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The genetic background to PTSD

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Abstract

Objective
Although extensive research has already been done on the genetic bases of psychiatric disorders, little is known about polygenetic influences in posttraumatic stress disorder (PTSD).

Methods
This article reviews molecular genetic studies relating to PTSD that were found in a literature search in Medline, Embase and Web of Science.

Results
Association studies have investigated 8 major genotypes in connection with PTSD. They have tested hypotheses involving key candidate genes in the serotonin (5-HTT), dopamine (DRD2, DAT), glucocorticoid (GR), gamma aminobutyric (GABRB), apolipoprotein (APOE2), brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) systems. The studies have produced inconsistent results, many of which may be attributable to methodological shortcomings and insufficient statistical power.

Conclusions
The complex etiology of PTSD, for which experiencing a traumatic event forms a necessary condition, makes it difficult to identify specific genes that substantially contribute to the disorder. Gene-finding strategies are difficult to apply. Interactions between different genes and between them and the environment probably make certain people vulnerable to developing PTSD. Gene-environmental studies are needed that focus more narrowly on specific, distinct endophenotypes and on influences from environmental factors.

1. Introduction

Posttraumatic stress disorder (PTSD) is a condition widely prevalent in people who have had one or more traumatic experiences. It is characterized mainly by symptoms of re-experiencing, avoidance and hyperarousal. PTSD is one of the few mental disorders in DSM-IV for which criteria include an etiological agent, in this case experiencing a traumatic event. Not everyone that has undergone a traumatic event develops PTSD (Costello et al., 2002; Monroe et al., 1991). Although lifetime prevalence of exposure to traumatic events is thought to be between 40% and 90% in the general population, the overall lifetime prevalence of PTSD is estimated at 7–12% (Breslau, 2001; Kessler et al., 1995; Olff and de Vries, 2005). This means that exposure to a traumatic event does not entirely explain the etiology of the disorder. The suspicion is that individuals with an existing genetic vulnerability have a higher risk of developing PTSD once they experience a trauma.

Two sources of evidence for a genetic component have been transgenerational research and epidemiologic studies on twins. Transgenerational studies have reported that PTSD is more likely to occur in certain families (Koenen et al., 2003; Yehuda et al., 1998, 2001, 2002). Twin studies have found that monozygotic twins are more concordant for developing PTSD after trauma exposure than dizygotic twins (Skre et al., 1993; Stein et al., 2002; True et al., 1993; Xian et al., 2000). Other research, including twin studies, has shown that PTSD may also be related to structural brain abnormalities. Smaller hippocampal volume and abnormalities in the septum pellucidum have both been linked to a higher susceptibility to PTSD (Gilbertson et al., 2002; Gross and Hen, 2004; May et al., 2004; Talbot, 2004). Possible confounding factors in twin and adoption studies are unique, non-shared environmental factors, such as personal life events and other psychosocial stress factors. Early prenatal and perinatal environmental factors may also play a role (Figee et al., 2004), as was also suggested in a recent study by Yehuda et al. (2005). The latter authors studied babies of mothers who were pregnant during the 9/11 World Trade Center attack and who later developed PTSD. Like their mothers, the babies were found to have low salivary cortisol. This suggests that stress-induced elevations of glucocorticosteroids during pregnancy may affect foetal brain development and thereby induce permanent changes in the glucocorticoid (GR) programming of the offspring. Hence, although evidence exists for a genetic vulnerability to PTSD, the vulnerability develops in interaction with environmental factors such as stressful life events or a mother's prepartum or even pre-fertilization hormonal status (de Kloet et al., 2005). The fact that monozygotic twins share a more similar intrauterine and postnatal environment than dizygotic twins makes it very difficult to estimate heritability from twin studies. Therefore, the genetic component in twin studies will be overestimated to an unknown degree (Joseph, 2002; Kamin and Goldberger, 2002; Robert, 2000). Although the discordance between monozygotic and dizygotic twin pairs is still seen by many as a 'gold standard', evidence for a genetic contribution is more likely to come from (molecular) association studies such as those summarized in this present review.

1.1. Endophenotypes

The etiology of PTSD is complex and multifactorial (Olff and de Vries, 2005). Like most other mental disorders, the heritable part of PTSD can be viewed as polygenetic. This means that different genes are assumed either to interact or to play an additional role in the disorder's ultimate onset.

The complexity of psychiatric disorders like PTSD makes it difficult to find specific genes that substantially contribute to the disorder. For this reason, genetic research often has its focus on endophenotypes – more elementary underlying traits or facets of clinical phenomena – whereby the number of genes required to produce variations in these traits may be fewer than those involved in producing a psychiatric diagnostic entity. Such basic traits may consist of neurophysiologic, biochemical, endocrinological, neuroanatomical, cognitive and neuropsychological measures (Gottesman and Gould, 2003). Radant et al. (2001) have argued that genes associated with certain endophenotypes may be implicated in PTSD development. These endophenotypes for PTSD include the deregulation of the hypothalamic pituitary adrenal (HPA) axis, the physiology of hyperarousal and the exaggerated acoustic startle response. Other important response measures include autonomic reactivity, such as heart rate variability, and psychological variables like memory problems (Segman and Shalev, 2003).

As PTSD is a complex disorder consisting of different types of symptom clusters, genetic research may be simplified by investigating specific symptoms or clusters. This, too, would improve the chances of identifying distinct contributions by specific genes. Most studies so far have supported the hypothesis that certain facets of PTSD have implications for prognosis and treatment strategy or for comorbidity (Perkonigg et al., 2005). Different symptom clusters have also been associated with different physiological parameters. The specific dimension of emotional numbing, for example, has been linked to low cortisol levels (Asmundson et al., 2004; Hawk et al., 2000; Mason et al., 2001), whereas hyperarousal symptoms show more connections to sympathetic nervous system activation (Olff et al., 2005b; Schell et al., 2004; Woods and Wineman, 2004). Several studies have shown that trauma victims with peritraumatic dissociation, and to a lesser extent with peritraumatic emotional responses, may be at greater risk for developing PTSD (Birmes et al., 2001, 2003; Fullerton et al., 2000, 2001; Koopman et al., 1994; Ozer et al., 2003).

