



## Review

## Translating the evidence for gene association with depression into mouse models of depression-relevant behaviour: Current limitations and future potential

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## ABSTRACT

Depression is characterised by high prevalence and complex, heterogeneous psychopathology. At the level of aetio-pathology, considerable research effort has been invested to identify specific gene polymorphisms associated with increased depression prevalence. Genome-wide association studies have not identified any risk polymorphisms, and candidate gene case–control studies have identified a small number of risk polymorphisms. It is increasingly recognised that interaction between genotype and environmental factors ( $G \times E$ ), notably stressful life events, is the more realistic unit of depression aetio-pathology, with  $G \times E$  evidence described for a small number of risk polymorphisms. An important complementary approach has been to describe genes exhibiting brain region-specific expression changes in depression. Mouse models of depression informed by the human evidence allow for the study of causality, but to-date have also yielded limited insights into depression aetio-pathology. This review of the translational evidence integrates human and mouse research approaches and evidence. It also makes specific recommendations in terms of how future research in human and mouse should be designed in order to deliver evidence for depression aetio-pathology and thereby to inform the development of novel and improved antidepressant treatments.

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## 1. Introduction

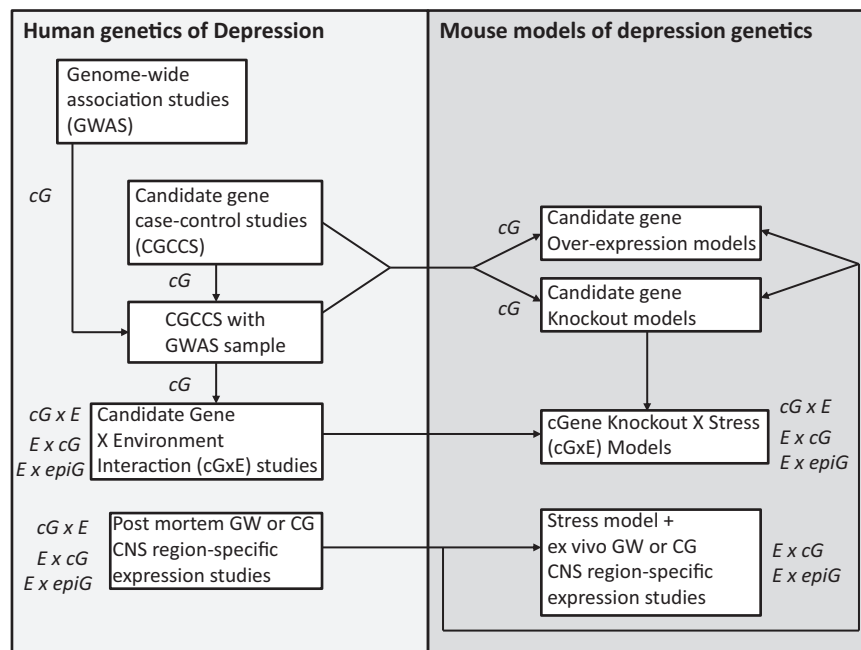
Major depressive disorder (hereafter depression) is the most prevalent disease of the central nervous system (CNS) and is one of the ten leading global causes of disease burden (Lopez et al., 2006). In the absence of a definitive understanding of its pathophysiology, depression is diagnosed exclusively on the basis of symptoms, course and outcome. According to the major diagnostic system for psychiatry (APA, 2000), depression constitutes one or both of the core symptoms, depressed mood (sadness, emptiness) and anhedonia (loss of interest or pleasure). The core symptoms must co-occur with at least four of the common symptoms, namely weight loss, insomnia, psychomotor retardation, fatigue, feelings of worthlessness/guilt, diminished ability to think/concentrate, recurrent thoughts of death or suicide, and suicide attempt/plan, for at least two weeks. Therefore, depression is a disease defined by a heterogeneous constellation of symptoms that are quite uninformative relative to the psychological dysfunctions that underlie them. The latter, in turn, are poorly understood in terms of their mediating pathophysiological processes at circuitry, cellular and molecular levels, and there is currently no pathophysiology input to the diagnosis. For the two core symptoms, depressed mood and anhedonia, neuropsychological dysfunction can be attributed, respectively, to hyper-sensitivity of the brain's punishment system and hypo-sensitivity of the brain's reward system (Pryce and Seifritz, 2011). Dysfunctional emotional-cognitive processing of punishing (aversive) stimuli/events is, at least in broad terms, a neuropsychopathology common to both depression and anxiety disorders e.g. generalized anxiety disorder. As would be expected therefore, there is a high prevalence of anxiety disorders in patients diagnosed with depression (APA, 2000).

Given the above situation, then an increased understanding of the genetics of depression is clearly vitally important. At the same time, it needs to be accepted that, given the heterogeneity of the disorder in terms of its diagnostic symptoms and the current absence of a pathophysiology basis to diagnosis, the obtaining of such increased understanding is bound to be extremely challenging. The heritability–liability estimate for depression, based on analysis of its relative concordance in monozygotic versus dizygotic twins, is 30–40%, with the remaining liability (60–70%) attributable to individual-specific environmental factors (Sullivan et al., 2000). Accordingly, aetiological models of depression emphasise the importance of both the genetic and the environmental contributions and indeed their interaction (Duncan and Keller, 2011). Gene–environment interaction ( $G \times E$ ) is itself complex and

potentially includes additive, synergistic and protective effects. Furthermore, additional factors including the potential for  $G \times E$  effects to be developmental-stage specific (Ansorge et al., 2007) and for their mediation by epigenetic mechanisms rather than specific DNA nucleotide sequences (Petronis, 2010), add to the complexity of understanding depression aetiology (see Section 2.6). One important consequence of these various levels of complexity has been the recognition that it will be essential to study aetiology in terms of specific markers or dimensions of depression in addition to – or quite possibly even instead of – its heterogeneous entirety. This will include analysis of the inter-relationships between genes and depression-relevant endophenotypes and between  $G \times E$  and depression-relevant state markers or intermediate phenotypes, with both of these approaches conducted at the level of cells, neurocircuits and behaviour.

The present review aims to present the case that progress can be made in understanding the genetics of depression by focussing on those genes for which there is robust (e.g. with independent replication) evidence for association with depression and then studying these same genes in valid mouse models of depression. The review sets the scene by summarizing the current status of the evidence for the genetics (i.e. genetic aetiology) of depression<sup>1</sup>. This evidence is presented under the methodological sub-headings: genome-wide association studies, candidate gene case–control studies, gene–environment interaction studies,  $G \times E$  – state marker and  $G$  – endophenotype studies, *post mortem* gene expression studies, and mediating mechanisms. For each gene for which one or more of these methods has provided robust evidence for an association with depression (specifically, with replication in the case of association studies), the current evidence for the impact of this gene in mouse models, is presented. The mouse evidence is presented in sections corresponding to those used to present the human data, with descriptions of the effects of manipulation of the relevant genes on depression-relevant behaviour and of the effects of depression-relevant environmental events on the brain expression of the relevant genes. Fig. 1 illustrates the approach used. This review of the current evidence is followed by a critical assessment of the experimental designs used and the evidence obtained to date. The review concludes with proposals for future experimental designs with the aim of maximizing the potential in mouse models for increasing understanding of

<sup>1</sup> As stated at the outset, here we are deploying the generic term depression to refer to major depressive disorder and are not addressing bipolar disorder.



**Fig. 1.** Schema to illustrate the inter-relationships between human and mouse studies aimed at increased understanding of the genetic aetio-pathophysiology of depression, and the overall approach used in this review. The methods of GWAS and CGCCS have been applied in humans to attempt to identify polymorphisms associated with depression (cG approach). Candidate genes have been studied in terms of their association with depression in interaction with environmental events, which can involve three major mechanisms: Polymorphism-specific predisposition to respond to the environment such that depression risk is increased ( $cG \times E$ ). Environmental events impacting on expression of a specific gene via altered transcription factor activity ( $E \times cG$ ), perhaps depending on the latter's genotype. Environmental events impacting on expression of a specific gene via altered epigenetic processes ( $E \times epi-cG$ ). The human descriptive evidence for the genetics of depression thus obtained has been applied to inform mouse experimental studies that are controlled and can yield cause–effect evidence. As in humans, studies of the mechanisms  $cG$ ,  $cG \times E$ ,  $E \times cG$  and  $E \times epi-cG$ , need to be applied in the mouse models. The current review is organised according to the study methods included in this figure.

the aetio-pathology of depression and its pharmacological treatment.

## 2. Current status of the human evidence for the genetics of depression

### 2.1. Genome-wide association studies

Genome-wide association study (GWAS) allows for hypothesis-free, population-level identification of those genes in which a variant, typically a single-nucleotide polymorphism, is associated with a phenotype, which can range from a specific character to a complex disorder. Genome-wide association studies are based on a case–control design in which a large sample of patients and controls are compared in terms of genotyping single-nucleotide polymorphisms (SNPs) across the entire human genome. The frequency of each polymorphism of each SNP is compared for differences between the patient and control cohorts. Given the enormous number of pair-wise statistical tests that this requires, the threshold of statistical significance is stringent and the required sample size correspondingly very large (Manolio, 2010). Of the eight GWAS studies for depression published to-date, seven have reported no loci and one has reported one locus of possible genome-wide statistical significance, namely the neuronal amino acid transporter gene *SLC6A15* (Kohli et al., 2011) (Table 1). A mega-analysis of these studies included more than 9000 subjects each for the depression and control groups, and 1.2 million single-nucleotide polymorphisms (SNPs); no locus of genome-wide statistical significance was identified (Sullivan and Consortium, 2012). Given the genetic architecture hypothesised for depression (e.g. a large number of contributing loci, small effect sizes,  $G \times G$  interaction effects and  $G \times E$  interaction effects), the heterogeneity of its symptoms and state markers, and its high prevalence, even such a mega-analysis

is proposed to be underpowered to detect “genes for depression” at the genome-wide level (Sullivan and Consortium, 2012).

### 2.2. Candidate gene case–control association studies

A considerable number of case–control association studies of specific candidate genes for depression have been conducted, stimulated by specific hypotheses for aetio-pathogenesis. Findings have been largely negative and inconsistent. Given that insufficient sample size/statistical power could be a limiting factor, meta-analysis of such studies has been carried out. An analysis of all depression case–control genetic association studies published up to 2007 revealed 22 polymorphisms that had been examined in at least three independent studies, and these were chosen for meta-analysis. This identified significant association of a polymorphism with depression for six genes: dopamine receptor 4 (*DRD4*), apolipoprotein (*APOE*), guanine nucleotide-binding protein subunit beta-3 (*GNB3*), methylenetetrahydrofolate reductase (*MTHFR*), dopamine transporter (*SLC6A3*), and serotonin transporter (*SLC6A4*) (Lopez-Leon et al., 2008) (Table 1). For at least one of these genes, namely *MTHFR*, a subsequent well-powered case–control study did not replicate the association (Gaysina et al., 2008).

Another approach has been to analyse polymorphism associations reported in more than one case–control study in a relatively large sample collected for GWAS; the focus on specific candidates allows for an increase in power in the statistical model used (Bosker et al., 2011). Of the 57 genes/92 SNP candidates analysed, three SNPs associated with depression were identified, one in the chromosome 5 open reading frame 20 gene (*C5orf20*) (also referred to as dendritic cell nuclear protein-1 (*DCNP1*)), one in the neuropeptide Y gene (*NPY*), and one in the tumor necrosis factor gene (*TNF*). In addition, the norepinephrine transporter gene (*NET*) exhibited significantly more SNPs with an association with depression than would be expected by chance (Bosker et al., 2011) (Table 1). It is

**Table 1**

Summary of genes for which one polymorphism has been reported to exhibit relatively high association with depression.

Gene	Risk polymorphism and biology	Study method	Reference
<i>SLC6A15</i> , Neuron-specific neutral amino acid transporter. Putative role in glutamate synthesis/transmission	SNP: <i>rs1545843</i> AA versus AG + GG. AA associated with > reduction in hippocampal volume in depressed versus control probands versus AG/GG	GWAS	Kohli et al. (2011)
<i>SLC6A4</i> , Serotonin transporter (5-HTT). Integral membrane protein for pre-synaptic removal of serotonin from synaptic cleft	2 × 22–23-bp insertion/deletion: <i>deletion</i> (S) versus insertion (L) in promoter region. (5-HTTLPR) S associated with < 5-HTT activity	CGCC, Meta-analysis	Lopez-Leon et al. (2008)
<i>DRD4</i> , Dopamine receptor 4 (D4). G protein-coupled receptor, activated by DA, inhibits adenylate cyclase, reducing cyclic AMP	48-bp VNTR: 2 repeats versus 4, 7 repeats in exon 3	CGCC, Meta-analysis	Lopez-Leon et al. (2005)
<i>APOE</i> , Apolipoprotein E. Catabolism of triglyceride-rich lipoproteins	Two SNPs: <i>rs429358</i> + <i>rs7412</i> allele $\epsilon 3$ versus allele $\epsilon 2$	CGCC, Meta-analysis	Lopez-Leon et al. (2008)
<i>GNB3</i> , Guanine nucleotide-binding protein, beta polypeptide 3. Beta-3 subunit of GNB proteins (G-proteins), which integrate signals between receptors and effector proteins	SNP: <i>rs5445</i> TT versus CT/CC T associated with > G protein activity	CGCC, Meta-analysis	Lopez-Leon et al. (2008)
<i>MTHFR</i> , Methylene tetrahydrofolate reductase. Enzyme for conversion of 5,10-MTHFR to 5-MTHFR. Low 5-MTHF (and low dietary intake of folic acid) leads to high homocysteine levels	SNP: <i>rs1801133</i> TT versus TC + CC TT associated with < MTHFR activity	CGCC, Meta-analysis	Lopez-Leon et al. (2008) but see Gaysina et al. (2008)
<i>SLC6A3</i> , Dopamine transporter (DAT1). Integral membrane protein for pre-synaptic removal of dopamine from synaptic cleft	40-bp VNTR: <i>rs28363170</i> 9/10 repeats versus 10/10 repeats in untranslated 3' region 9/10 associated with < DAT1 binding	CGCC, Meta-analysis	Lopez-Leon et al. (2008)
<i>C5orf20</i> , chromosome 5 open reading frame 20 or <i>DCNP1</i> , dendritic cell nuclear protein-1 (DCNP1). Expressed in dendritic cells (antigen-presenting cells that activate T cells and B cells)	SNP: <i>rs12520799</i> TT versus AT + AA T encodes premature termination of DCNP1 translation i.e. shorter protein	GWAS + CGCC	Wills-Owen et al. (2006), Bosker et al. (2011)
<i>TNF</i> , Tumor necrosis factor. Pro-inflammatory cytokine	SNP: <i>rs769178</i> C versus A	GWAS + CGCC	Bosker et al. (2011)
<i>NPY</i> , Neuropeptide Y. Neurotransmitter in CNS and autonomic NS	SNP: <i>rs16147</i> T versus C, T associated with < NPY; SNP: <i>rs16139</i> C versus T, C associated with < NPY	GWAS + CGCC	Bosker et al. (2011)
<i>SLC6A2</i> , Norepinephrine transporter (NET). Integral membrane protein for pre-synaptic removal of norepinephrine from synaptic cleft	SNP: <i>rs5558</i> G versus T, G associated with > NET; Many SNPs each with low association	GWAS + CGCC	Haenisch et al. (2008), Bosker et al. (2011)
<i>OPCML</i> , Opioid binding protein/cell adhesion molecule-like, Member of immunoglobulin superfamily, Essential for coupling between opioid receptors and G proteins, Co-localized with 5-HT, GABA	SNP: <i>rs12276491</i> AG versus AA	Family-based linkage analysis + GWAS for linked regions	Schol-Gelok et al. (2010)
<i>FKBP5</i> , FK506 binding protein 5. Inhibitor of corticosteroid via glucocorticoid receptor	Five SNPs each with a polymorphism that increases risk of depression in interaction with trauma	Prospective CG study	Zimmermann et al. (2011)
<i>CRHR1</i> , Corticotropin releasing hormone receptor 1. Stress-responsive neuropeptide and neurotransmitter	Multiple SNPs and one haplotype each with a polymorphism that increases risk of depression in interaction with child abuse	Retrospective CG study	Bradley et al. (2008)
<i>BDNF</i> , Brain-derived neurotrophic factor. CNS neurotrophin	SNP: <i>rs6265</i> A versus G, A associated with < BDNF increases depression score in children in interaction with 5-HTTLPR S and maltreatment	Prospective CG study	Kaufman et al. (2006)

