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REVIEW

Regulation of the glucocorticoid response to stress-related disorders by the Hsp90-binding immunophilin FKBP51

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Abstract

Immunophilin is the collective name given to a family of proteins that bind immunosuppressive drugs: Some immunophilins are Hsp90-binding cochaperones that affect steroid receptor function. Mood and anxiety disorders are stress-related diseases characterized by an impaired function of the mineralocorticoid and glucocorticoid receptors, two of the major regulatory elements of the hypothalamus-pituitary-adrenocortical axis. Genetic variations of the FK506-binding protein of 51-kDa, FKBP51, one of the immunophilins bound to those steroid receptor complexes, were associated with the effectiveness of treatments against depression and with a major risk-factor for the development of post-traumatic stress disorders. Interestingly, immunophilins show polymorphisms and some polymorphic isoforms of FKBP51 correlate with a greater impairment of steroid receptor functions. In this review, we discuss different aspects of the role of FKBP51 in such steroid receptor function and the impact of genetic variants of the immunophilin on the dysregulation of the stress response.

Keywords: depression, immunophilin, glucocorticoid receptor, Hsp90, Hypothalamic-Pituitary-Adrenal axis, tetratricopeptide repeat domain.

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One of the most abundant classes of transcriptional regulators in metazoans is the nuclear receptor family (Escriva *et al.* 2004). These receptors are phylogenetically related proteins clustered into a large superfamily (Evans 1988) that includes receptors for hydrophobic molecules, such as steroid hormones, retinoic acids, thyroid hormones, dioxin, sterols, fatty acids, leukotrienes, and prostaglandins. Steroid receptors are the best studied subfamily branch of that large tree and are characterized by the fact that they function not only as receptors able to recognize the hormone but also as transcriptional regulators. Actually, steroid receptors are a particular class of ligand-activated transcription factors.

Steroid hormones bind to each cognate soluble receptor to constitute a functional unit able to trigger specific biological responses that are subject to other kinds of non-hormonal- and/or non-receptor-dependent regulations (Vicent *et al.* 2002; Galigniana and Piwien Pilipuk 2004; Bain *et al.* 2007; Hilser and Thompson 2011). In addition, steroids can also function via membrane-associated receptors, also called non-conventional receptors, generating non-genomic effects (Groeneweg *et al.* 2012).

The major control mechanism of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoid steroids regulate the activity of this axis through negative feedback via the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Both steroid receptors mediate this regulation mainly in the hypothalamus and the pituitary, but also on the level of the hippocampus and other limbic structures (Ulrich-Lai and Herman 2009). The proper

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Abbreviations used: ACTH, adrenocorticotropic hormone or corticotropin; CRF, corticotropin-releasing factor; CsA, cyclosporine A; FKBP, FK506-binding protein; GR, glucocorticoid receptor; HIV, human immunodeficiency virus; HPA, hypothalamic-pituitary-adrenal axis; Hsp90, 90-kDa heat-shock protein; IMM, immunophilin; MR, mineralocorticoid receptor; PTSD, post-traumatic stress disorder; SNP, single nucleotide polymorphism; TPR, tetratricopeptide repeats.

negative feedback via the steroid receptor is critical for a healthy stress response (de Kloet 2003). Emerging literature is beginning to shed light on possible mechanisms of the association between glucocorticoids and mood. Accordingly, one of the most robust biological abnormalities observed in the majority of depressed patients is altered signaling via the GR, which often leads to an impaired negative feedback regulation and thus to partial glucocorticoid resistance (Holsboer 2000; Pariante *et al.* 2001). It has been suggested that the disruption in hippocampus-dependent regulation of the HPA axis, which leads to an abnormal increase in plasma corticosteroid hormone levels in response to stressors, is associated with the episodes of depressive disorders (Nestler *et al.* 2002). At the molecular level, GR polymorphisms have been associated either with receptor hypofunction or hyperfunction and could thus contribute to differential individual sensitivity to the effects of glucocorticoid treatment (van Rossum *et al.* 2006; Binder 2009; Claes 2009; Marques *et al.* 2009).

Among the members of the superfamily, the MR shows the highest homology with the GR. MR has a high affinity for endogenous corticosteroids and has been associated with regulation of cortisol circadian fluctuations (De Kloet *et al.* 1998). Conversely, GR has a lower affinity for endogenous corticosteroids, but a higher affinity for dexamethasone. Therefore, GR plays a role in regulating the peak morning response and stress response, as they are activated when endogenous glucocorticoid levels are higher. Interestingly, recent findings suggest that MR also shows polymorphic variants that appear to affect cortisol action (Klok *et al.* 2011). Moreover, the expression balance of both receptors, GR and MR, could be altered by gene variants of these receptors and by experience-related factors, which has been proposed to induce lasting epigenetic changes in the expression of them leading to the regulation of behavioral changes (Oitzl *et al.* 2010). Accordingly, prenatal stress increases the risk of depressive disorders in adult offspring by down-regulation of MR, which was linked to the development of depressive disorders with hippocampal dysfunction caused by attenuation of neuronal maturation and the development of dysfunctional neuronal networks in adulthood (Tamura *et al.* 2011). On the other hand, the specific elimination of GR homodimerization capability was found to cause a selective impairment of spatial memory that could not be rescued by glucocorticoids, whereas MR-related behaviors were left intact (Oitzl *et al.* 2001). This suggests that DNA binding and transactivation of the GR homodimer is required for independent glucocorticoid action on spatial memory in the face of unaltered functioning of the MR.

Interestingly, some individuals are vulnerable to affective disorders like depression, anxiety, and post-traumatic stress disorders, whereas others are resilient under similar stressful experiences. It is thought that the mechanisms underlying these inter-individual differences in coping with stress depend on the secretion and action of stress hormones (e.g., corticotrophin releasing hormone, vasopressin, endorphins, adrenocorticotrophic hormone, glucocorticoids, and adrenaline), which are largely shaped by gene-environment interactions throughout life. Responses to these hormones can also be shaped by associations with other regulatory factors. Thus, sensitivity to glucocorticoid action at the level of steroid receptors can be determined by the number of receptors, steroid affinity, ability of the steroid-receptor complex to translocate to the nucleus, its interaction with other signal transduction pathways, and the regulatory action in all these aspects by those chaperones and cochaperones bound to the receptor heterocomplex. Among the latter regulators, the 90-kDa heat-shock protein Hsp90 and its associated immunophilins (IMMs) play a key role. Although Hsp90 is absolutely required for the steroid binding capacity of these receptors (Pratt and Toft 1997; Galigniana *et al.* 2004c), FKBP51 shows proven inhibitory actions on both corticosteroid receptors, GR (Denny *et al.* 2000) and MR (Gallo *et al.* 2007) and is a positive regulator of the androgen receptor (Makkonen *et al.* 2009; Periyasamy *et al.* 2010).