The endophenotypic characteristics or symptom clusters referred to above could contribute to PTSD susceptibility, and each of them separately is likely to better reflect the underlying genetic constitution of a person with PTSD. Hence, genetic research ought to focus primarily on specific, distinct endophenotypes of PTSD (Smoller and Tsuang, 1998).

1.2. Molecular genetic research on PTSD

The major types of molecular genetic research are linkage studies, association studies and microarray analyses (Figee et al., 2004; Vonk et al., 1998; van Waarde et al., 2002). Linkage studies investigate at random the entire genomes of individuals, using DNA markers to locate chromosomal areas that are continuously passed on together with a particular disorder. Natural genetic variants are sought and their prevalence is assessed in subjects with a disorder like PTSD against healthy control groups. No prior hypothesis is required as to which gene could be causing the disorder. Linkage analyses are mainly successful in investigating monogenetic disorders, and they are often carried out on large families. This model is difficult to apply in PTSD research, because the essential condition for PTSD onset – exposure to trauma – is a variable that cannot be influenced. Nonetheless, experimental animal studies in laboratories have already made significant contributions to research on the relative roles that genetic and environmental factors play in stress responses. Most of the hypotheses on candidate genes implicated in PTSD derive from such animal studies (Gass et al., 2001). Candidate genes identified so far are the serotonin transporter gene (5-HTT), the dopamine

receptor gene (DRD2) and the dopamine transporter gene (DAT), the glucocorticoid receptor gene (GR), the GABA (A) receptor gene, the apolipoprotein E gene (APOE), the brainderived neurotrophic factor gene (BDNF) and the neuropeptide Y gene (NPY).

A next step in molecular genetic research is to investigate in association studies whether a connection exists between a genetic variant (polymorphism) and an endophenotype in a disorder. Studies like these can uncover smaller genetic effects. They, therefore, seem the method best suited for molecular genetic studies on complex disorders like PTSD (Sullivan et al., 2001). The presence of genetic polymorphisms in candidate genes in a group of individuals with a particular disorder is compared to a group of healthy individuals. Single nucleotide polymorphisms (SNPs) are small changes in DNA that have no visible effects but which do affect vulnerability. A distinction is made between functional and non-functional SNPs. Functional SNPs have effects on gene expression and/or protein function. No such effects are known from non-functional SNPs, but they can still be of use for association studies. Although they probably do not cause the disorder, they lie on a chromosome near the 'pathogenic' mutation.

Candidate genes investigated in association studies should preferably have already been identified in linkage analyses or should be part of a well-defined prior hypothesis. Because linkage analysis is difficult to perform in PTSD, candidate genes not identified in prior human linkage analyses are often selected for association studies on the basis of hypotheses about their putative functional relationship with PTSD (or after identification in animal studies). Should an association then be found, it merely shows that the polymorphism in question is somehow connected to a particular endophenotype of PTSD, but it does not demonstrate a causal relationship (van Rossum et al., 2005). Other yet undiscovered factors, such as a link between that polymorphism and another polymorphism in the same gene or a nearby gene, could explain the association. The genetic research conducted at present in relation to PTSD involves this type of association studies on candidate genes preselected without prior human linkage analysis (Figee et al., 2004; Vonk et al., 1998; van Waarde et al., 2002).

Another drawback of association studies specific to PTSD is that it remains unclear how many individuals in the 'healthy' group might themselves have a vulnerability to developing PTSD, but have not done so yet because they have never experienced a traumatic event, or for other reasons. This increases the danger of false-negative results in this type of research (Segman and Shalev, 2003). That is why it is important to compare the PTSD group not only with healthy controls but also with trauma-exposed controls without PTSD.

A relatively new method of genetic research is to study expression of genes in specific tissues by using microarray analysis (by using an RNA or cDNA chip). This was recently done for the first time in PTSD. Segman et al. (2005) used oligonucleotide microarrays to measure peripheral blood mononuclear cell (PBMC) gene expression in trauma survivors directly after they presented to a casualty department and four months later. The results showed an overall reduction in expression of transcription activators of PBMC in psychologically distressed victims. This demonstrates the possibility of stress-induced reduction of gene expression.

Despite the tremendous advances in knowledge in the past 5 years in the neurobiology and genetics of other psychiatric disorders like depression, genetic research on PTSD is still rare. Identifying genes that mediate susceptibility to PTSD would greatly improve our un-

derstanding of the disorder and could further uncover the molecular basis of those genes (Segman and Shalev, 2003). This article explores the current molecular genetic findings of association studies on PTSD by reviewing the major candidate genes investigated so far in relation to PTSD, followed briefly by a discussion about the key research results for each of the neurotransmission systems involved and suggests directions for future research.

2. Methods

We carried out searches in Medline, Embase and Web of Science databases (1966–October 2006) using the following medical subject heading terms: "genetics AND PTSD OR serotonin OR dopamine OR glucocorticoid OR GABA OR apolipoprotein OR BDNF OR NPY".

We have chosen those terms because candidate genes from these neurotransmitter systems had already been identified previously in animal research. We also entered the names of the candidate genes themselves as search terms. Additional articles were found by consulting reference lists and publications cited as related articles. We confined ourselves to association studies in humans and to articles published in English. No strict inclusion or exclusion criteria were specified, because our objective was to compile a broad review of publications on the genetics of PTSD.

Placing particular emphasis on literature reviews over the period 1966–2006, we obtained the following results:

-PTSD and genetics: 105 Medline hits 1949–2006, 13 Embase hits 1980–2006 (3 new), 9 Web of Science hits 1988–2006 (3 new).

-PTSD and genetics and review: 27 Medline hits 1949–2006, 7 Embase hits 1980–2006 (1 new), 3 Web of Science hits 1988–2006 (1 new).

-Serotonin and PTSD and genetics (without animal studies): 7 Medline hits 1949–2006 (3 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).