striking that *C5orf20*, *NPY* and *TNF* each has an important function in the immune system: *C5orf20* is important in antigen presentation, *NPY* is involved in T helper type 1 cell differentiation, and *TNF* is a pro-inflammatory cytokine. Limitations of this study were that one-third of the candidate SNPs were not present on the microarray chip, and certain length polymorphisms associated with depression, including the 22–23-bp insertion/deletion polymorphism in the promoter region of *SLC6A4*, were not detectable (Bosker et al., 2011; Lopez-Leon et al., 2008).

The final gene-depression association to be presented in this section was reported in a genome-wide linkage analysis conducted in a family-based depression study (Schol-Gelok et al., 2010). A linkage to depression was determined for four chromosomal regions. In one of these, 11q25, a follow-up GWAS study combined with a high-power statistical model identified a significant association of the opioid binding protein/cell adhesion molecule-like (*OPCML*) gene with depression (Schol-Gelok et al., 2010) (Table 1).

In summary, as for GWAS, the candidate gene case-control (CGCC) approach has identified only a small number of genes in

which one polymorphism exhibits differential association with depression. The approach of Lopez-Leon et al. (2008), of considering only those polymorphisms in which evidence for association had been replicated is to be commended and, indeed, the criterion of replication is advocated for all approaches to identifying gene-depression association (e.g. Duncan and Keller, 2011). However, it should be noted that the last replication meta-analysis was conducted with studies published up to 2007 (Lopez-Leon et al., 2008) and it is therefore possible that replication of initial CGCC association findings has been obtained for some additional genes in the mean time.

### 2.3. Gene-environment interaction studies

According to the G × E aetiology model (e.g. Caspi and Moffitt, 2006), depression is the consequence of bi-directional interaction between specific genetic traits in the brain or body and environmental events (e.g. stressors) that induce physiological changes. Specifically, the model proposes that the altered



genomic-proteomic expression resulting from  $G \times E$  is an aetiological trigger for depression. The  $G \times E$  model acknowledges the complexity of the aetio-pathology of depression. The most informative interaction would be that which involves an allele of a gene that is associated with a marked (synergistic) increase in the prevalence of depression in individuals that have experienced environmental stress, whilst other alleles of the gene are without effect (i.e. they exert a relative resilience effect).

The candidate gene (cG) for which  $G \times E$  (or  $cG \times E$ ) provides the most convincing evidence to-date for an aetiological association with depression is *SLC6A4* (serotonin transporter), which is also one of the small number of genes that has been linked with depression using candidate gene case-control association (i.e.  $G$  main-effect; Section 2.2, Table 1). In a longitudinal prospective study, the percentage of probands diagnosed for depression at age 25 years depended on genetic status in terms of the 22–23-bp insertion/deletion (“long”, L/“short”, S) polymorphism in the promoter region of *SLC6A4* in interaction with the number of stressful life events (SLE) experienced in childhood/young adulthood. Those carriers of the S allele who had experienced  $\geq 3$  SLEs were 2–3 times (35% prevalence) more likely to be depressed than the probands in each of the other  $G \times E$  groups, among which the likelihood of depression was equable (10–15% prevalence) (Caspi et al., 2003). A meta-analysis of this and the many subsequent studies of this  $G \times E$  interaction provided positive evidence when the SLEs were experienced in childhood (Karg et al., 2011), although the methodology used for this meta-analysis has received some criticism (Duncan and Keller, 2011).

Additional examples of candidate genes for which evidence for  $G \times E$  association with depression have been reported include the FK506 binding protein 51 gene (*FKBP5*) and the corticotropin-releasing hormone receptor type 1 gene (*CRHR1*) (Table 1). These genes encode, respectively, FKBP5, a member of the protein complex that modulates function of the glucocorticoid receptor, and *CRHR1*, the major receptor mediating the neuroendocrine and neurotransmitter stress responses caused by CRH. These stress-relevant genes were selected as candidates for  $G \times E$  association with depression based on the potential for specific polymorphisms to increase reactivity to environmental stress. For *FKBP5*, young-adult subjects who were homozygous for minor SNP alleles and who had experienced traumatic events were at increased risk of depression (Zimmermann et al., 2011). For *CRHR1*, adult depression was associated with interaction between SNP alleles and childhood exposure to parental abuse (Bradley et al., 2008), with high cortisol titres also being associated with the same  $G \times E$  interaction (Tyrka et al., 2009).

As is the case for  $G$  main effects, it is also likely that  $G \times E$  studies have been underpowered to-date. There are several reasons for this, including the vicious circle of the low probability of selecting the correct genes and environmental factors for analysis, because of the current poor understanding of depression aetiology (Duncan and Keller, 2011). Genome-wide  $G \times E$  association studies would be a valuable approach to overcome this current deficit. Another reason is that depression aetiology is likely to be polygenic and poly-environmental. The  $G \times E$  model can be built-up to, in principle, include a large number of genes and several age-specific environmental stressors i.e. the  $G^n \times E^n$  model. Given the heterogeneity of the diagnostic entity of depression, then it is probably unrealistic to expect to identify the  $G^n \times E^n$  terms that explain its aetiology. Focussing on specific depression symptoms, or associated neural or psychological state markers/intermediate phenotypes, and ensuring that the study sample is homogeneous for the symptom/state marker under investigation, is predicted to be more conducive to the discovery of  $G^n \times E^n$  models that account for a substantial proportion of the prevalence of those symptoms/state markers. Even further focus, to

the level of genetic traits or endophenotypes that predict symptoms/state markers, is the realistic level for identification of main effects of specific alleles (Hasler and Northoff, 2011), as discussed next.

#### 2.4. $G \times E$ – state marker and $G$ – endophenotype association studies

Referring to the association of  $G \times E$  with specific symptoms/state markers of depression, it is again the serotonin transporter gene – *SLC6A4* – that has received most attention. Healthy probands homozygous for the S allele with a history of stressful life events (high SLE) exhibited increased bilateral amygdala activation in response to fearful faces (Alexander et al., 2012), and increased cortisol response to a laboratory social stressor (Alexander et al., 2009), relative to each of the other  $G \times E$  groups. Furthermore, the S 5-HTTLPR  $\times$  high SLE probands were characterized by an increased functional coupling between the right amygdala and the hypothalamus, which would be consistent with a link between neural and neuroendocrine hyper-reactivity (Alexander et al., 2012). In another study, healthy probands were screened for negativity bias, i.e. the extent to which the person focuses emotionally and cognitively on negative life events. Within the group displaying high negativity bias, probands carrying the S allele who experienced early life stress exhibited particularly high negativity bias, associated with increased activation (fMRI BOLD) in the vmPFC (Brodmann's area (BA)11) during exposure to fearful stimuli (Williams et al., 2009). Focussing on specific markers in this way also facilitates advancing beyond black-box aetiology to pathophysiology. For example, recently it has been proposed that S 5-HTTLPR  $\times$  high SLE leads to a level of activity in the circuitry involving amygdala, anterior cingulate cortex and dorsal raphe nucleus that is commensurate with depression (Disner et al., 2011; Jasinska et al., 2012). In line with the evidence that the S allele is a risk genotype in interaction with SLE in early life particularly, the interaction between the 5-HTTLPR polymorphism and early childhood adversities was studied in terms of negative feedback sensitivity to reward omission using the probabilistic reversal learning test (see Section 3.2) (Owens et al., 2012). There was a significant  $G \times E$  interaction for negative feedback sensitivity (NFS) due to the relatively low and high NFS in S carriers without and with early life SLE, respectively, relative to the L-carrier groups (Owens et al., 2012).

Also at the level of endophenotype, i.e. without taking SLEs into account, the 5-HTTLPR polymorphism has been the major focus. The association of the S and L alleles with amygdala activation in response to aversive stimuli e.g. photographs of fearful faces, as assessed using functional imaging, has been investigated in a number of studies. The first such study reported that healthy S allele carriers exhibit relatively increased amygdala activation by aversive stimuli (Hariri et al., 2002). A meta-analysis of 5-HTTLPR genotype and amygdala activation included both healthy probands and patients, and reported that overall the S allele is associated with a small increase in amygdala activation by aversive stimuli relative to the L allele (Murphy et al., 2012). In the negativity bias study (Williams et al., 2009), the S allele was associated with increased activation in the midbrain, dmPFC (BA6) and ACC (BA32) in response to fearful faces (Williams et al., 2009). A meta-analysis of association between 5-HTTLPR genotype and cortisol reactivity to acute psychosocial stress reported increased reactivity in carriers of the S allele (Miller et al., 2012). It has been hypothesized that the life-span phenotypes of S 5-HTTLPR are a consequence of its effects on the 5-HT system during early life development of corticolimbic circuitry, and that S allele carriers will therefore be sensitive to  $G \times E$  during early life development, with long-term consequences (Caspi et al., 2010).

**Table 2**Summary of *post mortem* and translational studies of brain region-specific gene expression in depression.

Gene or functional gene group	Study design	Brain region of interest	Direction of expression change	Reference
Oligodendroglia/Myelination	MDD versus Control	Temporal cortex, BA21	Down-regulation	Aston et al. (2005)
Axonal growth/Path finding	MDD: M/nM, S/N		Down-regulation	
Synaptic proteins	Microarray		Down-regulation	
Signal transduction protein kinases			Down-regulation	
Protein phosphatases			Down-regulation	
Other phosphoregulation-related receptors			Down-regulation	
Chromatin/gene regulation			Down-regulation	
Protein synthesis/Degradation			Down-regulation	
Other			Down-regulation	
Transcriptional activation	MDD versus Control	vPFC, BA44	Down- or Up-regulation <sup>a</sup>	Klempan et al. (2009)
Ion transporter activity	MDD: nM, S		Down- or Up-regulation <sup>a</sup>	
Cell cycle control and division	Microarray		Down- or Up-regulation <sup>a</sup>	
GABA neurotransmission			Down- or Up-regulation <sup>a</sup>	
Glutamate neurotransmission			Down- or Up-regulation <sup>a</sup>	
Second messenger systems			Down- or Up-regulation <sup>a</sup>	
Tricarboxylic acid cycle	Microarray	vPFC, BA45	Down-regulation	
Cell cycle regulation			Down- or Up-regulation <sup>a</sup>	
Cell maturation			Down- or Up-regulation <sup>a</sup>	
Astrocyte	Microarray	vPFC, BA46	Down- or Up-regulation <sup>a</sup>	
Presynaptic proteins			Down- or Up-regulation <sup>a</sup>	
GABA neurotransmission			Down- or Up-regulation <sup>a</sup>	
Glutamate neurotransmission			Down- or Up-regulation <sup>a</sup>	
Cell maturation	Microarray	vPFC, BA47	Down- or Up-regulation <sup>a</sup>	
Synapse formation			Down- or Up-regulation <sup>a</sup>	
Tricarboxylic acid cycle			Down- or Up-regulation <sup>a</sup>	
Glutamate transporters	MDD versus Control	dACC, BA24	Down-regulation	Choudary et al. (2005)
Glutamine synthetase ( <i>GLUL</i> )	MDD: M/nM, S/N		Down-regulation	
AMPA Glutamate receptor ( <i>GRIA1</i> )	Microarray		Up-regulation	
Glutamate transporters		dIPFC, BA9	Down-regulation	
Glutamine synthetase ( <i>GLUL</i> )			Down-regulation	
Ionotropic glutamate receptors			Up-regulation	
GABA <sub>A</sub> receptor subunits			Up-regulation	
Stresscopin ( <i>UCN3</i> )	MDD versus Control	dIPFC, BA9	Up-regulation	Kang et al. (2007)
Forkhead box 3 ( <i>FOXO3</i> )	MDD: M/nM, S/N		Up-regulation	
Cannabinoid receptor 2 ( <i>CNR2</i> )	Microarray		Up-regulation	
Intracellular signal transduction			Down- or Up-regulation <sup>a</sup>	
Extracellular signal transduction			Down- or Up-regulation <sup>a</sup>	
Cell cycle regulation			Down- or Up-regulation <sup>a</sup>	
Cell differentiation			Down- or Up-regulation <sup>a</sup>	
Apoptosis			Down- or Up-regulation <sup>a</sup>	
Chromatin/gene regulation			Down- or Up-regulation <sup>a</sup>	
Pro-inflammatory cytokine pathway (incl. <i>TNFRSF11B</i> (TNF-family receptor), <i>IFNAR1</i> (interferon receptor 1))			Up-regulation	
Y-box-binding protein 1 ( <i>YBX1</i> )	MDD versus Control	ant. PFC, BA10	Up-regulation	Shelton et al. (2011)
Caspase-1 dominant-negative	MDD: nM, S/N/A		Up-regulation	
Inhibitor pseudo-ICE ( <i>COP1</i> )	Microarray		Up-regulation	
Apoptosis inhibitor ( <i>FKSG2</i> )			Up-regulation	
Pro-inflammatory cytokine pathway (incl. <i>IL-6</i> , <i>IFN<math>\gamma</math></i> , <i>TNF</i> )			Up-regulation	
Anti-inflammatory cytokine pathway (incl. <i>IL-10</i> )			Up-regulation	

Table 2 (Continued)

Gene or functional gene group	Study design	Brain region of interest	Direction of expression change	Reference
Neuron structure/function, e.g.: Potassium channel tetramerisation domain containing 12 ( <i>KCTD12</i> )	Familial MDD versus Control + Mouse UCMS versus Control MDD: M/nM, S/N	Amygdala	Up-regulation	Sibille et al. (2009)
Calcium channel, voltage-dependent, Microarray beta 2 subunit ( <i>CACNB2</i> )			Up-regulation	
Calcium/calmodulin-dependent protein kinase II delta ( <i>CAMK2D</i> )			Up-regulation	
Oligodendrocyte structure/function, e.g.: Plasma membrane proteolipid ( <i>PLLP</i> )			Down-regulation	
Myelin-associated oligodendrocyte basic protein ( <i>MOBP</i> )			Down-regulation	
G-protein coupled receptor 37 ( <i>GPR37</i> )			Down-regulation	

M/nM: proband prescribed (M)/not prescribed (nM) antidepressant medication at time of death. Cause of death: S suicide, N natural, A accidental.