In this review, we will focus on the genomic actions of steroid receptors and will analyze the regulation of their effects in stress-related events by high molecular weight immunophilins. In particular, we will focus on the product of the *fkbp5* gene, the Hsp90-binding immunophilin FKBP51. Therefore, as a manner to introduce the reader in the matter, we will first provide an overview of the molecular properties of IMMs and their influence on steroid receptor mechanism of action. Then, we will discuss the emerging picture related to the involvement of polymorphic isoforms of the Hsp90-binding immunophilin FKBP51 in stress-related disorders.

Immunophilins

Immunophilins (IMMs) comprise a family of intracellular proteins with peptidyl-prolyl-*cis/trans*-isomerase (PPIase) activity, i.e., the reversible *cis*↔*trans* interconversion of Xaa-Pro bonds. IMMs are classified by their ability to bind immunosuppressant drugs – CyPs (cyclophilins) bind cyclosporine A (CsA), and FKBPs (FK506-binding proteins) bind FK506 (Pratt and Toft 1997; Kang *et al.* 2008). The signature domain of the IMM family is the PPIase domain, which is in turn the drug binding domain. Only the low molecular weight immunophilins FKBP12 (gene name *fkbp1A*, chromosome location 20p13) and CyPA (gene name *pp1A*, chromosome location 7p13) are related to the immunosuppressive effect when the drug IMM complex inhibits the Ser/Thr-phosphatase activity of PP2B/calcineurin. This prevents the activation and subsequent nuclear translocation of NFAT (nuclear factor of activated T cells), a transcription factor that stimulates the production of interleukines and interferon-gamma (see (Li *et al.* 2011) for a recent review).

High molecular weight IMMs have a more complex architecture and are not related to the immunosupression process. The archetype of this subfamily is the 52-kDa FK506-binding protein, FKBP52 (Fig. 1). In addition to the PPIase domain, there are three additional domains – the nucleotide-binding domain, (also called FKBD2 in FKBP proteins) where ATP binds, the calmodulin-binding domain, a poorly characterized domain able to interact with calmodulin, and TPR domains, sequences of 34 amino acids repeated in tandem through which they bind to Hsp90 (Davies and Sanchez 2005). The TPR-domain IMMs are abundant and ubiquitous proteins that were first discovered associated to steroid receptors. Four of them have been relatively well characterized because of their association with these receptors, i.e. FKBP52 (gene name *fkbp4*, chromosome location 12p13.33), FKBP51 (gene name *fkbp5*, chromosome location 6p21.31), the cyclophilin CyP40 (gene name *ppID*, chromosome location 4q31.3), and the FKBP-like protein phosphatase PP5 (gene name *ppp5C*, chromosome location 19q13.3). These IMMs are highly ubiquitous and are also able to form complexes (many of them still to be characterized) with other factors, although their biological functions in these complexes are poorly understood.

In 2001, it was first reported that the steroid-dependent retrotransport of the glucocorticoid receptor (GR) is favored by the association of PPIase domain of FKBP52 with the dynein/dynactin motor complex (Galigniana *et al.* 2001) (Fig. 2). Both FKBP52 and dynein are recruited by the steroid receptor Hsp90 heterocomplex upon steroid binding, whereas FKBP51, an IMM that binds inefficiently to dynein and also impairs ligand binding and receptor-dependent transcriptional activity, is released (Gallo *et al.* 2007, Davies and Sanchez 2005). Even though the disruption of the receptor-Hsp90-FKBP52 complex increases the nuclear translocation half-time by 10-fold, the receptor still reaches the nucleus, suggesting the existence of an alternative, Hsp90-FKBP52-independent mechanism of movement (perhaps caused by simple diffusion).

Complexes of IMMs and motor proteins have also been related to trafficking of other soluble proteins, such as mineralocorticoid receptor (MR) (Galigniana *et al.* 2010), androgen receptor (AR) (Thomas *et al.* 2006), p53 (Galigniana *et al.* 2004a), the AIF/Rac3 complex (Colo *et al.* 2008), adeno-associated virus 2 (Zhao *et al.* 2006), etc., suggesting that most factors able to interact with the Hsp90-FKBP52 complex could share a similar molecular machinery of movement. Interestingly, while FKBP12 does not interact with motor proteins, CyPA, the other low molecular weight IMM involved in immunosupression, also associates with dyenin via its PPIase domain (Galigniana *et al.* 2004b), and

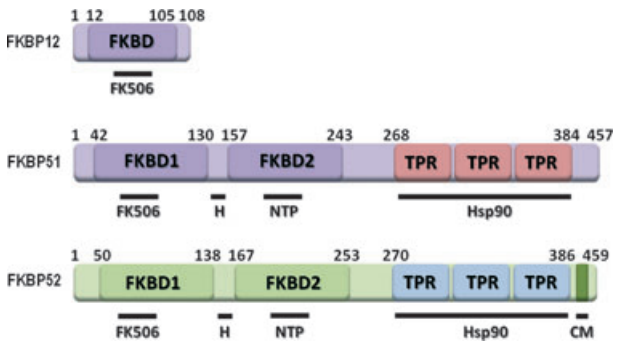

Fig. 1 Comparative domain maps of hFKBP12 and the tetratricopeptide repeats (TPR)-domain immunophilins hFKBP51 and hFKBP52. hFKBP12 is a one domain immunophilin able to bind the immunosuppressive macrolide FK506 triggering immunosupression by inhibition of calcineurin activity. Both high molecular weight immunophilins, hFKBP51 and hFKBP52, share high homology between them and are major components of steroid receptors. FKBD are the FK506 binding domains. In the TPR domain immunophilins, FKBD1 shows high homology with hFKBP12 and is the domain that shows peptidyl-prolyl-(*cis/trans*)-isomerase activity; FKBD2 is a PPIase-like domain that binds ATP and GTP, but not FK506. TPR is the tetratricopeptide repeat domain where Hsp90 binds. CM is the calmodulin-binding domain present in hFKBP52 only. H is the hinge domain, a region reach in polar amino acids able to interact directly with steroid receptors, and NTP represents the nucleoside triphosphate binding site.

