHR1 polymorphisms with stressful life events on suicide attempts in depression. Our

group (177) demonstrated a strong interaction of CRHR1 SNPs with child abuse predicting adult depressive symptoms in an inner city African-American sample of over 400 individuals. We reported protective effects of two 3-SNP CRHR1 haplotypes, whereby individuals with high levels of child abuse and individuals homozygous for the protective haplotypes had significantly less current depressive symptoms than abused individuals carrying other haplotype combinations. In fact the latter groups' scores on the Beck Depression Inventory were similar to the non-abused group, in which the SNPs had no effects on depressive symptom severity. This result was supported by similar findings in a sample of mostly Caucasian women described in the same report. All three SNPs were intronic and one of the two associated haplotypes contained rs479887 for which interaction with life events and suicide attempts had previously been reported (178). These early trauma x CRHR1 interactions on depression have been replicated and extended in independent studies. Polanczyk et al (2009) report a replication of the interaction on past-year and recurrent major depression using the same 3-SNP haplotype in a female cohort retrospectively assessed for childhood maltreatment (179). The interaction was, however, not observed in the prospective Dunedin cohort and the authors argue that this might be due to differences in the assessment of childhood trauma. Furthermore, two reports corroborate the putative functional role of these same variants using data from an endocrine challenge test, the combined dexamethasone/CRF test. Here the protective alleles are associated with a lower cortisol response in this test, either in interaction with early life events (180) or as a main genetic effect (181). The CRHR1 x environment interaction on depression and HPA-axis regulation are further strengthened by the fact that the effects seem to replicate across different ethnicities. As mentioned above, gene x gene interactions are likely important in the genetics of depression and anxiety. Ressler et al (2009) have reported a significant gene x gene x environment interaction, with these CRHR1 polymorphisms interacting with the serotonin transporter linked polymorphic region (5-HTTLPR) and child abuse to predict adult depression (182).

Blomeyer et al (2007) (183) described the interaction of another CRHR1 tagging polymorphism (rs1876831) and negative life events on adolescent alcohol consumption. In the presence of a large number of negative life events, individuals homozygous for the C allele of this SNP reported about twice as much life time heavy drinking as well as drinks per occasion than carriers of the T allele. This difference was not seen in the presence of a low number of life events. These findings have been extended to young adults, where this tagging SNP and stressful life events interact to predict progression of heavy alcohol use in young adulthood (184). The interaction of genetic variation of CRF system genes and stressful life events on alcohol consumption is further supported by data from rhesus macaques. A functional promoter polymorphisms in the CRH gene interacts with prior stress exposure to predict voluntary alcohol consumption in these animals (185).

In humans, further fine-mapping and re-sequencing will be needed to identify the putative functional variants responsible for these gene x environment interactions and whether the SNPs interacting with life events to predict alcoholism, suicide attempts or depression in fact represent distinct functional loci. Overall, these recent human genetic data strongly support a role for the CRF_1 receptor in depression and anxiety, most likely by moderating the effects of early trauma or life events in general.

Conclusions and future directions

Even though the majority of human genetic studies investigating genes encoding the CRF system are limited by small sample sizes or insufficiently dense coverage, the sum of these findings, especially those related to variants within *CRHR1*, support the concatenation of preclinical and clinical data implicating the CRF system in the pathophysiology of depression and anxiety. Together with the serotonergic and neurotrophic system (111), the

CRF system thus represents an example of how human genetic studies can contribute to specific pathophysiologic hypotheses in depression and anxiety.

It has to be noted though that the presented genetic association studies, including data in GWAS are only powered to detect associations with common genetic variants. Rare, functional variants in these genes may, however, also have a relevant impact for these disorders. Next generation sequencing methods will allow to catalogue and compare all sequence variation in these loci and to identify, rare but functionally relevant polymorphisms, including copy number variations.

In addition to expanding genetic association studies of this system in larger samples with sufficiently dense genetic markers as well as re-sequencing, the next generation of CRF-related genetic studies should focus on system genetics, analyzing the impact of the whole system including ligands, receptors as well as other genes upstream and downstream of this signaling pathway. There is already evidence for the association of genetic variants in genes intricately tied to the CRF system with depression and anxiety, such as the glucocorticoid receptor gene (186) and the gene encoding the glucocorticoid receptor co-chaperone FKBP5 (187) for example.