<sup>a</sup> Dependent on specific gene.

### 2.5. Post mortem gene expression studies

In terms of using a life span approach to research into the genetics of depression,  $G \times E$  – particularly early life  $E$  – aetiology studies are at one extreme, and *post mortem* studies at the other. *Post mortem* quantification of gene expression in target brain regions allows for identification of consistent down- or up-regulation of gene expression relative to an appropriate control group. As for polymorphism-depression association studies, either a GWAS or candidate gene approach is used. Indeed, in principle (although to date rarely in practise), quantification of gene expression can be combined with genotyping in order to assess whether changes in gene expression associated with depression are polymorphism-specific. The major findings of these studies are summarised below and in Table 2. In *post mortem* gene expression studies, the cause of death for probands in the depressed cohort are suicide, natural causes and accidental, with the latter two also being the causes of death in the control cohort. Given that suicide is most common in people who have experienced repeated depression episodes and are treatment-resistant, gene expression studies typically include subjects with this severe form of depression. The brain regions of interest are based on current understanding of the neurocircuitry of depression (e.g. Disner et al., 2011), and include temporal cortex, frontal cortex, subgenual anterior cingulate cortex, and amygdala (Table 2).

For temporal cortex, a GWAS study (Aston et al., 2005) identified significant expression changes for families of genes involved in neurodevelopment, signal transduction and cell communication. A number of genes related to oligodendrocyte function were decreased in their expression in depression, including genes encoding structural components of myelin and genes encoding enzymes for synthesis of myelin constituents (Table 2). Decreases in multiple myelination-related genes could contribute to the reduction in white matter observed in depression, which is proposed to cause deficient axonal function and synaptic degeneration (Aston et al., 2005).

For frontal cortex, a study of dorsolateral prefrontal cortex (dlPFC, Brodmann's area (BA) 9) and ventral PFC (BA47) did not identify any significant gene expression-depression associations, neither by GWAS nor by a candidate-gene approach based on specific aetio-pathology hypotheses (e.g. 5-HT receptors) (Sibille et al., 2004). In a GWAS study of primarily vPFC (BA44, 45, 46, 47), genes exhibiting altered expression in depression relative to controls were grouped according to their function (ontology). Genes involved in cell cycle control and cell division exhibited altered expression in BA44 in depression, as did genes involved in transcription in BA44 and BA47, genes involved in myelination in BA46, and genes for GABA and glutamate receptors in BA46; however, the direction of change in expression for each individual gene was unspecified (Klempner et al., 2009) (Table 2). A study of dorsal

anterior cingulate cortex (dACC, BA24) and dlPFC (BA9) also identified altered expression of genes involved in GABA and glutamate neurotransmission in depression (Choudary et al., 2005). Another study of BA9 identified dysregulation of the genes stresscopin (*UCN3*), Forkhead box 3 (*FOXO3*) and cannabinoid receptor 2 (*CNR2*), in depression (Kang et al., 2007) (Table 2).

A GWAS study of anterior PFC (BA10) identified up-regulation of genes involved in apoptosis. Furthermore, analysis of genes according to functional groups provided evidence for up-regulation of a number of pro- and anti-inflammatory cytokine genes (Shelton et al., 2011) (Table 2). The study of BA9 also identified increased expression of a TNF receptor and an interferon receptor in depression (Kang et al., 2007). In addition to this post-mortem evidence and the identification of depression-association of polymorphisms of immune function genes in case-control studies (Section 2.2; Bosker et al., 2011), a meta-analysis of studies of blood levels of cytokines in depression reports increased levels of the pro-inflammatory cytokines TNF and interleukin-6 (IL-6) (Dowlati et al., 2010). These various lines of evidence have contributed to the increasing interest in the pro-inflammatory cytokine hypothesis of depression (Dantzer et al., 2008). Both descriptive human and experimental animal studies demonstrate the association between psychosocial stress and increased cytokine levels in the periphery (human, animal) and CNS (animal) (Miller et al., 2009). Increased cytokine activity in humans, related to autoimmune disease, cytokine therapy or an experimental procedure, is associated with a number of depression symptoms including dysphoria, anhedonia, fatigue, decreased appetite and weight loss. In rodents, induced increases in cytokine levels lead rapidly to a period of sickness (fever, anorexia, hypokinesia, hypersomnia) that subsides and is followed by a period of depression-relevant behaviour (e.g. reduced sucrose preference, see Table 3) (Dantzer et al., 2008; Schiepers et al., 2005).

For subgenual anterior cingulate cortex (ACC, BA25) and amygdala, a study was conducted using a cross-species approach: Microarray was conducted in a depression versus control human sample and in mice exposed to chronic unpredictable stress (CUS, a composite environmental stressor over weeks involving different manipulations e.g. placement in another animal's cage, reversal of light/dark cycle to activate stress systems) versus control handling. Genes exhibiting a change in expression in the same direction in depressed humans and CUS mice were regarded as significantly affected (Sibille et al., 2009). For amygdala, there were reciprocal changes in gene expression in depressed humans and CUS mice, and according to various criteria, a core set of 32 genes, expressed in either glia or neurons, was identified. Examples of up-regulated genes were those encoding: potassium channel tetramerisation domain containing 12 protein (*KCTD12*) of the GABA receptor B; calcium channel, voltage-dependent, beta 2 subunit (*CACNB2*); and calcium/calmodulin-dependent protein kinase II delta (*CAMK2D*).

**Table 3**

Important depression psychopathologies, relevant human neuropsychological tests and corresponding mouse tests.

Psychopathology (relevant symptom)	Human test	Mouse test
Loss of pleasure/enjoyment of reward (Anhedonia)	Emotional reactivity/sensitivity to positive (visual) stimuli e.g. photos of happy faces	Relative reactivity to palatable stimulus versus water e.g. sucrose preference test
Loss of interest in/incentive for reward (Anhedonia)	Motivational reactivity to rewarding stimuli e.g. performing cognitive task for money	Operant responding for palatable stimulus on effortful motivation schedule e.g. variable-interval, progressive ratio reinforcement
High reactivity to aversive stimuli (Depressed mood)	Emotional reactivity/sensitivity to negative (visual) stimuli e.g. photos of sad or fearful faces	Emotional reactivity to conditioned stimuli associated with aversion e.g. fear conditioned freezing
Stress uncontrollability (Depressed mood, Helplessness)	Emotional-cognitive reactivity to aversive uncontrollability e.g. Learned helplessness effect	Escape behaviour in two-way shuttle box e.g. Learned helplessness effect
High negative feedback sensitivity (Depressed mood, Catastrophization)	Emotional-cognitive response to negative feedback e.g. Probabilistic reversal learning	Emotional-cognitive response to negative feedback e.g. Probabilistic reversal learning
High bias to negative expectancy (Depressed mood, Pessimistic outlook)	Emotional-cognitive reactivity to ambiguous stimuli e.g. Ambiguous-stimulus operant test	Emotional-cognitive reactivity to ambiguous stimuli e.g. Ambiguous-stimulus operant test
Fatigability (Fatigue)	Physical effort to complete a manual task e.g. Grip strength test	Effort-reward operant behaviour e.g. Treadmill running to avoid aversive stimulus

Modified from Pryce and Seifritz (2011).

Examples of down-regulated genes were: plasma membrane proteolipid (*PLLP*); myelin-associated oligodendrocyte basic protein (*MOBP*); and G protein-coupled receptor 37 (*GPR37*) (Sibille et al., 2009) (Table 2).

Summarizing the findings of *post mortem* studies of depression-associated changes in gene expression in specific brain regions, there is currently evidence for prefrontal cortex, anterior cingulate cortex, temporal cortex and amygdala. For a number of functional classes of genes, there is evidence that expression levels of genes in the same functional class are changed in the same direction in at least two different brain regions. Examples of such functional classes with potential aetio-pathological importance for depression include astrocyte- and oligodendrocyte-specific genes, synaptic protein genes, cytokine pathway genes, GABA-signalling/function genes and glutamate-signalling/function genes.

## 2.6. Mediating mechanisms: From polymorphic risk to epigenetic response

The major aim of this review is to assess the extent to which mouse models provide supportive causal evidence for the human descriptive evidence that depression is associated with specific gene polymorphisms and with (*post mortem*) altered levels of expression of specific genes. Nonetheless, it is also important to consider the mechanisms via which these inter-relationships can be mediated. There are three major categories of mediating mechanism: Firstly, a polymorphism in a specific gene can result in increased/decreased expression of its protein product, which in turn predisposes the individual to respond to SLEs such that depression risk is increased ( $cG \times E$ ). Second, an environmental event (e.g. SLE) results in increased/decreased levels of physiological-neurobiological factors that lead to increased/decreased activity of specific transcription factors and increased/decreased expression of specific genes, in turn leading to increased depression risk ( $E \times cG$ ). The impact of the increase/decrease in the level of a physiological-neurobiological factor might depend on polymorphism in relevant genes. Third, an environmental event (e.g. SLE) results in increased/decreased levels of physiological-neurobiological factors that lead to increased/decreased activity in epigenetic processes, in turn leading to increased depression risk ( $E \times \text{epi-}cG$ ). The epigenetic processes are altered methyltransferase activity and level of methylation of DNA, leading to decreased transcription-factor binding in promoter regions of specific genes; and de/acetylation of the histone proteins that regulate chromatin density, leading to, respectively, decreased/increased accessibility of DNA in promoter

regions of specific genes. An example for each of the three mechanisms via which altered gene expression can be linked to increased risk for depression is provided here.

$cG \times E$ : The *SLC6A4* short variant provides a well-studied example of a polymorphism associated with a distinctive protein phenotype that predisposes its carriers, via  $cG \times E$ , to increased depression prevalence. The  $cG \times E$  interaction is with early life/developmental stressors (see Section 2.3). Serotonin has important functions as a neurotransmitter and trophic factor during the maturation of the CNS, including modulation of neuronal division, differentiation and migration, growth cone elongation, synaptogenesis and dendritic pruning. It has been proposed that high levels of 5-HT during CNS development, as facilitated by reduced 5-HTT levels in 5-HTTLPR S carriers, will impact on these processes (Ansorge et al., 2007). Developmental stressors might interact with high basal 5-HT and/or might act *a posteriori* on the consequences of high basal 5-HT, to increase the depression risk of 5-HTTLPR S carriers.

$E \times cG$ : The evidence that SLEs lead to increased levels of pro-inflammatory cytokines and that the cytokine treatments used for certain illnesses often lead to depression, is consistent with environment-induced expression of pro-inflammatory cytokine genes providing an example of the second mechanism via which altered gene expression increases depression risk. That polymorphism at the *TNF* gene is associated with depression (Table 1) suggests that, for this cytokine,  $cG \times E$  and  $E \times cG$  mechanisms could act in unison.

$E \times \text{epi-}cG$ : Despite the justifiable growing interest in SLEs leading to brain region-specific changes in gene expression in depression via effects on epigenetic processes, to-date there is very limited evidence for epigenetic markers in the *post-mortem* depressed brain. One relevant study has reported that in hippocampal tissue from adult suiciders with a developmental history of abuse, decreased glucocorticoid receptor (GR) expression co-occurred with increased DNA methylation in the GR promoter (McGowan et al., 2009). Nevertheless, animal studies have yielded evidence that genes associated with human depression do exhibit changes in expression of depression-relevant genes that are associated with epigenetic changes in response to environmental factors. Examples include: decreased hippocampal GR expression and increased GR-promotor DNA methylation in adult rats that experienced low levels of maternal behaviour (Weaver et al., 2004); decreased hippocampal BDNF expression and decreased acetylation levels on histone residues in adult mice and rats that experienced social defeat and exhibited depression-relevant behaviour in adulthood (Sun et al., 2013); and increased acetylation levels on histone residues in adult mice that experienced



environmental enrichment and improved cognition in adulthood (Fagiolini et al., 2009). Clearly, epigenetic factors are at the interface between environmental stimuli and long-lasting – indeed even inter-generational – changes in gene expression that impact on molecular, cellular, regional and behavioural phenotypes (Petronis, 2010).