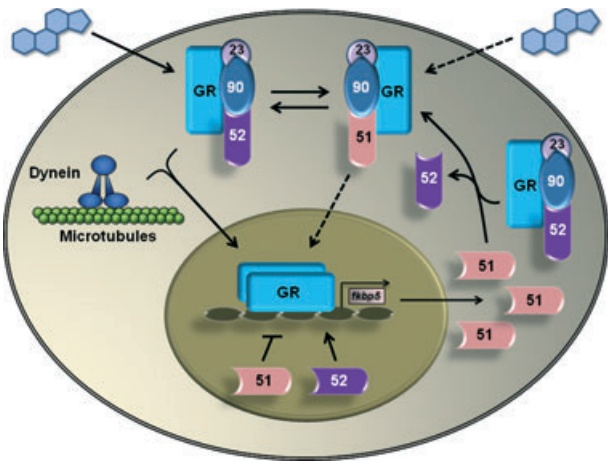

Fig. 2 Schematic model for GR regulation by steroid binding via FKBP signalling. Glucocorticoids bind with higher affinity to the GR Hsp90 heterocomplex containing FKBP52. In turn, ligand binding stimulates the recruitment of this immunophilin and dynein motors to facilitate GR retrotransport on cytoskeletal tracks. Upon receptor transformation (i.e., Hsp90 dissociation) in the nuclear compartment, GR dimers interact with specific promoter sites. Although FKBP52 favors GR action, FKBP51 impairs steroid binding affinity, GR retrotransport, and transcriptional activity. One of the genes activated by GR is fkbp5, whose product, FKBP51, competes with FKBP52 for the acceptor binding site on Hsp90. Therefore, the circular activation-inhibition cycle of glucocorticoid action is closed. Dashed lines represent weaker activity.

it is thought that this IMM participates in the early events of HIV infection favoring viral particles retrotransport (Sherry *et al.* 1998).

The biological function on protein trafficking of IMMs associated to heat-shock proteins is not limited to the cytoplasm. Once the cargo has reached the nuclear envelope using microtubules as molecular tracks, it must traverse the nuclear pore. The Hsp90 complex, including FKBP52 but not the highly homologous partner FKBP51, is able to interact with the nuclear import machinery, including nucleoporins and importins, facilitating the nuclear import of cargoes (Echeverria *et al.* 2009). FKBP52, Cyp40 and PP5 interact with motor proteins (Galigniana *et al.* 2002), and the steroid-dependent functional exchange between FKBP51 and FKBP52 has been demonstrated for GR (Davies *et al.* 2002) and MR (Gallo *et al.* 2007). These two IMMs have 60% identity and 75% similarity (Cox *et al.* 2007), they bind to the same TPR acceptor site in Hsp90 (Silverstein *et al.* 1999; Galigniana *et al.* 2010), and adopt similar PPIase domain conformations according to X-ray crystallography images (Sinars *et al.* 2003; Wu *et al.* 2004). Nevertheless, their functions differ drastically. Although FKBP52 is regarded as a positive regulator of the activity of corticosteroid receptors, FKBP51 is a negative regulator. Accordingly, its over-expression prevents the positive regulation by FKBP52 because of the competition of FKBP51 for the same binding site on Hsp90. Importantly, glucocorticoids self-regulate their own effects by inducing the expression of FKBP51 (Vermeer *et al.* 2003)(Fig. 2). On the other hand, although FKBP52 itself is able to interact with cytoskeletal structures (Czar *et al.* 1994; Perrot-Applanat *et al.* 1995; Chambraud *et al.* 2007; Quintá *et al.* 2011) and links steroid receptor with microtubules, FKBP51 does not show these properties. Perhaps related to these features, it was shown recently that the macrolide FK506 favors neuronal differentiation via FKBP52, whereas FKBP51 antagonizes these effects (Quintá *et al.* 2010; Quintá and Galigniana 2012). Thus, binding of either TPR protein to Hsp90 complexes could show inhibitory or stimulatory action. In turn, this property and the relative availability of each IMM in each particular tissue regulate the various aspects of the cell physiology (Gallo *et al.* 2007; Galigniana *et al.* 2010).

Peptidyl-prolyl-isomerase domain

Most of the peptide bonds of nascent polypeptides emerge from the ribosome in the *trans*-conformation and the majority retains that energetically favored state. Nevertheless, there is a significant amount ranging from 5% to 7% of the proteins with structures of peptidyl-prolyl bonds (i.e., Xaa-Pro bonds) that switch to the *cis*-conformation during folding, transport, and assembly. The *cis-trans* isomerization of Xaa-Pro bonds is one of the rate limiting steps of protein folding (Fischer and Aumuller 2003; Schiene-Fischer *et al.* 2012). The obvious influence of CyPs and FKBPs on the conformations, oligomeric states and consequent biological activities of these proteins cannot be explained on the sole bases of the PPIase activity alone. Actually, IMMs also act as molecular chaperones in an analogous manner to certain members of stress protein families. Historically, the first Cyp was identified in 1984 by its affinity purification via a CsA column (Handschumacher *et al.* 1984), not until 1989 was its PPIase activity simultaneously identified by two groups (Fischer *et al.* 1989; Takahashi *et al.* 1989). Since then, Cyps and FKBPs have been shown to influence the folding of a number of synthetic peptides and natural proteins such as collagen, chymotrypsin inhibitor, carbonic anhydrase, and ribonuclease T1, the latter considered the standard protein substrate of PPIases. It has been investigated structurally during refolding by NMR techniques and the results suggest that the presence of a non-native *trans*-prolyl bond at Pro39 in a folding intermediate that may represent the targeted species for enzyme catalysis propagates the effect of prolyl isomerization not only to regions adjacent to the proline but also to remote parts of the polypeptide chain (Balbach *et al.* 1999). Nevertheless, the exact catalytic mechanism of the PPIase activity still remains hazy. An interesting feature of IMMs is that although both Cyp and FKBP families possess PPIase activity, the sequence and structure of the two families are dissimilar, although in both proteins the substrate and the inhibitory immunosuppressants compete for binding to the PPIase active site. Thus, the PPIase domain has become synonymous with drug-binding domain. The rest of the IMM sequence often plays a family specific role, such as interacting with specific subsets of client proteins.

These mechanistic properties are not exclusive for PPIase domain proteins, but are a reminiscence of the strategies adopted by other families of proteins, for example, Ser/Thr phosphatases. In this case, all members of the family catalyze the same basic reaction of dephosphorylating P-Ser and P-Thr residues. However, the phosphatases of the PPP class share a highly conserved sequence and structure in their catalytic core (Ansai *et al.* 1996), whereas the metal-dependent PPM phosphatases have a very different sequence and a novel protein fold (Das *et al.* 1996). Finally, both high molecular weight IMMs and the IMM-like phosphatase contain protein-protein interaction domains outside the catalytic core, such as the TPR domains.

Emergence of *fkbp* genes

Because of their association to steroid receptors, two of the best characterized IMMs are FKBP52 and FKBP51, which are products of the *fkbp4* and *fkbp5* genes, respectively. Likely, these genes emerged by a gene duplication event from a common, ancestral invertebrate gene. Although eight pseudogenes for *fkbp4* have been identified, seven on chromosome 9 and one on chromosome 4, pseudogenes for

fkbp5 have not been reported yet (Cioffi *et al.* 2011). Although expression of *fkbp4* and *fkbp5* is quite different, the gene structures are homologous and the protein sequences are highly similar.