-Dopamine and PTSD and genetics (without animal studies): 8 Medline hits 1949–2006 (3 reviews), 0 Embase hits 1980–2006 (0 new), 1 Web of Science hit 1988–2006 (1 new).

-GR and PTSD and genetics (without animal studies): 1 Medline hits 1949–2006 (0 reviews), 3 Embase 1980–2006 (2 new), 1 Web of Science hit 1988–2006 (0 new).

-GABA and PTSD and genetics (without animal studies): 2 Medline hits 1949–2006 (0 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).

-APOE and PTSD and genetics (without animal studies): 1 Medline hit 1949–2006 (0 reviews), 1 Embase hit 1980–2006 (0 new), 0 Web of Science hit 1988–2006 (0 new).

-BDNF and PTSD and genetics (without animal studies): 1 Medline hit 1949–2006 (0 reviews), 2 Embase hits 1980–2006 (1 new), 0 Web of Science hits 1988–2006 (0 new).

-NPY and PTSD and genetics: 1 Medline hit 1949–2006 (0 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).

3. Results

3.1. Genes involved in the serotonin system

The literature search produced 7 hits in Medline, 3 of them reviews; Embase and Web of Science yielded 0 hits. We thus found a total of 7 articles, including 3 reviews. As two of the articles mainly involved the dopamine receptor (Comings et al., 1996; Lawford et al., 2003), we discuss them in that section below. One study is a receptor binding study instead of an association study (Bonne et al., 2005).

Table 1 summarizes the articles relating to candidate genes for PTSD. Reviews on genes involved in the serotonin system include Gross and Hen (2004); Stahl (2005) (state-of-the-art); Talbot (2004).

Evidence exists that the genes regulating the serotonin system play a role in susceptibility to PTSD or depression in response to various types of stressors over the life course (Stahl, 2005). The ascending serotonin pathway, originating in the dorsal raphe nucleus and innervating the amygdala and frontal cortex, facilitates conditioned fear. The dorsal raphe-nucleus-periventricular pathway inhibits inborn fight-or-flight reactions to impending danger. And finally the pathway connecting the median raphe nucleus to the dorsal hippocampus promotes resistance to chronic unavoidable stress (Graeff et al., 1996). Serotonin may have an inhibitory effect on norepinephrine (NE) neurons at the level of the locus ceruleus. In addition, serotonin terminals from the dorsal raphe and NE terminals from the locus ceruleus converge on the amygdala to mediate fear responses. It is unclear exactly how the serotonin circuits are disrupted in PTSD (Davis et al., 1997). The serotonin transporter gene has been identified in relation to the serotonin system and PTSD. A great deal of research has already been done on genes involved in the serotonin system, and which thereby affect serotonergic transmission. The serotonin transporter, also called 5-HTT (or SERT), is now the best studied biological substrate of depression (Kalia, 2005). Abnormal serotonergic activity may mediate susceptibility to affective disorders, and a relationship has also been found to stress reactions (Caspi et al., 2003; Mendlewicz et al., 2004). Initially, considerable experimental research was done on animals which supported the hypothesis of a 'gene-by-environment' interaction. It showed that variation in the 5HTT transporter gene was linked to altered serotonergic function following stressful early life events (Bennett et al., 2002; Murphy et al., 2001). For example, serotonin played a part in HPA-axis alterations in animals exposed to early life stress (Lauder, 1983).

Although the role of serotonergic transmission in the pathophysiology of PTSD is still unclear, serotonin is thought to be involved in the onset of the PTSD symptoms relating to mood, arousal and sleep. Allelic variation in human 5-HTT expression is caused by functional gene promoter polymorphisms with 2 predominant variant alleles, which are likewise associated with various anxiety responses to stressful events (Glatz et al., 2003). In vitro studies have shown the basal 5-HTT activity in carriers of the 5-HTTPR long (L) allele to be twice as high as in carriers of the short (S) allele, indicating that 5-HTT gene transcription may be modulated by these variants.

An association study in individuals with PTSD versus a healthy control group has found only marginal differences in genotypes between the 2 groups, except that the frequency of the SS genotype was significantly higher in the PTSD group. A limitation was that the controls had never experienced a trauma, so it was unknown whether some might have de-

veloped PTSD on trauma exposure (Lee et al., 2005). The sample was also small and might have been subject to population bias.

A number of studies have found associations between PTSD and depression in the year following trauma (in about 45% of PTSD subjects) (Shalev et al., 1998). Because far more genetic research exists on the impact of a stressful event on the etiology of depression, we will also mention those results here. They are inconsistent. Several studies, including a recent one in a large group of twins, found that individuals with two short alleles (SS or SL) of the 5-HTT polymorphism were more sensitive to depressogenic effects of stressful life events than those with one or two long alleles (LL) (Caspi et al., 2003; Hamet and Tremblay, 2005; Kendler et al., 2005). In contrast to those significant findings, Gillespie et al. (2005) found no associations, nor did a meta-analysis of 14 studies by Lasky-Su et al. (2005) find any clear relationship between the serotonin transporter gene and the onset of affective disorders after stress, except for a weak association between bipolar disorder and the 44-bp polymorphism of the 5-HTT genotype.

A review of neuroimaging studies found that people with SS or SL alleles showed greater amygdala activation in response to fearful stimuli than those with the LL allele (Hariri et al., 2002; Wurtman, 2005). In another study, neurochemical processes were associated with genetic variations in serotonergic neurotransmission relevant to anxiety. Significantly lower hippocampal N-acetylaspartate (NAA) concentrations were found in SL carriers than in individuals with a LL genotype, and NAA concentrations correlated negatively with anxiety traits on the Spielberger State-Trait Anxiety Inventory (STAI; Gallinat et al., 2005). Other studies have looked further into links between the 5-HTT gene and temperament. However, as Schinka et al. (2004) noted in a meta-analysis of 26 studies since March 2003, studies of the association between polymorphisms of the serotonin transporter gene (5-HTT) and trait anxiety have produced inconsistent results, raising questions about the strength of the relationship and the methodological conditions under which the relationship holds. They concluded that no link had been demonstrated between the 5-HTTLPR and trait anxiety.