### 3. Mouse models for the genetics of depression

#### 3.1. Genetic tools

The study of the genetic regulation of brain function and behaviour in mice in order to increase understanding of the contribution of expression of specific genes to the aetio-pathogenesis of depression can be divided into two broad methodological strategies. These strategies are complementary to those used in human gene polymorphism-depression association studies and depression-gene expression studies, respectively. Firstly, the effects in mice of manipulation of genes for which specific polymorphisms have been demonstrated to be differentially associated with depression in human, can be studied i.e. the reverse genetics approach. One major method here is homologous recombination of a gene with non-transcriptional DNA to generate a null mutant (knockout) or heterozygous mutant (partial knockout) mouse (Jackson and Abbott, 2000). For a human gene in which a polymorphism leading to reduced biological activity of the encoded protein is associated with depression, mice that are partial knockout for the homologous gene could provide an appropriate genetic model in comparison with the wildtype. Another major reverse genetic method is gene knock-in: With transgenic knock-in a specific additional allele and its regulatory regions are incorporated into the genome to generate “over-expressing” mice. With targeted knock-in, an allele already present in the mouse is targeted with a specific mutation that impacts on its transcription or translation (Jackson and Abbott, 2000). For a human gene in which a polymorphism leading to increased biological activity of the encoded protein is associated with depression, mice that over-express the protein (transgenic knock-in) or express the protein in its depression-risk amino acid sequence (targeted knock-in) could provide an appropriate genetic model in comparison with the wildtype (Jackson and Abbott, 2000). Such heterozygous-knockout and knock-in mouse models and their wildtype controls can be studied in depression-relevant readouts in order to investigate genetic aetiology of endophenotypes and their mediating mechanisms. Furthermore, such mice can be exposed to environmental-stress manipulations followed by study in depression-relevant readouts in order to investigate  $G \times E$  interaction aetiology of state markers and their mediating mechanisms. The second broad methodological strategy is to study the expression of genes in specific brain regions in mice modified genetically and demonstrated to exhibit depression-relevant endophenotypes, or in mice (possibly modified genetically) exposed to environmental stress and demonstrated to exhibit depression-relevant state markers, relative to their respective control groups i.e. the forward genetics approach (Causton et al., 2003).

Some of the major evidence obtained to-date from mouse models aimed at increased understanding of the genetics of depression is summarised below. The evidence is presented according to the two methodological strategies of reverse (Section 3.3) and forward genetics (Section 3.4) used to obtain it.

#### 3.2. Behavioural readouts

As noted in the Section 1: depression is classified as a heterogeneous group of mental and physical symptoms; the neuropsychological processes and their pathological states that underlie

the mental symptoms need to be characterized thoroughly; specific depression-relevant pathological states will demonstrate a much closer causal relationship with the expression levels of depression-relevant genes than will the diagnostic entity of depression. Major examples of psychopathological states associated with depression are given in Table 3, together with the symptom which each state underlies/is associated with. Another major advantage of the focus on specific neuropsychological processes is that it renders the study of depression considerably more amenable to animal model studies. In this regard, also given in Table 3 are some major examples of the behavioural tests that have been established to measure depression-relevant neuropsychological processes in humans, together with the analogous tests that have been developed in mice.

To recall, the major rationale for this review is to take those genes for which there is descriptive evidence for an association with depression and then to review the experimental (causal) evidence obtained in mice for the involvement of these genes in the regulation of depression-relevant behaviour. Accordingly, it would of course be optimal if the mouse studies conducted into behavioural effects of the genes in question had focused on translational depression-relevant behavioural tests, as given in Table 3. In reality, the behavioural effects of manipulation of the identified depression-associated genes have been studied to-date in terms of sucrose preference (Willner, 1997), fear conditioned freezing (Stiedl et al., 1999), the specific learned helplessness effect (or unconditioned stimulus pre-exposure effect) (Pryce et al., 2012), and the probabilistic reversal learning test (Ineichen et al., 2012). In the sucrose preference test, mice are presented with two bottles, one filled with sucrose (or saccharin) solution and one with water and the proportion of consumption of the sweet-tasting solution relative to total consumption is measured across several hours/days. The proportion of consumption of the sweet-tasting solution is typically 80–90% and decreases are therefore detectable and interpreted as reduced reward sensitivity. In fear conditioned freezing, mice are exposed to a specific context and a specific discrete stimulus (e.g. tone) that predicts an aversive unconditioned stimulus, typically inescapable electro-shock. During this exposure, mice develop freezing behaviour and they also express this fear behaviour on subsequent days, when re-exposed to the context or the discrete stimulus. Freezing in this test provides a measure of reactivity to aversive stimuli and is sensitive to the mouse's reactivity to the aversive unconditioned stimulus as well as its reactivity in terms of learning and memory about the context and discrete stimulus that predict the unconditioned stimulus. In the specific learned helplessness effect test, two groups of mice are pre-exposed to the same number, duration and intensity of aversive unconditioned stimuli, typically electro-shocks, and in one group these stimuli are escapable and in the other group they are inescapable. The mice pre-exposed to inescapable aversive stimuli exhibit a deficit in escape behaviour when, at the test phase, they are exposed to escapable e-shocks. In the unconditioned stimulus pre-exposure effect version, mice are pre-exposed to either inescapable or no aversive stimuli. In the probabilistic reversal learning test, mice are trained to exhibit operant behaviour to obtain reward and to exhibit serial spatial reversal learning. Super-imposed on the spatial reversal schedule, a low proportion of correct responses are punished in the form of non-delivery of reward. The major readout is, on the next trial, whether mice exhibit appropriate lose-stay or inappropriate lose-shift behaviour, with high levels of the latter indicating high negative feedback sensitivity. As stated in Table 3, the specific learned helplessness effect test and the probabilistic reversal learning test can be applied in both humans and mice and, furthermore, learned helplessness is increased (Pryce et al., 2011) and probabilistic reversal learning is deficient (Taylor Tavares et al., 2008) in depression.

A significant number of the relevant genetic mouse model studies have been conducted with four additional categories of behavioural test, and these are also included in this review. The first additional category that has been frequently used to study the effects of depression-associated genes is the current-antidepressant screening tests, namely the forced swim test (FST) (Lucki et al., 2001; Porsolt et al., 1977) and the tail suspension test (TST) (Steru et al., 1985). In both of these tests, motor inactivity develops across time in the test, and this increase in inactivity is antagonised by acute or chronic administration of reference antidepressant drugs, primarily selective serotonin reuptake inhibitors (Cryan et al., 2005; Lucki et al., 2001). The neuropsychological processes underlying both the development of inactivity and its pharmacological antagonism are unclear. Whilst behavioural despair resulting from uncontrollable stressor exposure is often cited as the mediating mechanism, this is a subjective interpretation that cannot be tested and such tests are deficient in the face and construct validity that are essential to conferring depression-relevance (Pryce and Seifritz, 2011). The justification for nonetheless including these studies is that the findings for effects or non-effects of depression-relevant genotypes in these tests might potentially increase understanding of the neuropharmacological mechanisms mediating current-antidepressant effects. The second additional category of behavioural test that has been used to study the effects of manipulation of depression-associated genes in mice is cognitive tests, represented by the delay discounting test of impulsivity, the Go/No-go test of response inhibition/impulsivity (Helms et al., 2008), and the continuous performance test of sustained attention incorporating a No-go response inhibition component (Young et al., 2011). The inclusion of tests of cognitive function is justified by cognitive deficits being common in depression, by these tests being applicable in both humans and mice, and by human depression being associated with deficits in these tests (Koetsier et al., 2002). The third additional category of behavioural test that has been frequently used to study the effects of manipulation of depression-associated genes in mice is the anxiety/anxiolytic screening tests, represented by the elevated plus maze test (Dawson and Tricklebank, 1995) and the light–dark box test (Crawley and Goodwin, 1980). These tests are based on the conflicting emotional-/motivational-state hypothesis of anxiety (Gray and McNaughton, 2000), with the conflict being approach–avoid relative to the respective physical properties of the different areas of the apparatus in the case of both the elevated plus maze and light–dark box tests. The justification for including studies reporting on these tests here is the high prevalence of anxiety disorders in depression (see Section 1) and the possibility that the findings for effects or non-effects of depression-relevant genotypes in these tests could increase understanding of the genetic aetiology of psychopathology relevant to anxiety disorders and depression. The fourth additional category of behavioural test that has been used frequently to study the effects of manipulation of depression-associated genes in mice is the general activity tests, represented by the open field test (Prut and Belzung, 2003). Measurement of general locomotion in a novel environment allows for assessment of the effect of a genetic manipulation on basic activity: given that other tests are activity dependent and their findings can be confounded by general activity effects, it is essential that basic activity is included in the behavioural phenotyping of the genetic model.

### 3.3. Mouse models informed by the human evidence for polymorphisms associated with depression

In this section, studies are presented that have addressed the effects of knockout or over-expression of genes for which it has been demonstrated, either by GWAS, CGCCS meta-analysis and/or G × E study, that specific polymorphisms are differentially

associated with depression. The relevant genes and their risk polymorphisms are given in Table 1.

#### 3.3.1. Serotonin transporter (mouse nomenclature: *Slc6a4*, 5-HTT, *SERT*; protein: 5-HTT, *SERT*)

Serotonin transporter (5-HTT) is synthesised by 5-HT neurons and is located on 5-HT axon terminals where it performs pre-synaptic reuptake of 5-HT and thereby is a major regulator of 5-HT level and post-synaptic receptor binding in the synaptic cleft. The serotonin transporter gene (*SLC6A4*) is associated with depression in terms of polymorphism in the copy number of a 22–23-bp repeat that constitutes the 5-HTTLPR, with either 14 ((S)hort/deletion) or 16 ((L)ong/insertion) copies of the repeat (Haddley et al., 2008). To summarize, the S allele was associated with increased risk of depression in meta-analysis (Lopez-Leon et al., 2008) (Table 1). The interaction of the S allele × multiple SLEs – particularly early life SLEs – was associated with increased risk of depression in meta-analysis (Karg et al., 2011). In healthy probands, the S 5-HTTLPR allele was associated with increased neuroticism (Lesch et al., 1996) and increased amygdala activation by fearful faces (Hariri et al., 2002). The interaction S allele × SLE was associated with increased bilateral amygdala activation in response to fearful faces (Alexander et al., 2012), increased cortisol reactivity to acute psychosocial stress (Alexander et al., 2009; Miller et al., 2012), and increased negativity bias (Williams et al., 2009) (see above, Sections 2.3 and 2.4). There is in vitro evidence that the S allele leads to reduced 5-HTT expression and function (Haddley et al., 2008). Human lymphoblast cell lines with different 5-HTTLPR genotypes were compared in terms of 5-HTT binding sites and 5-HT uptake: the S allele was associated with decreased 5-HTT activity in both the L/S and S/S genotypes (Lesch et al., 1996). The in vivo evidence is more equivocal: one SPECT study reported decreased 5-HTT binding in midbrain by S carriers but other SPECT studies reported no genotype effect. Also, positron emission tomography (PET) studies have not found any genotype differences in 5-HTT binding (Parsey et al., 2006; Shioe et al., 2003).

There is no mouse orthologue of the human 5-HTTLPR. *Slc6a4* KO mice have been generated and studied. Relative to WT, the 5-HTT KO mouse exhibited markedly increased extracellular 5-HT and reduced 5-HT clearance (Mathews et al., 2004; Montanez et al., 2003). Repeated testing in the FST led to a greater development of immobility in KO relative to WT mice (Wellman et al., 2007). KO mice spent less percent time on the open arms of an elevated plus maze and in the light compartment of a light–dark box, indicating increased anxiety, relative to WT (Holmes et al., 2003). Exposure to predator (cat) odour resulted in KO mice spending less percent time on the open arms of an elevated plus maze and in the light compartment of a light–dark box, relative to WT (Adamec et al., 2006). It is the HET–WT comparison that provides a mouse model for the reduced 5-HTT function associated with the S relative to L 5-HTTLPR genotype. 5-HTT HET mutant mice exhibited reduced 5-HT clearance and increased extracellular 5-HT levels, but otherwise normal 5-HT transmission, relative to WT (Jennings et al., 2010; Mathews et al., 2004; Montanez et al., 2003). HET mice exhibited increased fear-conditioned freezing to the context in which they experienced repeated moderate electro-shocks, relative to WT (Pryce et al., 2012). HET mice exhibited reduced negative feedback sensitivity following reward omission in the probabilistic reversal test (Ineichen et al., 2012), similar to human 5-HTTLPR S carriers (see Section 2.4). HET mice did not differ from WT in tests of anxiety (Holmes et al., 2003) including after exposure to predator odour (Adamec et al., 2006). A number of studies have investigated for differential effects of environmental stressors on HET versus WT mice i.e. G × E models. HET mice that received low levels of maternal care exhibited a reduced latency to immobility in the TST relative to HET mice that received high levels of maternal care but not

relative to WT mice (Carola et al., 2008). Postnatal exposure to repeated electro-shocks was without effect on adult behaviour in FST, elevated plus maze, light–dark box, and open field, both as a main effect and in interaction with genotype (Carroll et al., 2007). In a learned helplessness model, HET and WT mice were pre-exposed across consecutive days to either escapable or inescapable electro-shocks and then all mice were tested in terms of escape behaviour. Both HET and WT mice pre-exposed to escapable electro-shocks exhibited escape behaviour at test; the deficit in escape behaviour in the mice that had experienced inescapable electro-shocks was greater in HET than WT mice, that is, HET resulted in increased sensitivity to an aversive uncontrollable environment (Pryce et al., 2012) (Table 3). Mice over-expressing 5-HTT have been generated and, whilst not constituting a model for any depression-relevant human genotype, can be studied to assess whether such over-expression leads to depression-relevant phenotypes. Only anxiety tests have been applied in such mice to-date, including the elevated plus maze test: 5-HTT over-expression led to reduced anxiety (Jennings et al., 2006; Line et al., 2011).

The 5-HTT HET versus WT model provides a relevant analogue for the 5-HTTLPR S versus L polymorphism at the genotypic level. Furthermore, there is some evidence for analogy at the level of behavioural phenotypes: healthy S carriers exhibit increased neuroticism and reactivity to fearful stimuli; HET mice exhibited increased fear conditioning and developed increased learned helplessness. Further 5-HTT  $\times$  E mouse studies are required to increase understanding of the interaction between 5-HTT genotype and SLE, which appears to be specific to early life SLE, and human depression.