The genes for human (Scammell *et al.* 2003) and rabbit FKBP52 (Massol *et al.* 2003) were isolated from genomic DNA, and FKBP51 genes were isolated during the same time period (Scammell *et al.* 2001; Hubler *et al.* 2003). *Fkbp4* consists of 10 exons and 9 introns spanning approximately 10-Kb of genomic DNA, whereas *fkbp5* has 13 exons and 12 introns spanning more than 150-Kb. The organization of *fkbp5* is identical to that of *fkbp4* with the exception that non-coding exons 1–3 in *fkbp5* are absent in *fkbp4*. The exon-intron boundaries throughout the two genes are otherwise identical.

Using clustering analysis, it was postulated that both genes likely evolved before the emergence of fishes (Galat 2004). *Fkbp4* orthologues are found in chordates, *Drosophila melanogaster,* and *Caenorhabditis elegans* genomes, although the exon-intron boundary organization was conserved only among the chordates. *fkbp5* orthologues are found in chordates, but in neither *D. melanogaster* nor *C. elegans* genomes. Regarding the expression of both genes, the molecular interactions that mediate constitutive or regulated activity of *fkbp4* are largely unexplored, whereas our understanding of *fkbp5* expression is greater. There is increasing evidence that both genetic and epigenetic variations (Lee *et al.* 2010) in non-coding regions of *fkbp5* may influence basal and hormone-stimulated expression of this gene, such that single nucleotide polymorphisms in *fkbp5* are associated with elevated expression of FKBP51 impaired stress hormone regulation (see later).

Expression of *fkbp* genes and steroid receptor function

In the absence of ligand, members of the steroid receptor family remain sequestered in the cytoplasm and/or nucleus in complex with chaperone and co-chaperone proteins including, but not limited to Hsp90, Hsp70, a 23-kDa co-chaperone (p23), and one TPR-domain IMM. In the steroid-receptor heterocomplex, FKBP52 acts as a specific positive regulator of AR, GR, and PR function (Sivils *et al.* 2011). Despite the significant sequence, structural and/or functional homology that exists between some steroid hormone receptors (in particular, GR and MR) FKBP52 does not functionally affect neither MR nor ER. Thus, FKBP52 becomes an attractive therapeutic target for the treatment of diseases that depend upon a functional AR, GR, and/or PR signaling pathway. FKBP52 association with receptor-chaperone complexes results in an enhancement of receptor hormone binding (Riggs *et al.* 2003, 2007; Davies *et al.* 2005) and influences receptor localization within the cell (reviewed in Echeverria and Picard 2010). FKBP52 consists of a C-terminal Hsp90-binding TPR domain, an N-terminal FKBD1 subdomain that contains a functional PPIase active site, and a middle FKBD2 subdomain that is similar to FKBD1, but lacks PPIase activity (Wu *et al.* 2004). Based on previous biochemical and cellular studies, it could be predicted that *fkbp4*-deficient mice would display phenotypes related to hormone insensitivity syndromes. Indeed, *fkbp4*-deficient male mice display phenotypes consistent with partial androgen insensitivity syndrome including dysgenic seminal vesicles and prostate, ambiguous external genitalia, hypospadias, and nipples retained into adulthood (Cheung-Flynn *et al.* 2005, 2005; Yong *et al.* 2007). In addition to alterations in primary and accessory sex organs, the epididymis of FKBP52-deficient male mice have significantly depressed sperm counts and the sperm display abnormal morphology (Cheung-Flynn *et al.* 2005; Hong *et al.* 2007). Interestingly, in this same study FKBP52 was also reported to be present in epididymal sperm flagella, which agrees with FKBP52 association to dynein motor proteins (Galigniana *et al.* 2001). Therefore, FKBP52 could be involved in flagellar movement, which is an androgen-independent process.

The loss of FKBP52 in female *fkbp4*-deficient mice results in infertility (Tranguch *et al.* 2005, 2007). The mice appear morphologically normal; moreover, ovulation and fertilization are not overtly hindered. Infertility appears to be because of progesterone resistance and uterine defects resulting in embryonic implantation and decidualization failure (Yang *et al.* 2006; Tranguch *et al.* 2007). Implantation failure as a result of reduced progesterone signaling may be because of increased uterine oxidative stress in *fkbp4*-deficient female mice as a result of reduced levels of the anti-oxidant peroxiredoxin-6 (PRDX6) (Hirota *et al.* 2010).

Homozygous *fkbp4*-deficient mice were initially reported to have high serum corticosterone levels, which were hypothesized to result from the mice compensating for reduced GR activity (Cox and Smith 2007). Heterozygous *fkbp4*-deficient mice show increased susceptibility to high fat diet-induced hyperglycemia and hyperinsulinemia that correlates with reduced insulin clearance, hepatic steatosis and glucocorticoid resistance. This appears to be the outcome of reduced GR control of gluconeogenesis (Warrier *et al.* 2010).

On the other hand, FKBP51 has been regarded as a negative transcriptional regulator of steroid receptors, except in the case of the AR (Makkonen *et al.* 2009; Periyasamy *et al.* 2010). In addition, over-expression of FKBP51 impairs nuclear localization of corticosteroids receptors. Nevertheless, it should be pointed out that the subcelular localization of steroid receptors is also affected by other factors, such as phosphorylation status (Galigniana 1998; Piwien-Pilipuk and Galigniana 1998; Galigniana *et al.* 1999), redox milieu (Galigniana and Piwien-Pilipuk 1999; Piwien-Pilipuk and Galigniana 2000; Piwien-Pilipuk *et al.* 2002), nature of bound ligand (Vicent *et al.* 1997; Galigniana *et al.* 2000; Gallo *et al.* 2007), or protrusion of localization signals (Piwien Pilipuk *et al.* 2007).

IMM function is not limited to steroid receptor signaling. It has recently been suggested that FKBP52 catalyzes cis/trans isomerization of regions of the TRPC1, a non-specific Na^+ and Ca^{2+} channel located on the plasma membrane of numerous cell types, implicated in controlling neuronal channel opening (Shim *et al.* 2009). FKBP52 mediates stimulus-dependent TRPC1 gating through isomerization, which is required for chemotropic turning of neuronal growth cones and for axon guidance of commissural interneurons in the developing spinal cord. More recently, it was shown that the tyrosine kinase receptor RET51, an isoform of the proto-oncogene *RET* involved in the development and maintenance of the nervous system, forms complexes with FKBP52, and that the disruption of those complexes is a potential causative effect in the development of Parkinson's disease (Fusco *et al.* 2010).