To conclude, the serotonin transporter gene possibly plays a role in the degree of response to stressful events. Evidence has been found that variation in the 5-HTT gene moderates the sensitivity of individuals to the depressogenic effects of stressful life events. Little research has been done yet on 5-HTT and PTSD, but greater amygdala activation in response to fearful stimuli and lower hippocampal NAA concentrations were more likely in subjects who were homozygote SS or heterozygote SL than in those who were homozygote for LL.

3.2. Genes involved in the dopamine system

The literature search yielded 8 hits in Medline, 3 of which were reviews; Embase yielded 0 hits and Web of Science 1 additional new hit (a state-of-the-art article). The total came to 9 articles, including 3 reviews and 1 state-of-the-art article.

Reviews on genes involved in the dopamine system include Comings and Blum (2000), Gordon and Barnes (2003) (state-of-the-art), Noble (2000), Segman and Shalev (2003). See Table 1.

In animal studies, dopaminergic innervation of the basolateral nucleus of the amygdala, the medial prefrontal cortex and other limbic regions is highly responsive to stress and may be altered by stress (Goldstein et al., 1996; Inglis and Moghaddam, 1999). Also the enhancement of the acoustic startle response, which can be a symptom of PTSD, has been

related to the dopamine D1 receptor agonists in rats (Meloni and Davis, 1999). Genetically determined alterations in dopamine release and dopamine receptor expression in mice have been implicated in behavioural abnormalities induced by chronic stress (Puglisi-Allegra and Cabib, 1997). This finding was interpreted as suggesting that stress-induced alterations of central dopaminergic neurotransmission may be genotype-dependent and expressed in behaviour. Human studies showed that there was a relationship between urinary excretion of dopamine and plasma dopamine and (the severity of) PTSD symptoms (Hamner and Diamond, 1993; Yehuda et al., 1992). Together this has been seen as a relevant role for dopamine in the pathogenesis of PTSD.

There are 2 important PTSD candidate genes that directly affect the dopamine system: the dopamine receptor gene (DRD2) and the dopamine transporter gene (DAT). The D2 dopamine receptor (DRD2) minor (A1) allele (DRD2 A1) has already been linked to ADHD, Tourette's syndrome, conduct disorder and substance abuse (Noble, 2000).

This prompted suppositions that this gene may be involved in stress response in humans. The first study on the role of DRD2 in PTSD was published by Comings et al. (1996). Although this study reported a significant association between the presence of the DRD2 A1 allele and PTSD, this result was not confirmed in a later study (Gelernter et al., 1999). That could have been due to selection bias (from recruitment via a substance abuse treatment centre), comorbidity (with substance abuse), the small size of the earlier study and the difference in control groups. Comings compared the PTSD group with a trauma-exposed group without PTSD, while Gerlenter compared the PTSD group with a healthy control group. Alcohol continued to play a prominent role in the research that followed on DRD2 and PTSD. Although an association was found between the DRD2 A1 allele and PTSD, it was seen only in individuals that drank harmful daily amounts of alcohol (Young et al., 2002). The DRD2 A1 allele has further been linked to improved social functioning in individuals with PTSD being treated with paroxetine. Since only one study was involved, the clinical relevance is yet unclear (Lawford et al., 2003).

There has been only one study on the DAT gene in relation to PTSD. In a large group of twin pairs who had been in the war in Vietnam, it sought to establish a link between PTSD and the DAT SLC6A3 3'-variable number tandem repeat (VNTR), using a trauma-exposed control group without PTSD. Evidence was found that genetically determined changes had occurred in dopaminergic reactivity in the PTSD subjects (Segman et al., 2002). However, another study (not involving PTSD) on the effects of drugs on dopamine found that the VNTR polymorphism was not associated with any increase or decrease in expression of the DAT (Martinez et al., 2001).

To summarize, inconsistent results have been reported on the relationship between the dopamine receptor gene and PTSD. What does become clear from various studies is a connection between the presence of the DRD2 A1 allele and alcohol abuse, which could bias findings on the relationship of the former to PTSD. To gain more clarity about any link between PTSD and the DRD2 gene, it is therefore important to clearly document alcohol consumption in future association studies. Insufficient research has been done on the role of genotypes of the DAT gene in relation to PTSD; the functional role of the DAT SLC6A3 3'-VNTR is still unclear.

3.3. Genes involved in the GR system

The literature search produced 1 hit in Medline, 3 in Embase (2 new) and 1 in Web of Science (0 new), a total of 3 articles, including 2 reviews. Reviews on genes involved in the GR system include Grossman et al. (2002) and Radant et al. (2001). See Table 1.

GRs produced by the stress-responsive HPA axis, are well recognized for their regulatory role in peripheral metabolism and various brain functions. Increased GRs exposure in humans, including exposure to the endogenous GR cortisol, is associated with stress, and decreases memory and learning function. There is evidence that dysregulations in cortisol activity occur in PTSD, but it remains unclear whether these are a cause or a consequence of PTSD (Abelson and Curtis, 1996; Bjorntorp, 2002; Charney, 2004; Holsboer and Barden, 1996; Keck et al., 2004; Olff et al., 2005b; Olff and de Vries, 2005; Schreiber et al., 1996; Zobel et al., 1999). Hippocampal GR receptors play an important role in GR negative feedback. Abnormalities in negative feedback are found in depression and in PTSD and may be involved in the pathophysiology of these disorders (Liberzon et al., 1999). Brain imaging studies have demonstrated a strong relationship between PTSD and a reduction in the volume of the hippocampus. However, the mechanisms that cause such atrophy are not well understood. Recently, Zhang et al. (2006a) proposed the hypothesis that stress-induced changes of mitochondrial membrane potential are regulated by nongenomic and genomic actions of cortisol in hippocampal neurons (Zhang et al., 2006a).