### 3.3.2. Dopamine transporter (mouse nomenclature: *Slc6a3*, *DAT*; protein: *DAT*)

Dopamine transporter (*DAT*) is synthesised by DA neurons and is located on DA axon terminals where it performs pre-synaptic reuptake of DA and thereby is a major regulator of DA level and post-synaptic receptor binding in the synaptic cleft. The dopamine transporter gene (*SLC6A3*) is associated with depression in terms of a polymorphism in the copy number of a 40 base-pair (bp) variable number tandem repeat (VNTR), present in the 3' untranslated region (3'-UTR) (Lopez-Leon et al., 2008). The majority of *SLC6A3* alleles have either nine or ten 40-bp repeats in their 3'-UTR. Increased depression risk for carriers of the 9/10 genotype compared to the 10/10 genotype was demonstrated in a meta-analysis (Lopez-Leon et al., 2008) (Table 1). The evidence for the relationship between the 3'UTR VNTR polymorphism and *DAT* function is currently inconsistent: in vivo single-photon emission computed tomography (SPECT) studies variously reported that the 9/10 genotype is associated with lower levels of *DAT* expression in the striatal putamen compared to 10/10, or that a genotype with at least one 9 allele is associated with higher levels of striatal *DAT*, or the absence of an association between this genotype and *DAT* density/function (Haddley et al., 2008). In vitro studies of transfection of reporter plasmids containing one of the *SLC6A3* 3'-UTR VNTRs have also yielded conflicting results regarding transcriptional activity: depending on the cell line used, the 9 or the 10 3'-UTR VNTR yielded higher transcription levels or there was no difference between the two VNTRs (Haddley et al., 2008).

The human *SLC6A3* VNTR polymorphism is not present in the mouse *Slc6a3* (*DAT*) gene. Mice that were *DAT* KO exhibited markedly increased extracellular DA levels and slower clearance of synaptic DA relative to WT; HET mice were intermediate but also with increased extracellular DA and slower DA clearance relative to WT (Gainetdinov et al., 1998; Jones et al., 1998). The DA phenotypes of KO and HET mice co-occurred with down-regulation of both pre- and post-synaptic DA receptors (Haenisch and Bönisch, 2011). Behaviourally, KO mice exhibited increased locomotor activity relative to WT (Giros and Jaber, 1996; Pogorelov et al., 2005).

In a separate study (Perona et al., 2008), KO mice again exhibited hyper-locomotion relative to WT and relative to HET also. Furthermore, KO mice exhibited decreased immobility in the forced swim test (FST) and the tail-suspension test (TST) compared to WT; these phenotypes could reflect the hyper-locomotion phenotype. These *DAT* KO mice also exhibited increased sucrose preference relative to WT and HET. *DAT* HET mice also exhibited decreased immobility in the TST relative to WT and, in contrast to KO, this did not co-occur with hyper-locomotion. There was no difference between HET and WT in the sucrose preference test (Perona et al., 2008). In an open field, *DAT* HET mice spent more time in the centre than did WT (Pogorelov et al., 2005). C57BL/6 mice that had been reared in an enriched environment exhibited reduced *DAT* binding potential and reduced *DAT* expression per DA neuron, throughout the striatum (Bezard et al., 2003). However, to our knowledge, there has been no study of effects of environmental manipulations in *DAT* KO or HET mice relative to WT (i.e.  $G \times E$  study). Mice over-expressing *DAT* did not differ from WT in their initial (exploratory) locomotor activity when first exposed to a locomotor activity test, but after repeated exposures the *DAT* OE mice exhibited a more rapid decrease in activity, reflecting faster habituation to the test environment relative to WT mice (Donovan et al., 1999). Dopamine transporter knockdown (*DAT* KD) mice have also been generated that express 10% of WT *DAT* levels and have chronically increased extracellular DA in the striatum (Cagniard et al., 2006). These mice exhibit increased operant responding for sucrose on a progressive ratio schedule relative to WT, thereby demonstrating that *DAT* activity is an important regulator of reward incentive motivation.

Full knockout and almost complete knockdown of *DAT* constitute extreme situations that are unlikely to be of direct relevance to the role of *DAT* in mediating human depression risk, but are valuable in demonstrating the fundamental importance of *DAT* to psychological processes, such as reward processing, that are of direct relevance to depression. Meaningful interpretation of the above behavioural phenotypes of *DAT* KO, HET and KD mice relative to WT in terms of their relevance to the role of *DAT* in human depression will first require elucidation of the relationship between 9/10 VNTR *SLC6A3* genotype and *DAT*/DA phenotype. Extrapolating from the mouse evidence, it is to be expected that this risk genotype encodes increased mesocorticolimbic *DAT* activity and decreased sensitivity to reward.

### 3.3.3. Dopamine receptor 4 (mouse nomenclature: *Drd4*; protein: *D4*)

The dopamine receptor 4 (*D4*) belongs to the G protein-coupled receptor family and is expressed in the brain, at particularly high levels in prefrontal cortex and nucleus accumbens, regions central to emotional processing and depression (Murray et al., 2011). One function of *D4* is inhibition of adenylyl cyclase and reduced conversion of adenosine triphosphate (ATP) to the secondary messenger cyclic adenosine monophosphate (cAMP) (Lopez-Leon et al., 2005). In a case-control candidate gene meta-analysis, the dopamine receptor gene 4 (*DRD4*) was associated with depression in terms of a polymorphism in the copy number of a 48 base-pair (bp) VNTR present in exon 3. It is the 2 repeats genotype that has been associated with increased depression risk (Lopez-Leon et al., 2005) (Table 1). There is currently no definitive understanding of a relationship between this *DRD4* polymorphism and *D4* function. Effects on dopamine binding or G-protein activation have been proposed; for example, the third cytoplasmic loop of *D4*, which contains the sequence encoded by the VNTR, can act as a SH3 binding domain and the VNTR may determine its dopamine binding capacity (Oak et al., 2000).

Mice have been generated that are null mutant for *Drd4* (Falzone et al., 2002). In an elevated plus maze test, *Drd4* KO mice exhibited fewer open arm entries and spent less per cent time on the open



arms than WT, a difference that could not be attributed to altered general locomotor activity. In a light–dark box test, *Drd4* KO mice displayed an increased latency to first enter and spent less time in the lit compartment, relative to WT. There was no difference between *Drd4* KO and WT mice in terms of fearful freezing during conditioning or subsequent exposure to tone CS or context (Falzone et al., 2002). In one study reporting on tests of cognitive function, namely the delay discounting test and the Go/No-go test, there were no effects of *Drd4* KO on behaviour relative to WT (Helms et al., 2008). In another study, HET mice exhibited response disinhibition on a No-go test built into a continuous performance test, relative to WT (Young et al., 2011), which is of relevance given that depressed probands were also reported to exhibit response disinhibition in a continuous performance test (Koetsier et al., 2002). In a rotarod motor coordination test, *Drd4* KO mice displayed reduced spontaneous locomotor activity but fewer falls and remained longer on the rotating rod compared to WT. These KO mice exhibited increased dopamine synthesis and turnover compared to WT, indicated by higher DOPAC and L-DOPA titres in the dorsal striatum-caudate putamen, suggesting that one D4 function is as an inhibitory autoreceptor (Rubenstein et al., 1997).

Therefore, constitutional absence of *Drd4* led to adult mice that exhibit increased anxiety in novel environment tests; there was no effect on fear conditioning. Definitive understanding of the relationship between the *DRD4* polymorphism and D4 function will be necessary before depression-relevant mouse genetic models of altered *Drd4* function can be produced.

### 3.3.4. Apolipoprotein E (mouse nomenclature: *ApoE*; protein: ApoE)

Apolipoprotein E (ApoE) is synthesised widely in the periphery and the CNS and is essential for catabolism of triglyceride-rich lipoprotein constituents. The human apolipoprotein E gene (*APOE*) is polymorphic in terms of exon SNPs leading to three alleles, *APOE*- $\epsilon$ 2, - $\epsilon$ 3 and - $\epsilon$ 4, which encode different amino acids at two different positions of the ApoE protein. Allele- $\epsilon$ 3 is regarded as the neutral genotype. Allele- $\epsilon$ 4 is the largest known genetic risk factor for late-onset sporadic Alzheimer's disease. Allele- $\epsilon$ 2 is associated with both increased and decreased risk for atherosclerosis and with increased risk for hyperlipoproteinemia (Mahley and Rall Jr, 2000). In meta-analysis, the  $\epsilon$ 2 allele has also been demonstrated to be associated with a lower risk for depression compared to the  $\epsilon$ 3 allele (Lopez-Leon et al., 2008) (Table 1). There is growing evidence for the interaction of ApoE with immunological processes, including macrophage and T-cell functioning (Zhang et al., 2010a).

Transgenic female mice expressing human *APOE*- $\epsilon$ 2, - $\epsilon$ 3 or - $\epsilon$ 4 have been generated (Siegel et al., 2012). When compared on the elevated plus maze, the *APOE*- $\epsilon$ 2 mice spent less time on the open arms than did *APOE*- $\epsilon$ 3 mice, suggesting that *APOE*- $\epsilon$ 2 was anxiogenic. In the same study, there was a trend to increased ApoE levels in the amygdala, hippocampus and cortex in *APOE*- $\epsilon$ 2 relative to *APOE*- $\epsilon$ 3 mice (Siegel et al., 2012). Mice knockout for *ApoE* exhibited a phenotype of spontaneous atherosclerosis. When such mice were exposed to chronic unpredictable stress (CUS) (see below, Section 3.4), atherosclerosis was further increased (Zhang et al., 2010b). Furthermore, a number of blood pro-inflammatory biomarkers were also increased, including C-reactive protein and IL-6. Therefore, the G  $\times$  E interaction of *ApoE* KO  $\times$  CUS leads to high levels of atherosclerosis associated with a marked pro-inflammatory response (Zhang et al., 2010b). Extrapolating the mouse findings to the protective association of *APOE*- $\epsilon$ 2 with depression, one possibility is that this genotype leads to relatively high ApoE levels that are protective against psychosocial stress; given that psychosocial stress stimulates immune-inflammatory activation (Miller et al., 2009), a reduced psycho-immune response could reduce the risk

of depression. To our knowledge, no relevant behavioural studies of this G  $\times$  E have been conducted to-date.

### 3.3.5. Guanine nucleotide-binding protein, beta polypeptide 3 (mouse nomenclature: *Gnb3*; protein: GNB3)

The guanine nucleotide-binding proteins (G proteins) mediate signals between receptors and effector proteins, including the G protein-coupled receptors of several neurotransmitters in the CNS. G proteins are composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits and the *GNB3* gene codes for a  $\beta$  subunit. A SNP (C825T) in *GNB3* was associated with depression in a meta-analysis (Lopez-Leon et al., 2008). The T allele is the risk factor (Table 1). Carriers of this allele also had an increased risk of hypertension and obesity, both of which are co-morbid for depression. The T allele is associated with alternative splicing of the gene and the formation of a truncated but functionally active  $\beta$ 3 subunit which is referred to as G $\beta$ 3s. Expression of the splice variant results in an enhanced G protein activation on stimulation of G protein-coupled receptors (Siffert, 2001). To our knowledge, to-date there has been no study of mice with engineered changes in *Gnb3* expression in terms of depression-relevant neurobiology or behaviour.

### 3.3.6. Methylene tetrahydrofolate reductase (mouse nomenclature: *Mthfr*; protein: NAD(P)H)

The *MTHFR* gene encodes the enzyme methylenetetrahydrofolate reductase (NAD(P)H). NAD(P)H is important for a chemical reaction also supported by forms of the B-vitamin folate (folic acid, vitamin B9). Specifically, NAD(P)H converts 5,10-MTHF to 5-MTHF, the folate required for the multi-step process that converts homocysteine to methionine, an amino acid that is essential in neural tube development, for example. A common SNP in *MTHFR* is C677T, which results in an amino acid change. The TT genotype results in reduced NAD(P)H activity and elevated homocysteine levels in blood. It is the TT genotype that is associated with depression in case-control candidate gene meta-analysis (Lopez-Leon et al., 2008) (Table 1).

*Mthfr* null mutant mouse models have been generated; the heterozygous mutant (HET) would be expected to exhibit reduced NAD(P)H activity and therefore to provide an analogue of the depression-risk SNP of *MTHFR*. KO, HET and WT mice were studied in various behavioural tests. *Mthfr* KO mice exhibited increased activity in an open field relative to WT and HET, and spent increased time in the open arms of an elevated plus maze. Using an object recognition test, KO mice exhibited both medium- and long-term object memory impairment compared to WT and HET mice. These behavioural changes were associated with decreased total brain weight and hippocampal volume relative to WT and HET mice. There were no behavioural or brain differences between HET and WT mice (Jadavji et al., 2012). In a separate study, which compared HET and WT genotypes, HET male, but not female, mice exhibited increased open-field activity relative to WT (Levav-Rabkin et al., 2011).

Therefore, constitutional absence of *Mthfr* led to adult mice that exhibit increased activity and possibly even decreased anxiety in a novel environment test. *Mthfr* HET mice exhibit increased activity but no change in anxiety. Depression-relevant tests are still to be assessed in this mouse model, but it is noteworthy that there is no anxiety phenotype, at least in the absence of environmental manipulation, suggesting that the mouse does not provide a model for the study of variation in human *MTHFR* expression and function.

### 3.3.7. Neuron-specific neutral amino acid transporter (mouse nomenclature: *Slc6a15*; protein: SLC6A15)

Neuronal amino acid transport by SLC6A15 has been proposed to be important in neuronal metabolism by functioning as a provider of substrates for glutamate synthesis (Bröer et al., 2006). SLC6A15,



the gene encoding neuron-specific neutral amino acid transporter, was associated with depression in a GWAS (Kohli et al., 2011). The study identified a SNP occurring in the vicinity of *SLC6A15* and the AA genotype of the SNP was associated with both increased risk of depression relative to AG+GG and with reduced expression of *SLC6A15* in the hippocampus (Kohli et al., 2011) (Table 1). Expression of *SLC6A15* was also reduced in lymphoblastoid cell lines and peripheral blood monocytes derived from carriers of the depression-risk genotype relative to the other genotypes. These findings indicate that the SNP is affecting *SLC6A15* expression, via long-range regulatory mechanisms (Kohli et al., 2011). In the GWAS study that identified the *SLC6A15*-depression association, it was also reported that depressives exhibited reduced hippocampal volume relative to control probands and that this reduction was more pronounced in depressives with the AA risk genotype (Kohli et al., 2011). In a separate study, depressives carrying the risk genotype demonstrated increased plasma ACTH and cortisol activity relative to AG/GG carriers (Schuhmacher et al., 2012).