Because of their critical role in stress regulation, it is unlikely that detrimental variants in single genes within the CRF system will be common in the population. More likely, the sum of genetic variations with discrete functional effects in a number of genes within this system will impact stress axis regulation and susceptibility to stress-related psychiatric disorders. Similar to the number of studies now investigating the 5-HTTLPR and the BDNF Val66Met polymorphism (111), gene x gene x environment interactions of *CRHR1* variants with this polymorphism have been reported (182). It will now be important to identify and characterize the putative functional variants in *CRHR1* and their interaction with the other genes. From the above presented data it appears that loci within this gene moderate the impact of life stress on the development of psychiatric disorders. Stratifying individuals by the protective vs. susceptibility variants of these loci in neuroimaging and endocrine studies for example might yield a better understanding of the long term impact of trauma on neuronal circuitry and hormone systems, and more specifically which compensatory mechanisms are involved in the development of stress-related psychiatric disorders. The characterization of these variants may also be a tool to dissect possible epigenetic impacts of trauma on the CRF system. As described above, early life adversity has been shown to result in persistent effects on CRF neurotransmission and HPA axis regulation (in fact in an animal model of early prenatal stress, methylation of the *CRH* gene locus correlates with longterm changes in gene expression (188)). Genetic variations within the *CRHR1* locus appear to moderate this impact, possibly by enhancing or preventing epigenetic changes within this gene. In fact, early adversity and thus stress have been shown to lead to epigenetic changes, including difference in the methylation status of HPA-axis genes (189). One possibility would be that these putative variants could alter the epigenetic impact of stress, e.g. the methylation of CpG islands by changing a CG sequence. Indeed, a single SNP abolishing one CpG can be associated with global differences in methylation of the whole surrounding CpG island (190).

Despite some controversial results, the initial series of human genetics studies support the importance of this system in mediating stress-related psychopathology. In depth characterization of these variants could represent an important tool in elucidating the long term consequences of early life adverse experience.

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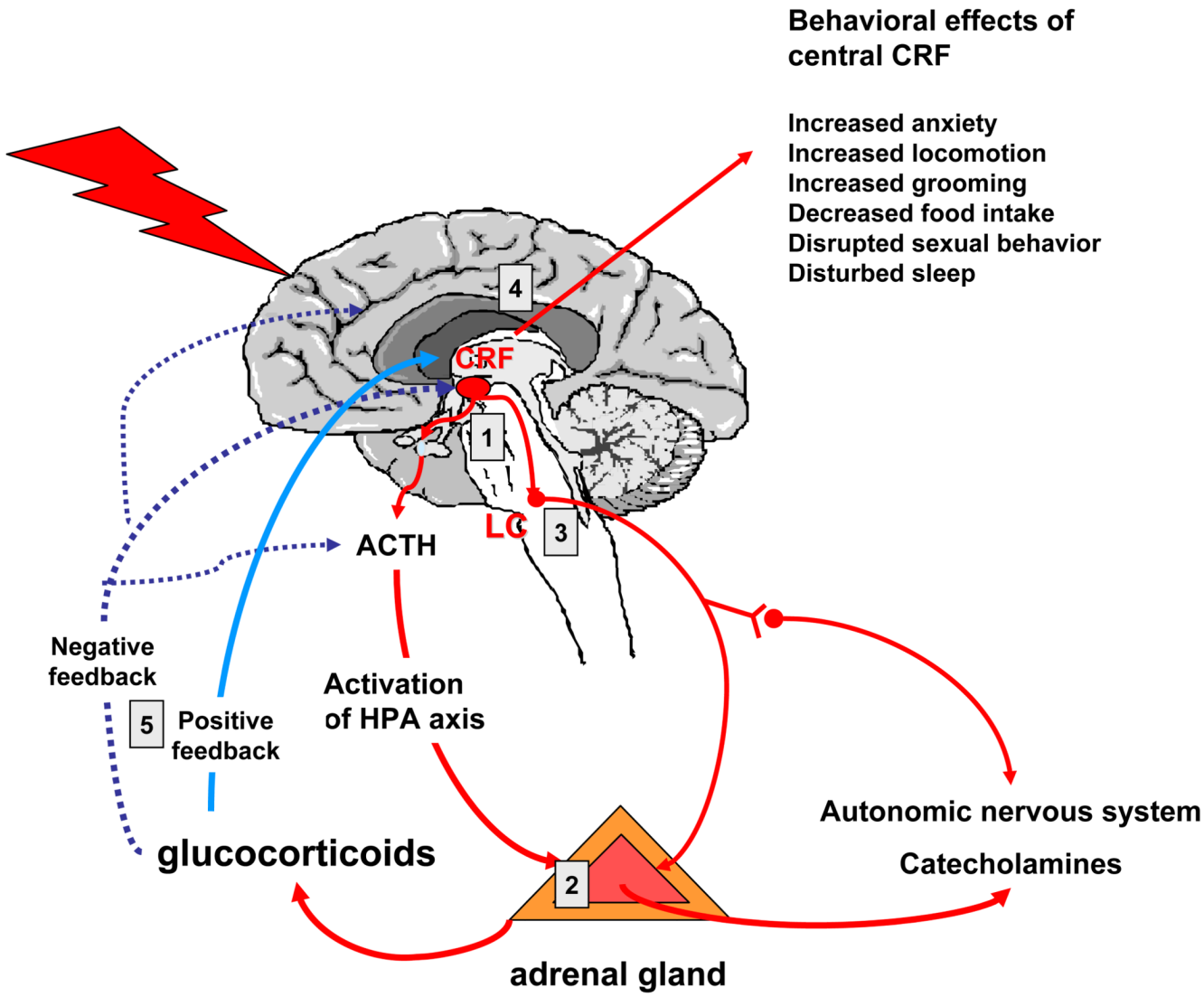