An earlier animal study found that 2 hippocampal GR receptors played a key role in regulating the HPA axis and the cortisol level (Liberzon et al., 1999). These were the mineralocorticoid receptor (MR or type 1) encoded on chromosome 5 and the glucocorticoid receptor (GR or type 2) encoded on chromosome 4 (de Kloet et al., 1998; Veldhuis et al., 1982). Because MRs have high affinity to cortisol (10 times greater than the GRs), it is mainly MRs that are occupied in the absence of stress (basal corticosteroid levels). When stress arrives, cortisol levels increase sharply and the GRs are also occupied. Aldosterone plays a part here; by MRs it can affect the expression of GR mRNA (Chao et al., 1998; de Kloet et al., 1998). The balance between the effects of these 2 corticosteroid receptor types is critical to the stress response and behavioural adaptation thereafter (de Kloet et al., 1998). Both MR- and GR-mediated effects of information processing facilitate behavioural adaptation and thereby stimulate higher brain centers to exert inhibitory control on HPA-axis activity (Gass et al., 2001). Individual differences in the number, the affinity and the efficiency of the signaling cascades activated by these receptors have direct effects on cortisol levels and biological activity (Gass et al., 2001; de Kloet and Derijk, 2004). Corticosteroid receptors function as transcription factors. Most evidence in relevant animal models points towards an involvement of altered GR rather than MR function (Liberzon et al., 1999; Kellner et al., 2002; Yehuda et al., 2004). Much research has already been done on the GR gene and on sensitivity to corticosteroids, and most studies have reported positive associations (Panarelli et al., 1998; Rosmond, 2002). Both the N363S and the BclI polymorphisms of this gene have been linked to GR hypersensitivity (Buemann et al., 1997; Di Blasio et al., 2003; Dobson et al., 2001; Huizenga et al., 1998; van Rossum et al., 2003). Bachmann et al. (2005) went on to test whether variations in the GR gene showed links to PTSD. In a group of PTSD and trauma exposed non-PTSD Vietnam War veterans, they screened for polymorphisms and assessed GR sensitivity using the low-dose dexamethasone suppression test (LD-DST) and the dermal vasoconstrictor assay (DVVA). The researchers concluded that the N363S

and BclI GG genotypes were not more common in PTSD subjects than in the control subjects or in the general population. However, they did find a significant association between the BclI GG genotype and low basal cortisol levels in PTSD. Subjects with PTSD and the GG genotype tended to be more responsive to the DVVA, and their DVVA response correlated with higher scores on the clinician-administered PTSD scale (CAPS). There was insufficient evidence, however, that variation in GR polymorphisms actually increases the susceptibility to PTSD.

In sum, no evidence was found of an association between common GR polymorphisms and PTSD. Only a subgroup of people with PTSD and the BclI GG genotype seemed more responsive to the DVVA and had higher CAPS scores, which in turn showed a significant negative correlation with basal plasma cortisol levels. As the group of individuals with a BclI GG genotype was small, these results will have to be verified in other groups.

3.4. Genes involved in the GABA system

The literature search in Medline delivered 2 hits, none of them reviews, and Embase and Web of Science yielded 0 hits. Of this total of 2 articles, one mainly involved the dopamine receptor (Comings et al., 1996); as it was discussed above, it will not be discussed here. See Table 1.

GABA plays a part in the pathogenesis of anxiety, affective disorders and insomnia; phenomena that also appear in PTSD. Evidence from animal studies suggests that alterations in the benzodiazepine/GABA(A) receptor complex can occur in response to stress and anxiety (Weizman et al., 1990). Clinical evidence in humans also supports a relationship between alterations in benzodiazepine receptor function and anxiety. Studies have shown the efficacy of benzodiazepines in the treatment of a variety of anxiety disorders, such as generalized anxiety disorder and panic disorder, but also in the treatment of symptoms of hyperarousal in PTSD (Braun et al., 1990). Although studies on the connections between GABA type A receptor alpha 6 subunit gene (GABRA6) and cortisol have reported that homozygotes for the T allele had generally higher diurnal cortisol levels (Rosmond et al., 2002a, b), only one study has sought links between GABA and PTSD (Feusner et al., 2001). It focused on the GABA(A) receptor beta 3 subunit gene (GABRB3). Individuals with PTSD who were heterozygote for the G1 polymorphism of the GABRB3 gene were found to have higher total scores than homozygote individuals on the General Health Questionnaire (GHQ), whose 4 subscales pertain to somatic symptoms, anxiety and insomnia, social dysfunction and depression. Comorbidity was not an exclusion criterion, the number of persons studied was small and there was no control group, as a consequence the results have to be interpreted with care. In sum, little is known about the influence of variation of genotypes involved in the GABA system in relation to PTSD.

3.5. Genes involved in the APOE system

The literature search in Medline yielded 1 hit and 0 reviews, and the same hit was found in Embase, and no hit was found in Web of Science, for a total of 1 article. See Table 1.

The APOE mediates the binding of lipoproteins to the low-density lipoprotein (LDL) receptor and plays an important role in the metabolism and redistribution of lipoproteins and cholesterol (Mahley, 1988). In animal studies Raber et al. found higher measures of anxiety following anxiety testing in adult APOE deficient male mice compared to wild-type

controls (Raber et al., 2000, Robertson et al., 2005). In human, APOE4 has been linked to a number of neuropsychiatric disorders, including Alzheimer's disease, stress and depression, as well as to smaller hippocampal volumes and to subjective and objective memory impairment (Cohen et al., 2001; Flory et al., 2000; Gallagher-Thompson et al., 2001; Kuller et al., 1998; Lippa et al., 1997). APOE2 has been linked to lower cortisol levels (Peskind et al., 2001). Since memory impairment also occurs in PTSD and smaller hippocampal volumes are sometimes found too, Freeman et al. (2005) assessed which role APOE genotypes might play in this disorder. They studied 54 male veterans with combat-related PTSD, recruited via a treatment centre. PTSD was assessed with 2 structured interviews, and genotypes were determined through a buccal swab. Memory tests were administered by staff members, who were blind to diagnosis and symptom severity. It was found that the carriership of allele 2, and not of allele 4, was associated with significantly lower scores on the memory test and with more severe re-experiencing symptoms. This study was limited by its small sample and by psychiatric comorbidity and there was no control group. There is, therefore, still insufficient evidence concerning the possible role of APOE genotypes in relation to PTSD.