To the best of our knowledge there have been no studies conducted with mice genetically-engineered for *slc6a15*. Mice that had been subjected to chronic social stress during adolescence exhibited decreased hippocampal expression of *slc6a15* relative to control mice (Kohli et al., 2011). Integrating the human and mouse findings, there is preliminary evidence indicating that low levels of *SLC6A15* in the hippocampus (and possibly other CNS regions), caused by genotype or stress or their interaction, could increase susceptibility to depression-relevant CNS function. Given that *SLC6A15* could be a limiting factor in glutamate synthesis, there could be a functional connection between *SLC6A15* polymorphism and the recent interest in the glutamate NMDA receptor-antagonist, ketamine, as an antidepressant (Aan Het Rot et al., 2012).

### 3.3.8. Chromosome 5 open reading frame 20 (mouse nomenclature: *C5orf20*; protein: DCNP1)

The *C5orf20* gene encodes dendritic cell nuclear protein-1, which is expressed in the dendritic cells – one type of the various antigen-presenting cells that activate T cells and B cells in the immune system – of the CNS and skeletal muscle. A T–A SNP was identified in *C5orf20*, and a T at this locus codes for premature termination of DCNP1 translation leading to a shorter protein. Carriers of the TT genotype are at increased risk of depression relative to A/T + A/A, according to a study that used a combined GWAS and candidate gene case–control approach (Bosker et al., 2011) (Table 1). To the best of our knowledge there have been no studies conducted with mice genetically-engineered for *C5orf20* to-date.

### 3.3.9. Tumor necrosis factor (mouse nomenclature: *Tnf*; protein: TNF)

The *TNF* gene encodes tumor necrosis factor, the pro-inflammatory cytokine that is synthesized by macrophages and T cells in the periphery and by neurons and glia in the CNS (Miller et al., 2009). A C–A SNP has been identified in *TNF* that was associated with depression (Bosker et al., 2011) (Table 1). The publication does not state whether C or A is the risk nucleotide and any association between the SNP and *TNF* expression and/or *TNF* function also remains to be described. A meta-analysis of blood levels of cytokines in depressed relative to control probands reported that *TNF* levels were increased in depression (Dowlati et al., 2010).

Mice that are null mutant for *Tnf* have been generated, as have mice null mutant for the *TNF* receptors, *TNF* receptor superfamily member 1a (*Tnfrsf1a* (*Tnfr1*)) and *TNF* receptor superfamily member 1b (*Tnfrsf1b* (*Tnfr2*)). Relative to WT, *Tnf* KO mice exhibited reduced time spent in locomotion and increased time spent grooming in an open field, and reduced percent time on the open arms of an elevated plus maze; these phenotypes are consistent with *Tnf*

KO leading to increased anxiety. The same study reported that, relative to WT, KO mice exhibited decreased immobility in the FST (Yamada et al., 2000). Mice that were null mutant for *Tnfr1* also exhibited decreased immobility in both the FST and TST, relative to WT (Kaster et al., 2012). Mice that were null mutant for *Tnfr1* and *Tnfr2* exhibited reduced anxiety in the light–dark box test (and reduced social aggression in the resident–intruder test), relative to WT (Patel et al., 2010).

When mice are treated with an infectious agent that stimulates a pro-inflammatory immune response and a period of sickness (see Section 2.5), this is followed by depression-relevant behaviours including decreased sucrose preference. *TNF* is proposed to be a major mediator of these neurobehavioural effects of the pro-inflammatory immune response (Dantzer et al., 2008). Sickness can be induced by intracerebroventricular (i.c.v.) infusion of *TNF*. That this effect of *TNF* is mediated by *TNFR1* was demonstrated by *Tnfr1* KO mice being resistant to i.c.v. *TNF*-induced sickness, whereas both *Tnfr2* KO and WT mice were fully responsive (Palin et al., 2009). Infusion of i.c.v. *TNF* led to increased immobility in the FST and TST without altering locomotor activity in an open field test (Kaster et al., 2012).

Therefore, it has not yet been established whether the *TNF* SNP associated with depression is functional and, if so, whether the risk polymorphism leads to increased or decreased *TNF* activity. At the protein level, there is evidence that *TNF* is increased in depression. In mouse, reduced *TNF* signaling has been demonstrated to either increase or decrease anxiety, to reduce immobility in FST and TST, and to reduce social aggression. Increased *TNF* activity is also a major characteristic of mouse models in which an induced pro-inflammatory response leads to depression-relevant behaviour.

### 3.3.10. Neuropeptide Y (mouse nomenclature: *Npy*; protein: NPY)

The *NPY* gene encodes neuropeptide Y, a 36-amino acid neuropeptide/neurotransmitter localized in the CNS and autonomic nervous system (ANS). Two SNPs have been identified in *NPY* that are associated with depression: One SNP (rs16147) is a T–C polymorphism in the promoter region of *NPY* that leads to inclusion of a different amino acid in preproNPY signal peptide; the T polymorphism is associated with depression. The other SNP (rs16139) is also a T–C polymorphism and here it is the C that is associated with depression. The study which described these associations also described reduced CSF *NPY* levels in the depressed patients relative to control probands, suggesting that the risk polymorphisms are associated with decreased *NPY* activity (Heilig et al., 2004) (Table 1). Another study demonstrated an association of the rs16147 SNP with depression in a G × E interaction, where the environmental factor was early life stress (Sommer et al., 2010). Measurement of *NPY* mRNA in postmortem ACC tissue from depressive and control probands demonstrated that the rs 16147 T-allele was associated with decreased *NPY* expression (Sommer et al., 2010). The endophenotype/state marker approach has been applied to *NPY* and its association with depression: in healthy subjects studied with fMRI, probands with a *NPY*-haplotype associated with low *NPY* expression demonstrated relatively high mPFC activation to negatively valenced words. Furthermore, probands with low-expression *NPY*-haplotype reported more negative affect in response to a pain-stress challenge (Mickey et al., 2011).

*NPY* is a key example of a gene for which investigation into its association with depression was stimulated by the evidence for *NPY* regulation of emotion/stress, as obtained in animal studies. *Npy* is expressed in rat and mouse CNS in hypothalamus, neocortex, hippocampus and amygdala, and is mainly located in inhibitory GABA interneurons. Furthermore, within the amygdala and hippocampus, exogenous *NPY* exerts an anxiolytic effect (Heilig et al., 2004). Neuropeptide Y levels are increased by repeated stress, suggesting that up-regulation of *Npy* expression could contribute to stress

coping (Heilig et al., 2004). Male *Npy* KO mice exhibited increased percent time spent on the open arms of an elevated plus maze relative to WT, whereas there was no effect of genotype in females (Painsipp et al., 2011). In the same study, in the FST, both male and female *Npy* KO mice exhibited increased floating/immobility and decreased swimming and climbing, relative to WT. There was no effect of *Npy* KO on locomotor activity in the open field in this study (Painsipp et al., 2011). Neuropeptide Y exerts its anxiolytic effects through post-synaptic receptor 1 (NPY1R) in the amygdala; however, it also exerts anxiogenic effects via pre-synaptic receptor 2 (NPY2R) in the amygdala. Accordingly, the effects of *Npy* KO on mouse emotional behaviour would be expected to be complex, as indeed would NPY modulation of human emotional behaviour. Using a combination of conditional *Npy2r* KO mice and viral vectors to induce knockout, the effects of *Npy2r* KO in specific amygdala nuclei could be investigated (Tasan et al., 2010). Deleting *Npy2r* expression in the central amygdala also resulted in reduced NPY2R, presumably pre-synaptic, in central amygdala projection regions, including bed nucleus of the stria terminalis, nucleus accumbens and locus coeruleus. Behaviourally, such a deletion of NPY2R resulted in mice spending increased percent time on the open arms of an elevated plus maze, increased percent time in the light compartment of the light–dark test, and decreased immobility in the TST. These findings suggest that pre-synaptic NPY2R inhibits GABA and/or NPY release from interneurons and/or projection neurons of the central amygdala and thereby exerts a net anxiogenic effect on behaviour under acute environmental challenge (Tasan et al., 2010).

Integrating the human and mouse evidence suggests that decreased NPY levels are a risk factor for anxiety and depression and that a net increase in NPY2R–relative to NPY1R–signaling increases anxiety- and depression-relevant behaviour.

### 3.3.11. Noradrenaline transporter (mouse nomenclature: *Slc6a2*; protein: NET)

Noradrenaline transporter (NET) is synthesised by noradrenaline (NE) neurons and is located on NE axon terminals where it performs pre-synaptic reuptake of NE and thereby is a major regulator of NE level and post-synaptic receptor binding in the synaptic cleft. Association of *SLC6A2* with depression has been reported using the candidate gene approach: A T–G SNP (rs5558) leads to inclusion of a different amino acid, and the G allele leads to increased NET density and NA uptake. The G allele was also associated with increased risk of depression (Haenisch et al., 2008). When all identified *SLC6A2* SNPs were assessed in a GWAS–depression sample, the proportion of SNPs that exhibited an association with depression was statistically greater than would be expected by chance (Bosker et al., 2011) (Table 1).

Extrapolating from the human evidence for an association between genetically-determined high NET activity and depression, it can be hypothesised that *Slc6a2* over-expression and *Slc6a2* KO will increase and decrease depression-relevant phenotypes, respectively. To our knowledge, there are no studies of *Slc6a2* over-expressing mice to-date, whereas a null mutant *Slc6a2* mouse has been generated. In two studies it was demonstrated that *Slc6a2* KO mice exhibited reduced immobility in the FST (Perona et al., 2008; Xu et al., 2000) and TST (Perona et al., 2008) relative to WT and one study reported no effect of the null mutant in FST (Haller et al., 2002). Using a G  $\times$  E design, the latter study also reported that *Slc6a2* KO  $\times$  chronic social defeat mice exhibited decreased immobility in the FST relative to WT  $\times$  chronic social defeat mice (Haller et al., 2002). A similar approach was used in a second study (Haenisch et al., 2009): KO and WT mice were included in G  $\times$  E experiments with either physical restraint stress or chronic social defeat as stressor and a non-stressed control condition. Using FST as readout, in the non-stressed group, KO mice exhibited reduced

immobility relative to WT mice. Within genotype, restraint stress or chronic social defeat stress increased immobility in WT but not in KO mice. Using sucrose preference test as a read out, in the non-stressed group, KO mice exhibited increased sucrose preference relative to WT mice. Within genotype, restraint stress or chronic social defeat stress decreased sucrose preference in WT but not in KO mice. Using a social interaction test as readout, chronic social defeat decreased time spent in the social interaction zone in WT but not in KO mice. These studies indicate that *Slc6a2* KO led to endophenotypes of reduced acute stress-induced immobility and increased reward sensitivity, and to stress resistance in terms of maintaining these phenotypes under the chronic stress conditions that led to depression-relevant phenotypes in mice expressing *Slc6a2* (Haenisch et al., 2009).

The evidence for *Slc6a2* KO mice is striking in terms of the magnitude of the G and G  $\times$  E effects demonstrated. Null mutants display endophenotypes of increased interest in sucrose and decreased immobility (antidepressant-like) in the FST and TST. Furthermore, it confers resilience against the effects of chronic stress on interest in sucrose and mobility in the FST. Studies with *Slc6a2* HET mice will be important, as will development of a *Slc6a2* over-expressing model, to link mice findings to the human evidence that NET hyperactivity is a risk factor for depression, and indeed that blocking of NET (Bymaster et al., 2005) is a current antidepressant mechanism of action, with stimulatory effects on psychomotor activity, arousal and attention being proposed to be important in this respect (Dell'Osso et al., 2011).

### 3.3.12. Opioid-binding protein/cell adhesion molecule-like (mouse nomenclature: *Opcml*; protein: OPCML)

Opioid-binding protein/cell adhesion molecule-like (OPCML) protein is concentrated in the CNS and often co-localised with 5-HT and GABA. OPCML protein is necessary for coupling between opioid receptors and G-proteins and is therefore integral to opioid signalling and the acute and chronic effects of opioids (Schol-Gelok et al., 2010). An association between *OPCML* polymorphism and depression was reported based on a combined linkage-analysis and GWAS approach. An A–G SNP in the intronic region of *OPCML* was differentially associated with depression, with the AG genotype conferring increased depression risk (Schol-Gelok et al., 2010) (Table 1). To our knowledge, there have been no mouse studies of the effects of manipulation of the *Opcml* genotype on brain or behaviour to-date.

### 3.3.13. FK506 binding protein 5 (mouse nomenclature: *Fkbp5*; protein: FKBP5)

Based on the corticosteroid hypothesis of depression (De Kloet et al., 2005; Holsboer, 2000), a considerable number of studies have been conducted into the association of the two corticosteroid receptors, mineralocorticoid receptor (MR) and glucocorticoid receptor (GR), with depression. Whilst there have been original reports of SNP association with the disorder for each of the receptors (e.g. Spijker and van Rossum, 2009), replication studies have not yet been conducted to an extent that would justify inclusion in candidate gene meta-analysis. Corticosteroid receptor function is regulated by chaperone proteins forming a molecular hetero-complex, and one such protein is FK506 binding protein 5 (FKBP5), coded by *FKBP5*. FKBP5 acts as an inhibitor of GR function. Several SNPs in *FKBP5* have been demonstrated to be associated with altered post-stress recovery of cortisol levels to baseline via the GR-mediated negative feedback loop. Furthermore, there are reports that the same SNP variants that predispose to such hypercortisolism are associated with depression risk, although other studies have not replicated this association (Zimmermann et al., 2011). In a prospective study, five *FKBP5* SNPs were demonstrated to be associated with depression, not as a genetic main effect but

in interaction with trauma, defined as events that caused intense fear, helplessness or horror (Zimmermann et al., 2011) (Table 1).