The HPA axis and the glucocorticoid response

The hypothalamic-pituitary-adrenal (HPA) axis is a complex pathway of interaction between the corporal systems that regulates reactions to stress and many body processes, including digestion, the immune system, mood and emotions, sexuality, and energy storage and expenditure. Upon stress exposure, corticotropin-releasing factor (CRF) is released from the hypothalamus and is transported to the anterior pituitary, where it stimulates the secretion of adrenocorticotropin (ACTH), which in turn stimulates the synthesis and release of glucocorticoids from the adrenal cortex. The neuroendocrine stress response is counter-regulated by circulating glucocorticoids via negative feedback mechanisms targeting the pituitary, hypothalamus, and hippocampus. This negative feedback loop is essential for the regulation of the HPA axis and, therefore, for the regulation of the stress response via steroid receptors (Carrasco and Van de Kar 2003).

Substantial evidence suggests that the attenuation of the HPA axis activity, which is an integral part of the adaptive response to stressors and challenges, is often impaired in patients suffering from major depression (Holsboer 2000; de Kloet *et al.* 2005). Major depression has been repeatedly shown to be associated with elevated levels of circulating glucocorticoids, decreased responsiveness to dexamethasone suppression and increased adrenocortical response to stimulation with CRF in the combined dexamethasone/CRF test. Although the exact physiological consequences of prolonged glucocorticoid elevation are not fully understood, it is intriguing that successful treatment of depression mostly goes along with a normalization of HPA axis reactivity. Moreover, remitted individuals with incompletely attenuated HPA axis overdrive have a higher risk of relapse, which has led to the above-mentioned hypothesis that GR dysfunction is connected with the major depression syndrome.

In the central nervous system, cortisol, the main glucocorticoid in primates, mediates its effects by binding to the MR and the GR (de Kloet *et al.* 2005). Although both receptors are negatively regulated by FKBP51 (Gallo *et al.* 2007, Wochnik *et al.* 2005), they differ in their affinity and distribution, but it still remains unclear how the nervous system discriminates the mineralocorticoid response mediated by MR from the glucocorticoid response mediated by GR. Because most of the effects associated with cortisol, especially those related to the central nervous system, have been attributed to GR, we will focus on the mechanism of this particular receptor type.

Glucocorticoid receptor has their highest density in the hippocampus, but is also prominent in the prefrontal cortex (de Kloet 2003), both brain regions being of substantial importance for memory function. Thus, acute administration of glucocorticoids impairs long-term memory retrieval (Buchanan 2007), whereas equivalent effects have been obtained using psychosocial laboratory stressors (Kuhlmann *et al.* 2005). On the other hand, memory consolidation seems to be positively influenced by cortisol (Wolf 2009). Several mental disorders are characterized by memory disturbances, these alterations not being just secondary symptoms, but must be regarded as key features of these disorders.

FKBP51 signaling

The discovery that FKBP51 was structurally and functionally similar to FKBP12 led to early speculation that it contributed to the immunosuppressive actions of FK506, perhaps by inhibiting the calcineurin-NFAT pathway of T-lymphocytes. This was fostered when it was shown that there is a high level expression of FKBP51 in T-cells (Baughman *et al.* 1997). Although FKBP51 can inhibit calcineurin in an FK506-independent manner, it has little to no effect on NFAT transcriptional activity in cell-based assays (Li *et al.* 2002, 2002; Weiwad *et al.* 2006). Studies performed in T-cells derived from mice deficient in FKBP12 were conclusive to demonstrate that there is no contribution by FKBP51 to the anti-proliferative actions of FK506 (Xu *et al.* 2002). Thus, the product of the *fkbp5* gene, like that of the *fkbp4* gene (Wiederrecht *et al.* 1992), is not relevant for FK506-mediated immunosuppressive effect in T-lymphocytes.

The importance of FKBP51 in GR signaling was first elucidated in New World Monkeys. These animals show increased plasma cortisol levels compared to other primates, including humans. In squirrel monkeys free cortisol levels are 50-100 times higher, but they do not show signs of hypercortisolism because of GR resistance in the target organs. This GR resistance is conferred by the overexpression of FKBP51 (Denny *et al.* 2005, Scammell *et al.* 2001). An important feature of the *fkbp5* gene is that its expression is induced by glucocorticoids as part of an intracellular ultra-short negative feedback loop for GR activity (Reynolds *et al.* 1998). GR induces *fkbp5* transcription by activation of at least two intronic steroid hormone

response elements (Hubler and Scammell 2004), and increased transcription and translation of *fkbp5* following steroid receptor activation reduces GR function. Importantly, an impaired signaling cascade via cortisol-activated GR leads to an impaired negative feedback regulation, and thus, to partial glucocorticoid resistance. Interestingly, this circuit appears to be one of the most robust biological abnormalities observed in mood disorders (Pariante and Miller 2001). As a consequence, the glucocorticoid-dependent induction of FKBP51 expression leads to GR resistance. In depressed patients, this is reflected by basal hypercortisolemia and cortisol escape from dexamethasone suppression, as well as an increased ACTH and cortisol release in the combined dexamethasone suppression/CRF-stimulation test (Watson *et al.* 2002). Animal data also support this hypothesis as a transgenic mouse strain, expressing an antisense mRNA directed against the GR gene leading to impaired GR expression displays not only neuroendocrine abnormalities similar to depressed patients, but also depression-like behavioral changes (Barden *et al.* 1997), as well as in forebrain-specific GR knock-out mice (Boyle *et al.* 2005) and heterozygous GR mutant mice with a reduced number of expressed GRs (Ridder *et al.* 2005). In other words, it appears that in this syndrome the lack of expression of the GR parallels its functional inhibition by FKBP51. If this is correct, the underlying molecular mechanism by which the GR is involved in this pathology relates to trans-activation rather than trans-repression events. Interestingly, in response to antidepressant treatment, partial GR resistance is restored, and a resolution of the HPA-axis hyperactivity and GR insensitivity precedes clinical improvement to antidepressant drugs in patients (Ising *et al.* 2008; Schule *et al.* 2009).