Figure 1. The actions of CRF at different levels of the stress response

1- CRF nerve terminal in the median eminence release CRF into the hypothalamo-hypophyseal portal system and stimulate ACTH release from the anterior pituitary.
2- CRF directly stimulates cortisol and cathecholamine synthesis from the adrenal gland
3- CRF stimulates noradrenergic neurons in the locus coeruleus
4- CRF mediates a series of behaviors through actions on cortical and limbic brain regions.
5- CRF transcription is negatively regulated by glucocorticoids in the hypothalamus, but positive regulation has been reported in limbic brain regions.

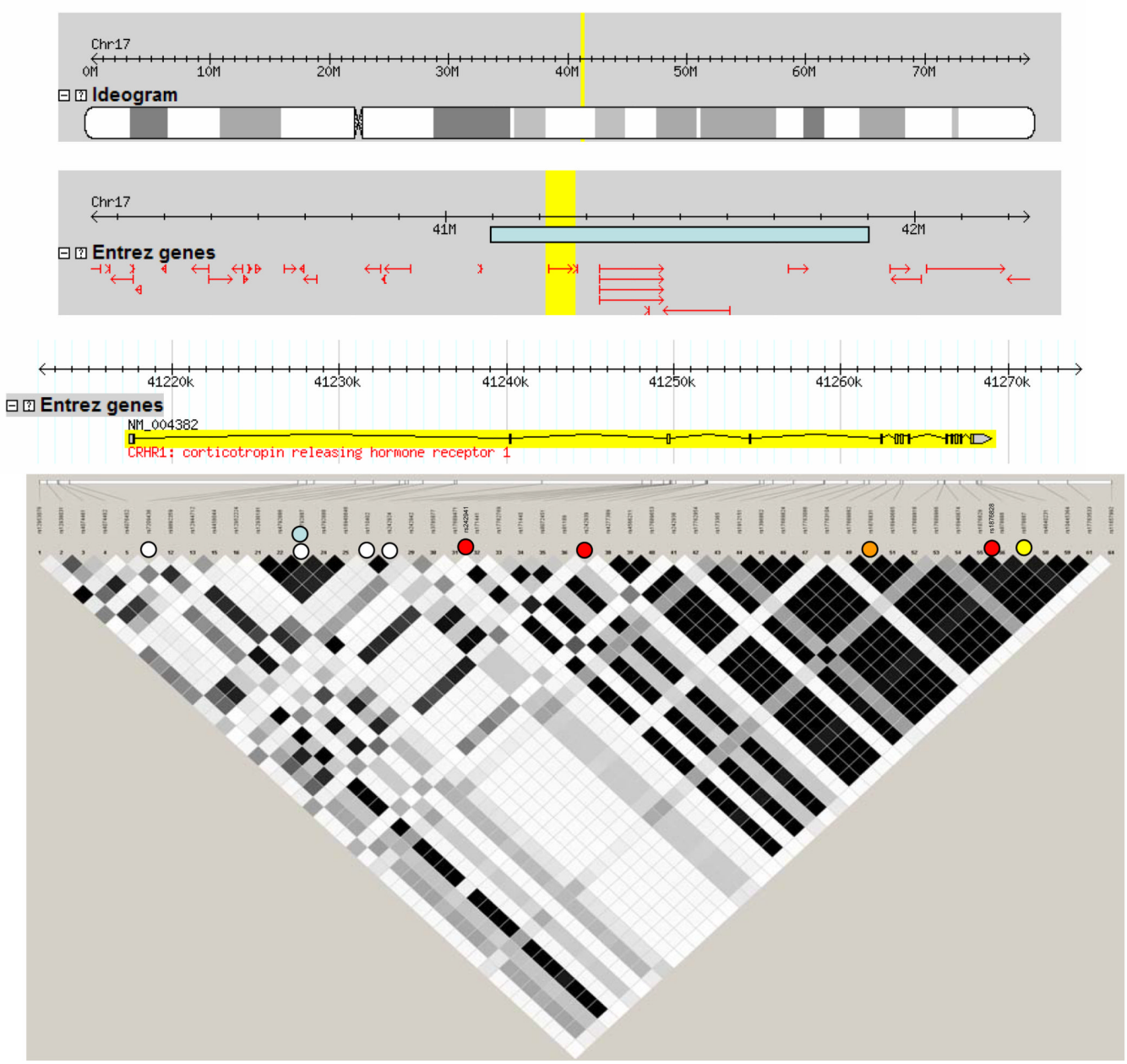

Figure 2. The genetic organization of the CRHR1 gene encoding CRF_1

All representations have been derived from www.hapmap.org. The top panel shows the position of the CRHR1 gene on chromosome 17 (yellow). The second panel shows the 2 mega bases (MB) surrounding the CRHR1 gene (highlighted in yellow) with the position of the neighboring genes (red) and the position of the inversion polymorphism (light blue). The next panel shows the position of the exons and introns of the CRHR1 gene, with the 5' end of the gene on the left. Below are the SNPs available for this gene in the HapMap project with the linkage disequilibrium (LD) structure for the Caucasian CEU population, as most studies on the genetic of CRF system genes have been performed in Caucasians. LD is represented with the r-squared measure with black squares connecting SNPs in complete LD (r-squared = 1) and white squares connecting SNPs with no LD (s-squared = 0). Shades of grey represent r-squared values between 0 and 1.