Given that FKBP5 inhibits GR function and that GR mediates the effects of the high cortisol levels induced by stress, including post-stress cortisol decreases via negative feedback, then the potential effects of SNPs that induce changes in FKBP5 structure-function are difficult to predict. On the one hand, decreased FKBP5 activity would be expected to increase GR transactivation; on the other hand, increased GR transactivation would be expected to accelerate the negative feedback loop leading to recovery of basal cortisol levels. Mice deficient of *FKBP5* have been generated and compared with WT littermates (Touma et al., 2011). In the FST, there was no effect of *Fkbp5* KO per se. However, when mice were exposed to restraint stress followed by a FST and then at 24 h to a second FST, KO mice exhibited increased swimming and decreased floating relative to WT at the second FST specifically. There was no effect of *Fkbp5* KO on behaviour in an open field test, elevated plus maze test or light–dark box test. The corticosterone response to restraint stress was reduced in *Fkbp5* KO mice relative to WT.

From these findings it can be tentatively concluded that a lack of FKBP5 leads to increased stress resistance, such that polymorphisms of *FKBP5* that increase FKBP5 activity could lead to increased stress sensitivity and be a risk factor for depression.

### 3.3.14. Corticotropin releasing hormone receptor 1 (mouse nomenclature: *Crhr1*; protein: CRHR1)

Intimately related to the corticosteroid hypothesis of depression (see above) is the hypothesis that corticotropin releasing hormone (CRH) is a causal factor in depression. CRH in the hypothalamus acts as a neurohormone to stimulate corticotrophin release from the pituitary gland, which in turn stimulates corticosteroid release from the adrenal gland. CRH is also synthesized in several limbic regions of the CNS and functions as a neurotransmitter (Berton and Nestler, 2006). Two G-protein coupled receptor proteins are receptors for CRH, namely CRH receptor 1 and 2. CRHR1 is expressed post-synaptically and mediates the excitatory effects of CRH on emotional and stress responses (Berton and Nestler, 2006). The reporting of increased CRH levels in the cerebrospinal fluid of depressed versus control subjects (Nemeroff et al., 1984) stimulated interest in CRH and its receptors with respect to depression aetio-pathology (Nemeroff, 1996). With respect to *CRHR1* polymorphism and its association with depression, there have been no reports for genetic main effects, but certain SNPs have been reported to be associated with depression in interaction with environmental stressors, most notably abuse experienced during childhood, in retrospective studies (Bradley et al., 2008; Grabe et al., 2010). Furthermore a  $G \times G \times E$  interaction on depression has been reported for *CRHR1* SNPs, 5-HTTLPR VNTR (S, L) and childhood abuse (Ressler et al., 2010) (Table 1).

Null mutant *Crhr1* mouse models have been generated. Null mutant *Crhr1* mice exhibited unchanged basal and decreased stress-induced corticotrophin and corticosterone levels relative to WT and HET (Smith et al., 1998; Timpl et al., 1998). The KO mice exhibited increased locomotor activity in an open field (Timpl et al., 1998) or no change in locomotor activity in an open field (Smith et al., 1998), relative to WT and HET mice. The KO mice exhibited reduced latency to enter and increased time spent in the light compartment in a light–dark box test relative to WT and HET mice (Smith et al., 1998; Timpl et al., 1998). The KO mice exhibited increased percent time on the open arms of an elevated plus maze relative to WT mice (Smith et al., 1998). There was no phenotypic differences between HET and WT mice on these endocrine and behavioural measures (Smith et al., 1998; Timpl et al., 1998). In a separate study (Lu et al., 2008), there was no effect of *Crhr1* KO relative to WT on percent time spent immobile in the FST. When the FST was preceded by restraint stress, WT mice exhibited

decreased immobility relative to non-stressed WT mice whereas there was no effect of the restraint stress in KO mice; furthermore, stressed *Crhr1* KO mice exhibited increased immobility relative to stressed WT mice. These findings suggest, somewhat unexpectedly, that *Crhr1* mediates active behavioural responses to acute stressors (Lu et al., 2008). Using contextual fear conditioning it was demonstrated that mice in which *Crhr1* KO was specific to the limbic forebrain exhibited reduced long-term fear memory relative to WT mice. In addition to this behavioural phenotype, the KO mice exhibited absence of the long-lasting increase in AMPA receptor signalling in the dentate gyrus of the hippocampal formation that was observed in WT mice (Thoeringer et al., 2012).

In line with the hypothesis that hyper-activity in CRH-CRHR1 signaling can lead to depression-relevant phenotypes, mice constitutively over-expressing *Crh* have been engineered. Such mice exhibit hyper-corticoidism and develop an overall phenotype that is analogous to Cushing's syndrome. To achieve more controlled increases in CRH-CRHR1 signaling, a conditional mouse model was generated in which *Crh* over-expression was specific to the CNS (Lu et al., 2008). Relative to control mice, homozygote and heterozygote CNS-*Crhr1* over-expressing mice exhibited, somewhat unexpectedly, reduced immobility in the forced swim test. Increased noradrenergic signaling due to CRHR1-mediated hyper-activation of the locus coeruleus was proposed as a mediating mechanism (Lu et al., 2008).

Despite the elegant molecular-genetic manipulation studies conducted with *Crhr1* and *Crh* mouse models to-date, there has been only limited progress in linking findings to the human evidence for increased CRH-CRHR1 signaling in depression, such that it is parsimonious to conclude that increased CRH-CRHR1 signaling is not causally involved in the pathogenesis of depression. However, it also needs to be stated that the lack of validity of the readout tests used to-date, such as the FST, means that concluding statements must be tentative.

### 3.3.15. Brain-derived neurotrophic factor (mouse nomenclature: *Bdnf*; protein: BDNF)

Brain-derived neurotrophic factor (BDNF) is the most abundant and widely-distributed neurotrophin in the CNS. BDNF promotes neurogenesis, neuronal survival, axonal and dendritic growth, and synapse formation, both in the developing and adult CNS. BDNF expression is decreased in the *post mortem* hippocampus of depressed (suicide) patients relative to controls (Duman and Monteggia, 2006). A common G-A SNP (rs6265) in the *BDNF* gene results in an amino acid change from valine to methionine in the prodomain at codon 66 (Val66Met). In neuronal culture systems, relative to BDNF<sub>Val</sub>, BDNF<sub>Met</sub> resulted in a decreased distribution of BDNF to neuronal dendrites and decreased activity-dependent BDNF secretion, possibly reflecting relatively reduced interaction of BDNF<sub>Met</sub> with a sorting protein (Chen et al., 2006). Humans carrying the G (Met) allele exhibit depression-relevant endophenotypes relative to A (Val) carriers, including smaller hippocampal volume and deficits on hippocampal-dependent memory tasks (Chen et al., 2006), and a deficit in the extinction learning of conditioned fear responses that is associated with greater amygdala reactivity (Soliman et al., 2010). With respect to association of this *BDNF* polymorphism with depression, there have been no reports for genetic main effects, but  $G \times G \times E$  interaction effects have been reported. In a child study of depression, assessed using the Mood and Feelings Questionnaire, in abused/neglected (maltreatment) children relative to control children (Kaufman et al., 2006), there was a significant interaction between *BDNF* Val/Met genotype, 5-HTT S/L genotype and maltreatment status. Depression scores were greater in Met  $\times$  SS carriers who had experienced maltreatment than in children in other  $G \times G \times E$  groups (Table 1).



There is no mouse orthologue of the *BDNF* polymorphism underlying Val66Met. *BDNF*<sup>Met</sup> knock-in mice have been generated (Chen et al., 2006). *Bdnf*<sup>Met/Met</sup> and *Bdnf*<sup>F<sup>Met</sup>/Met</sup> mice exhibited normal *BDNF*<sub>Met</sub> expression in the brain, but the regulated secretion of *BDNF*<sub>Met</sub> from neurons was defective, resulting in a decrease in available *BDNF* in *Bdnf*<sup>Met/Met</sup> mice. *Bdnf*<sup>Met/Met</sup> and *Bdnf*<sup>F<sup>Met</sup>/Met</sup> mice exhibited decreased hippocampal volume compared to WT, a finding that is analogous to human studies. *Bdnf*<sup>Met/Met</sup> mice exhibited a reduced number of entries into and percent time spent in the centre of an open-field, and reduced percent entries onto and percent time on the open arms of an elevated plus maze, in comparison to WT. *Bdnf*<sup>F<sup>Met</sup>/Met</sup> did not exhibit such increased anxiety-relevant behavior. Mice that were partial knockout for *Bdnf* (*Bdnf*<sup>F<sup>Met</sup>/(-)</sup>) exhibited anxiety phenotypes similar to those of *Bdnf*<sup>Met/Met</sup> mice (Chen et al., 2006). In a separate study, the effects of *Bdnf*<sup>Met</sup> knock-in were studied on extinction learning of conditioned fear (Soliman et al., 2010). Whilst there was no effect of genotype on fear conditioning, there was a dose–response inhibitory effect of *Bdnf*<sup>Met</sup> on extinction learning, with a greater continued expression of freezing behaviour to the CS in the absence of the US. *Bdnf* knock-out mice have also been generated. In one study, HET mice exhibited less locomotion than WT (Hall et al., 2003). In a separate study, no differences were observed between HET and WT mice in activity or anxiety behavior assessed in an open-field and elevated plus maze test, respectively. There was also no genotype effect in the sucrose preference test or FST (MacQueen et al., 2001). In the US pre-exposure test (one group of mice exposed to inescapable electroshocks (IS) and the other group to no shocks (NS)), HET-IS mice exhibited an increased escape latency relative to WT-IS mice. However, HET mice also exhibited reduced pain sensitivity in a hot plate test, such that reduced nociception could have contributed to the escape deficit observed (MacQueen et al., 2001). Mice with forebrain-specific knockout of the *trkB* receptor, the main mediator of *BDNF* signaling, exhibited stereotyped hyper-locomotion relative to WT, but no phenotype in the FST or elevated plus maze test (Zörner et al., 2003). Transgenic mice overexpressing the dominant-negative form of *trkB*, resulting in reduced activation of *trkB*, did not differ from WT in time spent immobile in FST (Saarelainen et al., 2003).

Thus, there is some converging evidence for humans and knock-in mice that *Bdnf*<sup>Met</sup> and the resultant decrease in *BDNF* availability is associated with decreased hippocampal volume and impaired extinction of conditioned fear responses. However, in studies with *BDNF* KO or *trkB* KO mice there was no phenotype in terms of sucrose preference or immobility in the FST. It will be important to conduct further studies to clarify this issue: firstly, *BDNF* is hypothesized to be an important mediator of current antidepressant mechanisms of action (Martinovich et al., 2007), and furthermore, for putative novel antidepressants, notably the glutamate NMDA-receptor-antagonist ketamine, a central role is proposed for *BDNF* in the mechanism of action (Duman et al., 2012).

### 3.4. Mouse models for the study of stress effects on brain region-specific gene expression

Post mortem transcriptome-level studies of altered gene expression in specific regions of the brain from depressed versus healthy control subjects have yielded important findings, as summarized above (Section 2.5, Table 2). Mouse models in which environmental stress has been demonstrated to lead to depression-relevant behavioural effects provide important opportunities for ex vivo transcriptome (mouse microarray) or candidate gene studies of altered gene expression. Such ex vivo mouse model studies can be aimed at replicating human findings as a validation step and at identifying novel genes and pathways that exhibit stress-dependent changes in expression in specific brain regions. In this

section we describe a prominent example of a study for each of these two approaches.

As described above (Section 2.5), one study (Sibille et al., 2009) assessed gene expression changes in parallel in depressed subjects and mice exposed to chronic unpredictable stress (CUS). The mice (BALB/c) were exposed to CUS for 7 weeks, and the regimen consisted of repetitions of single caging, exposure to rat or cat faeces or water in the home cage, tilting or shaking the home cage, placement in an empty cage of a conspecific, and altering length and time of the light–dark cycle. Behavioural effects of CUS were increased latency to begin feeding in a novelty-suppressed feeding test, and decreased latency to attack an intruder in a resident/intruder test. Gene expression effects were assessed using mouse microarray on mRNA isolated from cingulate cortex (including part of the prelimbic cortex), amygdala and dentate gyrus. Each of the three regions exhibited altered gene expression in 150–300 genes, but very few genes exhibited altered expression in two regions and none did so in all three regions (Sibille et al., 2009; Surget et al., 2009). A relatively large number of genes that exhibited altered expression have a demonstrated association with depression and/or antidepressant mechanism of action. This included genes coding for components of neurotransmitter systems (GABA, glutamate, and peptides), signal-transduction pathways (protein kinase C (PKC) genes, phospholipase C (PLC) genes, and mitogen-activated protein kinase (MAPK) genes) or second messenger systems (cyclic adenosine monophosphate), and a large number of oligodendrocyte markers (e.g. *Mobp*, *Pllp*, *Gpr37*) (see Tables 2 and 4). These genes exhibited altered expression in human depression and the mouse CUS model primarily in amygdala, and it is possible that the regions of cingulate cortex sampled were not analogous between the two species.

A second mouse model investigation into stress effects on gene expression studied chronic social defeat in C57BL/6 mice, and brain regions of interest were within the mesolimbic dopamine circuit, namely nucleus accumbens and ventral tegmental area (Krishnan et al., 2007). Chronic social defeat (CSD) involved 10-min per day physical exposure to and attack by an aggressive ex-breeder male CD1 mouse, and olfactory, visual and auditory exposure to the CD1 mouse for the remainder of the day. This was repeated over 10 days and a different CD1 mouse was used on each day. Behavioural effects of CSD were reduced time in proximity of a CD1 mouse in a social interaction test; approximately half of the CSD mice exhibited this effect relative to controls, and these mice were described as CSD susceptible (SUS), in contrast to CSD uns susceptible (UNS). The SUS mice also exhibited reduced sucrose preference in a sucrose preference test and a hyperthermic response when exposed to a CD1 mouse. Both SUS and UNS mice exhibited decreased percent time on the open arms of an elevated plus maze and increased serum corticosterone response to forced swimming, relative to control mice. *BDNF* was measured as a candidate protein and exhibited increased expression in nucleus accumbens in SUS mice relative to UNS and control mice. With respect to gene expression, of particular interest here are genes that exhibited altered expression in SUS versus control mice but not in UNS (or at least not in the same direction as SUS) versus control mice. In the nucleus accumbens, this included the genes adenylyl cyclase isoform 7 (*Adcy7*), ciliary neurotrophic factor (*Cntf*), histone deacetylase 2 (*Hdac2*), Homeo box B3 (*Hoxb3*), runt-related TF-1 (*Runx1*) and phospholipase C gamma-2 (*Plcg2*), which were up-regulated, and Homer homolog 3 (*Homer3*), which was down-regulated. In the ventral tegmental area, this included the genes galanin (*Gal*) and wingless-related MMTV site 2 (*Wnt2*) which were up-regulated, and NEL-2 like (*Nell2*), which was down-regulated, in SUS versus control mice specifically (Krishnan et al., 2007) (Table 4).