3.6. Genes involved in the BDNF system

The literature search produced 1 hit in Medline, 2 in Embase (1 new) and 0 in Web of Science, a total of 2 articles, without reviews. See Table 1.

BDNF, a member of the neurotrophin family, promotes neuronal survival and regulates the proliferation and differentiation of nerve cells in the peripheral and central nervous systems. It has important regulatory effects on the serotonergic, glutamatergic and dopaminergic neurotransmitter systems (Zhang et al., 2006b). BDNF is also involved in hippocampal long-term potentiating, which is related to learning and memory (Yamada et al., 2002). There is strong evidence that BDNF may contribute to the pathogenesis of several neuropsychiatric disorders and is also believed to be involved in PTSD. Data of an animal study suggest that psychological, as well as unconditioned physical stress, can decrease hippocampal BDNF mRNA, which could be relevant to the pathogenesis of stress-related disorders, such as depression and PTSD (Rasmusson et al., 2002). Zhang et al. observed the association of gene variants of the BDNF gene and several neuropsychiatric phenotypes. They compared 69 subjects with PTSD with a healthy control group. The SNPs G-712A and C270T and Val-66Met were genotyped. There was only an association between the newly described SNP G-712A and substance dependence, but no association of the SNPs with PTSD. Given the low heterozygosity or the low information content of SNPs C270T and G-712A, these 2 polymorphisms appear to require larger numbers of cases to ensure adequate statistical power. In addition, although the overall study sample was large, the sample for PTSD was much smaller and this limited the power to detect significant associations (Zhang et al., 2006b). In the Korean population Lee et al. (2006) analyzed the genotype and allele frequencies of the BDNF gene Val66Met polymorphism in 106 PTSD patients and 161 unrelated healthy controls using a case-control design. The genotype and allele frequencies for the BDNF gene polymorphism did not differ between the 2 groups (Lee et al., 2006). In summary at this moment there is no evidence concerning the possible role of gene variants involved in the BDNF system in relation to PTSD.

3.7. Genes involved in the NPY system

The literature search in Medline yielded 1 hit and 0 reviews in Medline, and Embase and Web of Science yielded 0 hits. See Table 1.

NPY is a 36-amino acid peptide neurotransmitter. Animal studies have suggested that NPY is involved in the regulation of appetite, reward, anxiety, and energy balance (Lappalainen et al., 2002). NPY is present in extensive neuronal systems of the brain and is present in high concentrations in cell bodies and terminals in the amygdala (Morgan et al., 2000). Morgan et al. found in 2 different studies with soldiers without a control group, that acute stress elicits NPY release and that this release is positively associated with cortisol and NE release. The finding that greater levels of NPY release are associated with less psychological distress suggests that NPY confers anxiolytic activity in humans (Morgan et al, 2000). Individuals with the Pro7/Leu7 genotype have higher maximal increases in the plasma concentration of NPY in response to maximal physiological stress as compared with Leu7/Leu7 individuals (Kallio et al., 2001). Lappalainen et al. tested whether the Leu7Pro allele associated with alcohol dependence in a population study compared to a healthy control group. Population stratification potential and diagnostic specificity was studied by genotyping individuals from additional populations and psychiatric diagnostic classes, such as PTSD. There were 77 PTSD Vietnam combat veterans. Consensus diagnoses were made with the Structural Clinical Interview for DSM-IV diagnosis (SCID). The main outcome measure was the difference in Leu7Pro allele frequencies between alcohol-dependent subjects and controls. There was no association with PTSD (Lappalainen et al., 2002). Until now there is no evidence for the possible role of gene variants involved in the NPY system in relation to PTSD.

4. Discussion

Our review shows that – in contrast to many other psychiatric disorders – no extensive genetic studies have been carried out on PTSD. Association studies have investigated 8 major genotypes in connection with PTSD. They have tested hypotheses involving key candidate genes in the serotonin, dopamine, GR, GABA, APO, BDNF and NPY systems. The results indicate that the serotonin transporter gene possibly plays a role in the degree of response to stressful events, in particular in the sensitivity of individuals to the depressogenic effects of stressful life events. As for the dopamine system, results have been inconsistent and may be dependent on confounding effects of alcohol abuse. GR receptor polymorphisms were not generally found to be more frequent in PTSD. Only in a small subgroup of PTSD patients with the Bcll GG genotype relations were found between PTSD symptoms and basal cortisol levels. For the GABA system and the APO system there is only little evidence associating these systems with PTSD. There was no association found between PTSD and BDNF or the NPY system.