White dots denote the position of SNPs interacting with child abuse to predict adult depressive symptoms (177, 179) or cortisol response in the combined dexamethasone/CRF test (180, 181). The light blue dot denotes the SNPs interacting with life events to predict suicide attempts (145). Red dots denote SNPs predicting response to antidepressants in patients with anxious depression (174, 175). The orange dot denotes the SNP interacting with life stress to predict heavy drinking (183, 184) and the yellow dot denotes the SNP associated with panic disorders together with a SNP in AVPR1B (143).

Table 1

Genomic position of the genes encoding the ligands and receptors of the CRF system.

Gene: Symbol	Gene Product Name	REFSEQ ID ENTREZ Gene ID	position on chromosome	Number of exons	gene size (bases)
CRH	corticotropin releasing factor (CRF) or hormone (CRH)	NM_000756, 1392	8q13.1	2	2,080
CRHBP	corticotropin releasing factor/hormone binding protein	NM_001882, 1393	5q13.3	7	16,619
CRHR1	corticotropin releasing factor/hormone receptor 1 (CRF1)	NM_004382, 1394	17q21.31	13	51,525
CRHR2	corticotropin releasing factor/hormone receptor 2 (CRF2)	NM_001883, 1395	7p15.1	12	29,278
UCN	urocortin	NM_003353, 7349	2p23.3	2	866
UCN2	urocortin 2/ stresscopin-related peptide	NM_033199, 90226	3p21.3	2	2,049
UCN3	urocortin 3/ stresscopin	NM_053049, 114131	10p15.1	2	9,194