Polymorphism of the *fkbp5* gene and its relation with stress disorders

All those preclinical and clinical studies suggest that one mechanism of action of anti-depressant drugs is to restore negative HPA-axis feedback through the GR leading to a decrease of the over-expressed peptides CRF. This led to analysis of potential polymorphisms in genes involved in HPA-axis regulation that may contribute to the susceptibility for developing depression and the onset of clinical response to antidepressant treatment. Genotyped single-nucleotide polymorphisms in the GR, CRF, and five cochaperones of the glucocorticoid receptor (*BAG1, STUB1, TEBP, fkbp4,* and *fkbp5*) that regulate GR activity yielded novel evidence that polymorphisms in the expression of the *fkbp5* gene are closely related. This led to analyze the potential increased recurrence of depressive episodes and the efficient response to antidepressant treatment (Binder *et al.* 2004). *fkbp5* gene is located on chromosome 6p21, a chromosomal region associated with bipolar disorder and psychosis, and several single nucleotide polymorphisms (SNPs) in *fkbp5* have been found (Koenen *et al.* 2005; Cheng *et al.* 2006; Binder 2009; Tatro *et al.* 2009b; Horstmann *et al.* 2010; Shibuya *et al.* 2010) and related to treatment response in patients with mood disorders. In Binder's pioneer study (Binder *et al.* 2004), a C/T single nucleotide polymorphism in the intron 2 of the *fkbp5* gene (rs1360780) was reported. The T allele of this polymorphism is associated with higher levels of FKBP51 protein and with less suppression of cortisol to the dexamethasone test, as well as to slower recovery of cortisol response to a psychological stress test in healthy subjects (Ising *et al.* 2008). Although the exact mechanism still remains unclear, it is accepted that this polymorphism influences FKBP51 protein levels through translation or protein stability. These studies suggest that the T allele is associated with higher levels of the IMM leading to impaired negative feedback of the HPA axis.

A strong association between polymorphisms and response to antidepressant drugs was also found in depressed patients. Functional polymorphisms in the GR gene have been identified that associate with different responsiveness to the dexamethasone-suppression-test after treatment for depression (van Rossum *et al.* 2006). So far several SNPs within the *fkbp5* locus have been associated with these functional outcomes. Because of the high extent of linkage disequilibrium, that is low number of recombination events in this region, it is difficult to pinpoint a single causative functional polymorphism. Linkage disequilibrium is high in most populations, including Caucasians, African Americans, Africans, and Asians (Ising *et al.* 2008; Binder *et al.* 2009).

Given the fact that these polymorphisms are associated with higher FKBP51 levels leading to the consequent GR resistance and impaired negative feedback, it could be speculated that FKBP51 alleles associated with a slower return to baseline of stress-induced cortisol levels also increase the risk for stress-related psychiatric disorders. Currently, there is evidence for the impact of *fkbp5* in both mood and anxiety disorders. Normally, there is a feedback loop that leads to an increase of *fkbp5* upon GR activation (Hubler and Scammell 2004; Paakinaho *et al.* 2010), thereby limiting excessive activation of GR. The lack of this feedback loop may be the basis of dysregulated responses to stress. In this respect, it is intriguing that *fkbp5* is relevant for the development of stress-related mental disorders only in combination with traumatic events (Binder *et al.* 2008; Xie *et al.* 2010).

Polymorphism of *fkbp5* in post-traumatic stress disorders

Exposure to a traumatic event is required for classifying a mental disorder as a post-traumatic stress disorder disease (PTSD). Symptoms reflect stress-induced changes in neurobiological systems and/or lack of adaptation to stressors (Yehuda and LeDoux 2007), in particular in the HPA axis. In

turn, the HPA axis is also affected by genetic and epigenetic variability that affect the sensitivity of the GR (Schmidt *et al.* 2011). It is accepted that functional differences in *fkbp5* activation are associated with a different risk profile to develop PTSD after trauma exposure. The extent of up-regulation of *fkbp5* mRNA in mononuclear cells a few hours after a trauma has been shown to correlate directly with the development of PTSD four months after (Segman *et al.* 2005). Peripheral blood *fkbp5* mRNA expression was reduced in patients with PTSD, consistent with enhanced the GR responsiveness in this disorder and FKBP51 expression. In addition, two studies have shown that the alleles associated with enhanced induction of FKBP51 with cortisol and impaired negative feedback in controls are associated with increased risk to develop PTSD or related symptoms. Two genotypes (rs3800373 and rs1360780) have been associated with higher levels of peritraumatic dissociation and increased peritraumatic dissociation after medical trauma, such that they are considered predictors of PTSD (Ozer *et al.* 2003). In a cohort of hundred subjects analyzed to predict the level of PTSD symptoms, a higher induction of *FKBP5* by cortisol and thus GR resistance of several genotypes (rs9296158, rs3800373, rs1360780, and rs9470080) were associated with a higher risk of actual PTSD symptoms. This was also paralleled by effects on GR sensitivity, as measured by the dexamethasone suppression test. The alleles associated with high FKBP51 protein/mRNA expression were associated with GR resistance in individuals without PTSD symptoms. This functional association appears to be switched in patients with PTSD symptoms. Here, these same alleles were associated with a higher dexamethasone suppression ratio and thus increased GR sensitivity which is associated with PTSD (Yehuda *et al.* 2004), while the protective genotype was associated with relative GR resistance in patients with PTSD symptoms. Thus, alterations in *fkbp5* function could conceivably be involved in abnormal GR-mediated signaling in neurons involved with stress response and memory formation. Those polymorphisms within the *fkbp5* gene that lead to altered GR responsiveness promote sensitization of the neural systems involved in stress response and emotional memory processing.

Mood Disorders

Several studies have also investigated the association of the *fkbp5* polymorphisms with mood disorders (Kato 2007). Although over-representation of the high-induction alleles has been reported in patients with unipolar depression (Lekman *et al.* 2008), over-transmission of a number of *fkbp5* alleles, including the high-induction alleles associated with unipolar depression, has also been reported in families with bipolar disorder (Willour *et al.* 2009). Similar to PTSD, major depression syndromes have also been associated with GR-supersensitivity in *fkbp5* high-induction allele carriers (Binder *et al.* 2004), as opposed to the GR resistance observed in healthy controls (Ising *et al.* 2008). In patients homozygous for the high induction alleles, the ACTH-response in the combined dexamethasone-CRF test was significantly lower than in carriers of the other genotypes. Nevertheless, their cortisol response was still significantly higher than the one of healthy controls.

In rodents, prenatal stress has been associated with a decrease in expression of *fkbp5* in the prefrontal cortex and this was restored by administration of antidepressant drugs (Szymanska *et al.* 2009). Unfortunately, studies on central nervous system *fkbp5* in mood disorders are limited to date, although the recent use of animal models may shed light in this field. In this regard, recent evidence showed that animals with a conventional *fkbp5* deletion are less responsive to the adverse effects of chronic stress with regards to physiological and neuroendocrine parameters. FKBP51KO mice displayed a diminished physiological and neuroendocrine response to chronic social defeat stress, evidenced by lower adrenal weights as well as lower basal corticosterone levels, an attenuated response to a novel acute stimulus and an enhanced recovery, as well as more active stress coping (Touma *et al.* 2011; Hartmann *et al.* 2012). Thus, it appears that FKBP51KO mice are less affected by chronic social stress on a number of parameters, because of an enhanced sensitivity of the GR.