Table 1. PTSD candidate genes

Gene	Function (known or hypothetical)	Chromosomal location	Subjects	Controls	Results	Main author / Year
5-HTT	Serotonergic neurotransmission	17q11.1-q12	100	197	Pos.assoc. betw. SS genotype and PTSD	Lee et al, 2005
DRD2	Dopamine D2 receptor expression	11q22-23	63	None	Pos. assoc. betw. DRD2 A1 allele and response to paroxetine	Lawford et al., 2003
DRD2	Dopamine D2 receptor expression	11q22-23	91	51	Pos. assoc. betw. DRD2 A1 allele and PTSD+alcohol	Young et al., 2002
DRD2	Dopamine D2 receptor expression	11q22-23	52	87	No assoc. betw. DRD2 and PTSD	Gelernter et al., 1999
DRD2	Dopamine D2 receptor expression	11q22-23	37	19	Pos. assoc. betw. DRD2 A1 allele and PTSD	Comings et al., 1996
DAT	Still unknown, possible role in dopaminergic neurotransmission	Unknown	102	104	Pos. assoc. betw. dopaminergic activity and PTSD	Segman et al., 2002
GR	Glucocorticoid receptor expression	5q31-q32	75	33	No assoc. betw. N363S/BclI GR polymorphisms and PTSD	Bachmann et al., 2005
GABR3	GABA(A) receptor beta 3 expression	15q11-13	20 G1G1	39 G1-nonG1 / 27 NonG1-nonG1	Pos. assoc. betw. heterozygosity of GABA(A) receptor beta 3 subunit gene and high scores for anxiety/insomnia, depression, somatic symptoms+social dysfunctioning	Feusner et al., 2001
APOE	34k Da protein mediating binding of lipoproteins to the LPL receptor	12q13-14	54 APOE2/2 APOE2/3 APOE2/4 APOE3/3 APOE3/4	None	Pos. assoc. betw. APOE2 and poorer memory scores+more severe re-experiencing	Freeman et al., 2005
BDNF	Proliferation and differentiation of nerve cells and regulatory effects on diverse neurotransmitter systems	Unknown	69 G-712A C270T Val66Met	250	No assoc. betw. BNDF G-712A, C270T and Val66Met and PTSD	Zhang et al., 2006a,b
BDNF	Proliferation and differentiation of nerve cells and regulatory effects on diverse neurotransmitter systems	Unknown	106 Val66Met	161	No assoc. betw. BDNF Val66Met and PTSD	Lee et al., 2006
NPY	Vasoconstriction and inhibition of catecholamine release	Unknown	77 Pro7/leu7 Leu7/leu7	267	No assoc. betw. NPY pro7/leu7 and leu7/leu7 and PTSD	Lappalainen et al., 2002

The association studies that have been done have serious limitations. In the first place, studies were based on hypothesis-driven searches for candidate genes rather than on candidate genes already identified in genetic linkage research. That means that any associations found could never provide causal explanations, because they might have been confounded by other factors. Second, some association studies involved candidate genes identified in animal studies. Notwithstanding the important contributions made by experimental studies with animals, a serious shortcoming is that they fail to address a range of other factors that influence the disorder, so that none of the mutations seen in animals can serve as full models for specific psychiatric disorders in humans (Gass et al., 2001; Shekar et al., 2001).

A further difficulty with genetic research on PTSD involves the complexity of this disorder, as seen in the many potential endophenotypes, the precondition of exposure to a traumatic experience, and the high prevalence of comorbidity with other psychiatric disorders. All of these compromise the reliability of the endophenotypes. In doing research on PTSD, it is essential that PTSD groups should be compared not only with a healthy control group, but also with a trauma-exposed group that did not develop PTSD, if the conditional risk of certain genetic markers is to be teased out. Genetic analysis in PTSD is additionally complicated by factors such as incomplete penetrance and pleiotropy (Radant et al., 2001). Incomplete penetrance means that a particular genotype will not always result in a particular endophenotype or phenotype. A gene shows a complete penetrance of 1 only if all individuals of a particular genotype have the same endophenotype. That is not usually the case, and that obviously makes it more difficult to establish a linkage between a particular genotype and an endophenotype. Pleiotropy means that a single gene influences different traits and may thus be linked to different endophenotypes.

Most studies are informed by an assumption of gene-environment interaction deriving largely from animal experiments (Caspi et al., 2003). This could be another reason for inconsistent outcomes. Environmental influences can contribute to the effects a polymorphism has on a particular endophenotype or phenotype (van Rossum et al., 2005). Different types of stressful events, for instance, can have different effects on the expression of genes (de Kloet and Derijk, 2004). Early developmental stress is thought to potentially induce neurobiological changes in humans and thereby to confer a higher risk of psychopathology, in particular anxiety and affective disorders (Heim and Nemeroff, 1999, 2002). Interestingly, though, there are also studies that suggest the possibility of underlying genes, which would actually increase a person's likelihood of being exposed to a trauma in the first place. That would heighten vulnerability to PTSD through a different route (True et al., 1993). An elevated risk of trauma involvement has been linked to factors such as personality traits, substance abuse, patient histories of mood or anxiety disorders, and familial psychopathology (Breslau et al., 1998; Breslau and Davis, 1995; Brewin et al., 2000; Stein et al., 2002). This leads to the conclusion that a genetic predisposition, combined with early stress in critical developmental stages, can result in a phenotype that is neurobiological vulnerable to stress, and which lowers the threshold of an individual for developing a depressive or anxiety disorder once additional stress exposure occurs.

Given the many limitations of the genetic research done on PTSD so far, the current findings provide only very modest explanations for why certain people seem more liable than others to stress-related disorders like PTSD or depression (Glatz et al., 2003). Why one person develops depression and the other PTSD or both is still unexplained, and a good

deal of future research will be necessary to clarify it. Not only is there evidence that both disorders could be linked to reactions to traumatic experiences, but twin research has also produced evidence that both disorders may be part of the same underlying dimension and that the two conditions may be genetically linked. Chantarujikapong et al. (2001) have argued that depression, generalized anxiety disorder and panic disorder could all be part of a 'post-combat response syndrome'. Clear vulnerability has also been found in certain families to the co-occurrence of PTSD with depression or dysthymia (Chantarujikapong et al., 2001; Koenen et al., 2003). The genetic risk factor for trauma exposure also correlates positively with the genetic risk factor for depression (Kendler and Karkowski-Schuman, 1997), which could be evidence for a genetically determined joint confounder (such as personality traits). By conducting further genetic research on both depression and PTSD in response to stressful or traumatic experiences, researchers would be able to study much larger groups than they could up to now, in which they can also seek evidence for a common underlying genetic susceptibility to the 2 disorders.

The distinction we have made here between the genetics of the serotonin, dopamine, GR, GABA, APO, BDNF and NPY systems is an artificial one. In reality, the neurotransmitter systems have considerable influence on one another, and there are presumably many more factors that have not yet been studied. Serotonergic neurotransmission, for example, is involved both in the HPA axis and in reactions to stressful events. In vitro studies suggest that elevated GR concentrations can precipitate depression by inducing an increase in 5-HTT expression, after which increased 5-HT uptake causes a reduction in synaptic 5-HT concentrations. Dexamethasone, a potent glucocorticosteroid hormone, has also been found to induce increased 5-HTT expression in immortalised human B-lymphoblastic cells. This suggests a 5-HTT-genotype-dependent dose response to a glucocorticosteroid hormone antagonist (Glatz et al., 2003). These examples illustrate how many interactions occur between neurotransmitter systems that further increase the complexity of the whole.