Psychosocial stress

In another study in humans, the potential relationship between polymorphic variants of the *fkbp5* gene and recovery from psychosocial stress in healthy individuals was analyzed, and it was found that three single nucleotide polymorphisms in the *fkbp5* region (rs4713916, rs1360780, and rs3800737) have the strongest effect (Ising *et al.* 2008). Thus, homozygous subjects for any of these *fkbp5* genotypes displayed an incomplete normalization of the stress-elicited cortisol secretion. This was also observed following a second test additionally accompanied by an increased self-reported anxiety. Subjects carrying those variants are at risk of displaying chronically elevated cortisol levels after repeated stress, constituting a risk factor for stress-related diseases. Remarkably, no genetic association was observed between *fkbp5* and the ACTH response to psychosocial stress. A possible explanation for this counter-intuitive observation could be the involvement of non-genomic regulatory mechanisms mediated by membrane steroid receptors (Groeneweg *et al.* 2011).

Bipolar disorder is another major mental disorder characterized by repeated episodes of mania and depression, often causing severe psychosocial impairment. A recent whole-genome association study carried out by a Wellcome Trust Consortium in 2000 patients with bipolar disorder (Consortium W. T. C. C 2007) did not reveal a strong genetic risk

factor. Thus, the molecular basis of bipolar disorder remains still unknown. Intriguingly, altered intracellular calcium levels are a consistent finding in studies of bipolar disorder, and recent studies pointing the role of mitochondrial dysfunction led to the possibility that mitochondrial calcium dysregulation is involved in the pathophysiology of the disease (Kato 2008). Interestingly, our group has recently reported that FKBP51 is a major mitochondrial IMM (Gallo *et al.* 2011), such that it raises the possibility that FKBP51, like other mitochondrial IMMs such as CyPD (Doczi *et al.* 2011), could regulate calcium availability in the brain.

Drug resistance associated to *fkbp5* polymorphism

In view of the fact that the product of *fkbp5* gene is a cochaperone associated to the GR, and because the HPA axis plays a cardinal role in the regulation of the biological responses that follow stress, it is not surprising that a number of studies have been focused on the association of *fkbp5* polymorphisms with response to antidepressant drugs. A first study showed a strong association between polymorphisms in *fkbp5* and response to antidepressant drugs in 280 depressed patients (Binder *et al.* 2004). Patients homozygous for the high-induction alleles responded over 10 days faster to antidepressant treatment than patients with the other genotypes. This effect appears to be independent of the class of antidepressant drug that is used as it was observed in groups of patients treated with either conventional tricyclic antidepressants, selective serotonin reuptake inhibitors, or mirtazapine, a tetracyclic antidepressant. This might suggest that the mechanisms in which *fkbp5* is involved in a positive response to the treatment are downstream of the primary binding profile of antidepressant drugs.

In a recent pharmacogenetic study of adolescent depression (Brent *et al.* 2010), an association was found between polymorphisms of the *fkbp5* gene and the occurrence of suicidal events, most of them consistent with a recessive effect that persisted after controlling for treatment effects, and clinical covariates that were related to a suicidal outcome. The same markers found in this work, rs1360780T and rs3800373G, are those reported in association with the development of PTSD with current or past exposure to trauma in children and adults (Koenen *et al.* 2005). Studies of adult populations have also found an association between these markers and a greater number of depressive episodes, more rapid response to antidepressants, and an elevated risk for bipolar disorder. However, although there is a significant association between the *fkbp5* genotypes and suicidal events, these studies lacked a placebo condition. Healthy adult subjects who are homozygous for each of these alleles (rs1360780TT, rs3800373GG) show a greater and more prolonged cortisol response compared with those who have complementary genotypes (Ising *et al.* 2008). This is consistent with the function of the *fkbp5* gene, which codes for a protein that decreases the sensitivity of the GR to the effect of corticosteroids.

The same *fkbp5* single nucleotide polymorphisms discussed above (rs1360780 and rs3800373) were also studied from the pharmacological point of view in a sample of 246 geriatric patients treated for 8 weeks in a double-blind randomized comparison trial of paroxetine, a selective serotonin reuptake inhibitor, and the tetracyclic antidepressant mirtazapine (Sarginson *et al.* 2010). A significant association was not found between these FKBP51 genetic variants and clinical outcomes, suggesting that *fkbp5* is unlikely to play a major role in determining antidepressant treatment outcomes in geriatric patients.

The high-induction alleles of *fkbp5* that are associated with GR resistance in healthy controls are sometimes associated with enhanced GR-sensitivity in depressed patients as compared to patients carrying the other alleles. In fact, in the patients carrying the genotypes associated with faster response to antidepressant treatment, HPA-axis hyperactivity as measured by the dexamethasone-CRF test at in-patient admission was significantly reduced compared to the other patients (Binder *et al.* 2004). This could facilitate the normalization of HPA-axis hyperactivity that is associated with clinical response to most antidepressant treatments.

Depressive disorders related to HIV infection

Patients infected with human immunodeficiency virus (HIV) have a higher risk of developing major depressive disorders than the general population. Using post-mortem brains from individuals suffering from HIV with or without major depression, it was observed that depression, but not HIV-status, was associated with an increase in *fkbp5* mRNA and FKBP51 protein expression in the frontal cortex (Tatro *et al.* 2009a). More recently, a study showed increased cortical expression of FKBP51 in the mid-frontal gyrus of 55 HIV-infected subjects free of cerebral opportunistic diseases whereas no significant alterations were observed for GR or FKBP52 (Soontornniyomkij *et al.* 2012), which would represent the negative feedback to reduce GR sensitivity in the setting of chronic stress-induced elevation of GR-mediated signaling inherent in HIV infection and could be related to maladaptive stress response and HIV-associated neurocognitive disorders.

Immune Mechanisms

Recent studies where two-dimensional gel electrophoresis with DIGE (fluorescence difference gel electrophoresis) technology (Friedman and Lilley 2008) were performed to study the effects of cortisol on human monocytes (Billing *et al.* 2007) and macrophages (Billing *et al.* 2011). These studies revealed a total of 28 modulated proteins in monocytes and 20 in macrophages, FKBP51 being the

strongest regulated among all of them. Interestingly, this IMM also showed the strongest synergism of the steroid with lipopolysaccharide-activated macrophages associated with enhanced expression of the immune response and metabolic proteins. It should be noted that most proteins analyzed in these studies were down-regulated.

Importantly, it has been showed (Oishi *et al.* 2003) that *fkbp5* belongs to the clock genes and follows a circadian rhythm of the HPA axis with rapid up- and down-regulation, which exerts influence in the GR-dependent mechanisms of the immune response. The GR is a natural candidate for glucocorticoid resistance in inflammatory diseases. A significantly lower expression of GR mRNA was reported in the intestinal mucosa of patients with steroid resistant ulcerative colitis (Raddatz *et al.* 2004; De Iudicibus *et al.* 2011), whereas steroid binding affinity in mononuclear cells has been found reduced in patients with glucocorticoid resistant diseases (Kam *et al.* 1993). FKBP51 is rapidly up- and down-regulated following the circadian rhythm of the HPA-axis, which in turn affects immediately the GR-dependent response. Recent studies were conducted in asthmatic patients where the role of *fkbp5* genetic variants in the response to glucocorticoids (rs3800373, rs9394309, rs938525, rs9470080, rs9368878, and rs3798346), and not correlation with FKBP51 isoforms were detected (Hawkins *et al.* 2009), but genetic variations in the *STIP1* gene that encodes for Hsp70/Hsp90 organizing protein, Hop. This co-chaperone, that possesses a TPR-domain able to compete with IMMs for the Hsp90 acceptor site, seems to have a role in identifying asthmatic subjects who were more responsive to GC therapy (Hawkins *et al.* 2009).

Regarding the potential actions of FKBP51 in microglia, cells that represent the resident macrophage population of the brain and spinal cord, it has recently been reported (Wohleb *et al.* 2011) that the number of CD11b+/CD45high/Ly6C high macrophages that trafficked to the brain was significantly increased in mice exposed to repeated social defeat. In addition, several inflammatory markers were also increased on the surface of microglia (CD14, CD86, and TLR4) and macrophages (CD14 and CD86), as well as levels of interleukin-1β were increased. In parallel, reduced levels of glucocorticoid responsive genes such as GILZ (glucocorticoid-induced leucine zipper) and FKBP51 were detected. All these stress-dependent changes in microglia and macrophages were prevented by propranolol, a β-adrenergic receptor antagonist, suggesting a potential regulation of the microglia by activation of β-adrenergic and interleukin-1 receptors in cases of induced anxiety-like behavior. Taken together, these findings suggest that microglia may become less sensitive to the anti-inflammatory effects of glucocorticoids, perhaps because of the known inhibitory action of this IMM on GR action. Within the context of the subjects addressed in this review, these observations may establish a mechanism by which social stress increases the pro-inflammatory state of the central nervous system and promotes behavioral-related complications.

Prolonged stress disturbs the HPA-axis, and HPA-dependent mediators suppress some parts of the immune system impairing the immune response. Therefore, it was speculated that this might promote the initiation and progression of some types of cancer (Reiche *et al.* 2004). Furthermore, DNA-damage, failure in DNA-repair leading to somatic mutations, and inhibition of apoptosis are also affected by prolonged stress and could be involved in the onset and outcome of some types of cancer. This raises the possibility that individual variations in glucocorticoid sensitivity by some *fkbp5* variants such as rs1360780, which appears to be related to increased rather than inhibited glucocorticoid sensitivity (Binder *et al.* 2004; Lekman *et al.* 2008; Lovas *et al.* 2009), may predict the susceptibility of an individual to cancer and also could be a risk factor for metabolic syndrome as well.

Cushing's Disease

To date, there is no information related to the potential role of *fkbp5* polymorphisms in Cushing's Disease. Dysregulation of the HPA axis leads to the lack of suppression for the effects of cortisol and/or ACTH. Thus, the glucocorticoid cascade model shows that deficiency of the HPA axis to regulate circulating cortisol results in a feed-forward mechanism of increased cortisol levels during stress and a greatly decreased ability to return to resting conditions (Sapolsky *et al.* 1986). As a consequence, all cells are subject to prolonged GR activation and its downstream effects. It is expected that FKBP51 may attenuate GR activation at the receptor level as well as at the resulting transcriptional response. A challenging possibility is that, according to the type of *fkbp5* isoform, the syndrome may course with better or worse consequences. Because all cells of the organism should be affected including neurons, it is likely that those previously discussed effects related to high exposure to glucocorticoids may be affected by these isoforms of FKBP51. Nevertheless, there are no studies to date addressing this point and what is speculated here remains in the field of the prediction.

Envoy

The experimental evidence supports a model in which the over-induction of *fkbp5* following cortisol release in response to a stressor would lead to an impaired negative feedback of cortisol release. In turn, this extends the elevation of cortisol following a stressor. This maladaptive and prolonged stress response may render individuals more vulnerable to psychiatric disease, as shown for the cases of unipolar and bipolar depression or post-traumatic stress diseases. Inasmuch as the expression of the *fkbp5* gene is responsive to cortisol, genetic and/or epigenetic modifications that may alter such interac-

tion could modulate the effects of environmental stressors on the HPA axis and, as a consequence, the risk for stress-related disorders. This raises the possibility that specific drugs targeting the function of *fkbp5* may be useful to prevent negative long-term effects of trauma or stress on GR function, as well as to make more efficient the restorations of the dysregulated GR pathway in some disorders.

A desirable strategy for future genetic studies could be to examine different genetic variations using available technologies of dense-marker genome-wide arrays, copy number variations, and dense sequencing of areas of interest, coupled with gene expression and extensive proteomic studies as to generate eventual pathways and networks of genes implicated in neuroendocrine pathways and psychiatric phenotypes. This is particularly important for the case of individuals with greater tendency to commit suicide and cases of childhood traumas. In this regard, recent evidence was presented that variation in the *fkbp5* gene of a large sample of 2122 haplotypes of *fkbp5* associates four SNPs (rs3800373, rs9296158, rs1360780, rs9470080) with suicide attempts, but increased risk was observed only among individuals reporting high levels of childhood trauma (Roy *et al.* 2010). Measures of cognitive and biological biomarkers of treatment response and behavior should also be incorporated into genetic studies. Such approaches may even help research to go beyond association to explicate biological mechanisms whereby antidepressants are associated with an increased risk for suicidal events. Pharmacogenomic approaches hold promise for being able to identify those individuals who are at greatest risk for experiencing mental syndromes. Furthermore, the identification of genetic markers associated with increased risk, as it is the case of polymorphic variants of *fkbp5*, may not only provide clues regarding the etiology of some mental events but also the development of possible therapeutic targets.

Not commented in this article because of length limitations, it should be noted that androgens are also a positive regulators of *fkbp5* (Makkonen *et al.* 2009; Stechschulte and Sanchez 2011), which adds one more player to an already complicated equation where the final effects are greatly influenced by sex (Heim and Nemeroff 2009; Kokras *et al.* 2011). Interestingly, many of the SNPs of *fkbp5* localize close to or overlap with the regions that encompass steroid-regulated enhancers. These polymorphisms are predicted to lead to inter-individual differences in the steroid sensitivity of the FKBP51. However, greater experimentation about the effects of these SNPs on such activity is needed to confirm their functional significance.

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