In sum, the research to date is still too scarce to warrant any firm conclusions. The relative lack of published studies on the genetics of PTSD could also partly be a consequence of publication bias against negative findings. The positive findings are often difficult to interpret because of the relatively small samples (increasing the chances of false negative outcomes) and the methodological shortcomings. Although some polymorphisms have been described for some genes, little is known about their prevalence or about the relevance of haplotypes (fixed combinations of polymorphisms or mutations) in the development of PTSD.

5. Future research directions

An interaction model appears to exist between genes and other factors, which results in a vulnerability to PTSD, which is in part genetically determined. The genetic contribution lies in 'susceptibility genes' or 'vulnerability genes'. Genetic research on PTSD can, therefore, best be conducted by gene-environmental studies.

Because the clinical picture of PTSD is so complex, further research is needed both on specific endophenotypes and on specific symptoms or subgroups of PTSD. In the field of psychiatric genetics, the concept of endophenotypes is becoming increasingly popular. Neurobiological correlates of the disease which are genetically influenced and stable over

time are considered to be more promising targets of investigation. They are more directly influenced by gene effects and presumably defined by a genetic determination, which is less complex than the phenotype of the disorder. The endophenotype strategy has already been successful regarding other complex disorders, such as alcoholism. For example, one possible endophenotype of alcohol dependence may be related to the P300 waveform of the event-related brain potential (ERP) (Carlson et al., 2004; Hesselbrock et al., 2001). Since endophenotypes are influenced by many different factors, it is more likely that associations can be found between a particular genotype and 'proximal endophenotypes' like gene expression and proteins, which are less subject to influence of environmental factors, than between a genotype and more 'distal endophenotypes' like cortisol levels or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) diagnoses, which undergo more environmental influences. The success of genetic research will stand or fall with the specificity of diagnoses (to minimize heterogeneity and false-positive results) and the sensitivity of diagnostic criteria (to maximize the statistical power of datasets). The most promising approach may be to focus on functional polymorphisms or genetic variants, which have already been shown to influence neurobiological parameters that play a part in particular endophenotypes or PTSD symptom clusters. Future genetic studies can be facilitated if more specific endophenotypes are first identified. Endophenotypes should be studied that are stable over time, that are more common in individuals with PTSD than without PTSD, and which are genetically associated with PTSD but are not a consequence of it (Radant et al., 2001). Another need is to study large groups of subjects in order to improve statistical power. Comorbid disorders should receive special attention, both as risk factors and as potentially confounding factors.

Gender, age and ethnicity must also be taken into consideration. Animal studies have found that genes, including 5-HTTLPR, modulate the effects of early life stress in female macaques (Barr et al., 2004a, 2004b). Findings like these could help explain why women are more prone to PTSD than men (Olff et al., 2007). Gender should be analyzed in future research where ever possible. Age-related associations with particular polymorphisms have been reported too, and polymorphisms may also have different effects in individuals from different ethnic backgrounds. Polymorphisms may vary in frequency, and different combinations of polymorphisms may result in different endophenotypes or phenotypes (van Rossum et al., 2005). Study samples should, therefore, be comparable in terms of gender, age and ethnicity. If all the above considerations are addressed, association studies can definitely make substantial contributions to the study of genetic variations in relation to PTSD.

The emerging field of epigenetics examens hereditary changes in gene function, which occur without actual changes in DNA structure. Neuroscientists have only recently begun to investigate the possible roles of epigenetic mechanisms in behaviour, physiology and neuropathology. As Levenson and Sweatt explain, "Epigenetics refers to a set of self-perpetuating, post-translational modifications of DNA and nuclear proteins that produce lasting alterations in chromatin structure as a direct consequence, and lasting alterations in patterns of gene expression as an indirect consequence" (Levenson and Sweatt, 2005). Studies in PTSD could clearly benefit from epigenetic research. For example, in animal models, prenatal stress, GR exposure or inhibition/knockout of 11 beta-hydroxysteroid dehydrogenase type 2 (11 beta HSD-2, the fetus-placental barrier to maternal GRs), reduces birth weight and causes permanent hypertension, hyperglycemia, increased HPA axis activity and

behaviour resembling of anxiety. Also in humans, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Besides that, in human pregnancy severe maternal stress affects the offspring HPA axis and associates with neuropsychiatric disorders. PTSD appears to be a variable in the effects. Intriguingly, some of these effects appear to be 'inherited' by a further generation, itself unexposed to exogenous GRs at any point in the lifespan from fertilization, implying epigenetic marks persist into subsequent generations (Seckl and Meaney, 2004, 2006). Longitudinal studies are needed to determine how the association between maternal PTSD symptoms and cortisol levels and infant temperament reflect genetic and/or epigenetic mechanisms of intergenerational transmission (Brand et al., 2006).

Ultimately, genetic studies in PTSD will help to clarify its etiology and to refine the encompassing notion of genetic susceptibility. People who are genetically vulnerable in one of the gene systems implicated in PTSD could have a greater vulnerability to environmental influences and traumatic experiences in particular. More knowledge of genetic and environmental influences and how they contribute to the more 'proximal' or 'distal' endophenotypes, and perhaps to PTSD as a whole, should eventually enable a more precise identification of risk factors. Information on the specific functions of particular genes could improve the treatment of the disorder.

Therapeutic options in the field of genetics are still in their embryonic stages, but they could have implications for both prevention and treatment strategies in the future (Lesch, 1999). If a multiplicity of gene variants could be identified which contribute to chronic or refractory PTSD, this would open unique opportunities for developing new treatment approaches like gene therapies and tailor-made drugs. But that goal is still a distant one. Far more research is needed before we can sufficiently understand the genetic contribution to PTSD.