Given that the two studies reviewed above were conducted with different mouse strains and investigated the effects of different



**Table 4**

Summary of ex vivo studies of brain region-specific gene expression in mice exposed to chronic stressors.

Gene or functional gene group	Study design	Brain region of interest	Direction of expression change	Reference
Brain-derived neurotrophic factor ( <i>Bdnf</i> ) Adenylate cyclase isoform 7 ( <i>Adcy7</i> ) Cadherin 9 ( <i>Cdh9</i> ) Histone deacetylase 2 ( <i>Hdac2</i> ) Homeo box B3 ( <i>Hoxb3</i> ) Runt-related transcription factor 1 ( <i>Runx1</i> ) Phospholipase C gamma-2 ( <i>Plcg2</i> ) Homer homolog 3 ( <i>Homer3</i> ) Galanin ( <i>Gal</i> ) Wingless-related MMTV site 2 ( <i>Wnt2</i> ) NEL-2 like ( <i>Nell2</i> )	CSD (SUS) versus Control Microarray	Nucleus accumbens	Up-regulation Up-regulation Up-regulation Up-regulation Up-regulation Up-regulation Down-regulation Up-regulation Up-regulation Down-regulation	Krishnan et al. (2007)
Potassium channel tetramerisation domain containing 12 ( <i>Kctd12</i> ) Calcium channel, voltage-dependent, beta 2 subunit ( <i>Cacnb2</i> ) Calcium/calmodulin-dependent protein kinase II delta ( <i>Camk2d</i> ) Oligodendrocyte structure/function, e.g. Plasma membrane proteolipid ( <i>Plip</i> ) Myelin-associated oligodendrocyte basic protein ( <i>Mobp</i> ) G-protein coupled receptor 37 ( <i>Gpr37</i> ) Urocortin 3 ( <i>Ucn3</i> )	UCMS versus Control Microarray	Amygdala	Up-regulation Up-regulation Up-regulation Down-regulation Down-regulation Up-regulation	Sibille et al. (2009)
	3 h physical restraint versus Control. In situ hybridization	Paraventricular nucleus	Down-regulation Down-regulation Up-regulation	Venihaki et al. (2004)
Cannabinoid receptor 2 ( <i>Cnr2</i> )	UCMS versus Control. RT-PCR	Amygdala Whole brain	No change Up-regulation	Onaivi et al. (2008)
Neuron-specific neutral amino acid Transporter ( <i>Slc6a15</i> )	CSD versus Control. In situ hybridization	Hippocampus	Down-regulation	Kohli et al. (2011)

CSD, chronic social defeat; SUS, susceptible to CSD UCMS, unpredictable chronic mild stress.

chronic stressors on the transcriptome in different brain regions, then the lack of concordance between their respective findings is not too surprising. The study by Sibille et al. identified genes from several functional classes (see Table 2) that exhibited similarly altered expression in the amygdala in Balb/c mice that had experienced CUS and in human depression relative to their respective comparison groups. It will be important to conduct further such studies using C57BL/6 mice, the background strain for most behavioural genetic models, given that the amygdala demonstrates significant mouse–human homology in both structure and function and is a brain region that is central to emotional processing and exhibits altered functioning in depression.

### 3.5. Mouse models for genes exhibiting brain region-specific altered expression in depression

So far, we have considered (1) mouse models for polymorphism–depression associations, based on gene knock-out, knock-in and overexpression approaches, and (2) mouse models for CNS region-specific up- or down-regulation of gene expression in depression, based on stress manipulations and ex vivo transcriptome quantification. In this third and final section, we consider (3) mouse models for CNS region-specific up- or down-regulated gene expression in depression, based on gene knockout and over-expression approaches. For this, three genes are presented that were identified as exhibiting significantly altered expression in the region of interest using transcriptome-level analysis with appropriate statistical stringency; namely, urocortin 3, forkhead box D3 and cannabinoid 2 receptor ((Kang et al., 2007); see Section 2.5 and Table 2).

#### 3.5.1. Urocortin 3 (*Stresscopin*) (mouse nomenclature: *Ucn3*; protein: *UCN3*)

Stresscopin is a member of the CRH neuropeptide family and a selective ligand for CRH receptor 2 (CRHR2). Expression of *UCN3*

was increased in dlPFC in depressed relative to control subjects (Kang et al., 2007) (Section 2.5 and Table 2). In mouse, *UCN3* is expressed in neurons mainly in brain regions involved in stress processing, including paraventricular nucleus of hypothalamus (PVN), bed nucleus of the stria terminalis (BNST) and basomedial nucleus of amygdala (Venihaki et al., 2004). In C57BL/6 mice, 3 h physical restraint induced increased *Ucn3* expression in the PVN with no change in the amygdala (Venihaki et al., 2004) (Table 4). Intracerebroventricular (i.c.v.) injection of *UCN3* in C57BL/6 mice resulted in an increase in amount of activity and percent time spent in the centre of an open field compared to mice injected with vehicle. It also led to a decreased latency to enter the light compartment in a dark–light box test compared to vehicle mice. These findings suggest that i.c.v. injection of *UCN3* is anxiolytic (Venihaki et al., 2004). Whilst to our knowledge there are no published studies of over-expressing or null mutant *Ucn3* mice, null mutant *Crhr2* mice have been generated to study the effects of neutralising the CRH neuropeptide family ligands of this receptor, including *Ucn3*. Relative to WT, in an open field test, *Crhr2* KO mice exhibited less activity and time spent in the inner quadrant. In an elevated plus maze test, *Crhr2* KO mice entered less frequently and spent less percent time on the open arms, compared to WT mice. No effect of genotype was detected in the light–dark box test. These findings suggest that *UCN3* and other CRHR2 ligands are anxiolytic. Male and female *Crhr2* KO mice exhibited increased immobility relative to WT in the FST, indicating that lack of CRHR2 exerts the opposite effect to that of current antidepressants (Bale and Vale, 2003). Given the mouse findings that *UCN3* is anxiolytic, that this effect is mediated by CRHR2, and that lack of CRHR2 increases immobility in the FST, the mouse evidence does not support a causal link between increased dlPFC *UCN3* expression in depression and pathogenesis. It is possible that increased *UCN3* expression in depression is a component of the mobilization response to stressful environments that are risk factors for depression.

### 3.5.2. Forkhead box D3 (mouse nomenclature: *Foxd3*; protein: FOXD3)

FOXD3 is a member of the forkhead family of transcription factors that is involved in the development of the vertebrate neural system, including the promotion of neural crest cell fate, migration and differentiation of neural crest cells, as well as in regulation of tissue-specific gene expression, embryogenesis and tumorigenesis (Kang et al., 2007). In a *post mortem* GW expression study of the DLPFC (BA9), *FOXD3* mRNA expression was increased in the gray matter of depressed subjects relative to controls (Kang et al., 2007) (Section 2.5 and Table 2). To our knowledge there are no studies of over-expressing or null mutant *Foxd3* mice. The study that reported the increased expression of DLPFC FOXD3 in depression also reported increased *Foxd3* expression in the PFC of Sprague Dawley rats exposed to chronic unpredictable stress relative to non-stressed controls (Kang et al., 2007).

### 3.5.3. Cannabinoid 2 receptor (mouse nomenclature *Cnr2*; protein: CNR2, CB2)

The cannabinoid receptor 2 (CNR2, CB2) is expressed by cells in immune tissues and also in the CNS, by microglia, astrocytes and certain neuronal subpopulations (Fernandez-Ruiz et al., 2006). CNR2 functions include regulation of CNS cell responses to inflammatory and degenerative stimuli, by reducing the production of various cytotoxic factors such as cytokines, nitric oxide and reactive oxygen species (Fernandez-Ruiz et al., 2006). In a *post mortem* GW expression study of the DLPFC (BA9), *CNR2* expression was increased in depressed relative to control subjects (Kang et al., 2007) (Section 2.5 and Table 2).

Mouse model studies have contributed to the evidence that *Cnr2* expression is induced by neuroinflammatory signals, with *Cnr2* levels increasing in microglia and astrocytes in response to inflammation (Kang et al., 2007). In mouse CNS cultures, CNR2 binding activates the MAPK/MAPKK signaling pathway and thereby reduces pro-inflammatory cytokine levels (Molina-Holgado et al., 1997) and increases neuronal survival (Benito et al., 2008). In BALB/c mice exposed to chronic mild stress, *Cnr2* expression was increased in whole brain preparations relative to those from control mice (Onaivi et al., 2008) (Table 4).

Mice have been generated that over-express *Cnr2* in neurons and glia specifically (CB2xP). There was no effect of over-expression in an open field test relative to WT in terms of total locomotor distance, but CB2xP mice did exhibit increased central locomotor distance and decreased peripheral locomotor distance relative to WT. In a light–dark box test, CB2xP mice spent more time in the light box than did WT. In an elevated plus maze, CB2xP mice spent increased percent time on the open arms relative to WT. In a tail suspension test (TST), CB2xP mice exhibited reduced immobility compared to WT. Following chronic mild stress, there was no difference between CB2xP and WT mice in either the TST or a sucrose consumption test (Romero-Zerbo et al., 2012). Null mutant *Cnr2* mice have been generated: such KO mice exhibited decreased total locomotor distance and decreased activity in the centre of an open field, relative to WT (Flake and Zweifel, 2012). In BALB/c mice, ICV injection of *Cnr2* antisense oligonucleotide led to increased percent time spent on the open arms in an elevated plus maze test relative to controls that were injected with sense or mismatch oligonucleotides, a finding consistent with *Cnr2* inactivation exerting an anxiolytic effect (Onaivi et al., 2006).

Therefore, there is evidence that CNS-specific *Cnr2* over-expression leads to anxiolytic and antidepressant-like effects in mice, which contradicts the interpretation that the increased DLPFC CNR2 expression observed in depression is directly relevant to depression aetio-pathology. However, it is nonetheless possible that increased CNR2 expression represents a biomarker

for pro-inflammatory processes that could be pathological in depression.

## 4. Mouse models of the genetics of depression: Critical assessment of the experimental designs used and evidence obtained

As reviewed above, current evidence for the genetic aetiology and pathogenesis of depression is scant and there are a number of reasons for this, major among them being: the complexity and heterogeneity of depression psychopathologies; the current lack of diagnostic neuropathologies; the small number of GWAS or CGCC studies to-date of depression-relevant endophenotypes in healthy subjects; the low penetrance of specific alleles for depression, specific symptoms of depression and probably even specific depression-relevant endophenotypes; the importance of environment-induced physiological-neurobiological processes (E) for depression aetio-pathology; the lack of studies to-date of GWAS  $G \times E$  interaction for depression and specific depression symptoms and state markers; the small number of studies to-date of  $cG \times E$  for depression and specific depression symptoms and state markers; the small number of studies to-date of GW up- or down-regulation of gene expression in depression-relevant brain regions/circuits and subsequent studies of gene hits.

Given the limited progress to-date in establishing which genes are central to depression aetio-pathology and via what mechanisms, it is only to be expected that there has been little major progress in terms of generating mouse models that provide convincing evidence for the involvement of specific genes in the regulation of depression-relevant phenotypes. Nonetheless, there are some examples of where mouse models of human descriptive evidence have provided convincing supportive causal evidence, and demonstrate that the translational-genetic approach does indeed have potential for increasing understanding of the aetio-pathogenesis of depression. Here we would highlight the evidence that a *SLC6A2* SNP that leads to increased NET activity is associated with increased risk of depression (Haenisch et al., 2008) (Table 1), and that *Slc6a2* KO mice exhibited endophenotypes of decreased immobility in the FST and increased sucrose preference, and resilience to the effects of environmental stress observed in WT mice in terms of reduced sucrose preference and increased social avoidance (Haenisch et al., 2009). Another highlight would be the evidence that a *BDNF* SNP leading to *BDNF*<sup>Met</sup> leads in human to endophenotypes of decreased hippocampal volume/function and impaired extinction of learned fear/increased amygdala reactivity and to increased levels of depression-relevant psychological state markers in interaction with the 5-HTTLPR S allele and early life stress (Chen et al., 2006; Kaufman et al., 2006; Soliman et al., 2010), and that *Bdnf*<sup>Met</sup> knock-in mice exhibited increased anxiety in an elevated plus maze and a deficit in extinction learning in fear-conditioned freezing (Chen et al., 2006; Soliman et al., 2010).

Of course, the human–mouse model research approach is not unidirectional and, in addition to the reverse genetics approach, study of the effects of aetiologically-valid environmental manipulations on brain region-specific transcriptome expression in mice i.e. the forward genetics approach, has great potential for candidate gene and signaling-pathway discovery in depression aetio-pathogenesis. One important example here is the study that compared microarray gene expression changes in the amygdala in depressed humans versus healthy controls and in mice exposed to CUS versus non-manipulated controls (Sibille et al., 2009) (see Sections 2.5 and 3.4 and Tables 2 and 4). Significant and bidirectional predictions of altered gene expression were demonstrated for the amygdala, and according to various criteria a core set of 32 genes, expressed in either glia or neurons, was identified (Sibille

et al., 2009). These genes would appear to be important candidates for future human and mouse model studies. A second important example is the study that measured microarray gene expression changes in the nucleus accumbens and ventral tegmental area of mice that had been exposed to and exhibited depression-relevant behavioural effects of the environmental manipulation, chronic social defeat (Krishnan et al., 2007).

When discussing the progress made with and the limitations of mouse models of the genetics of depression, it is appropriate to also refer to the validity of the environmental manipulations and the readouts – primarily behavioural – that alongside the genetic manipulations constitute such models (Pryce and Seifritz, 2011). With respect to environmental manipulations, the current understanding of the characteristics of life events that render them stressful is limited, although there is evidence that major loss, humiliation, entrapment and uncontrollability are of high importance (Kendler et al., 2003). Uncontrollability is the basis of learned helplessness, which is an important aetio-pathological concept in preclinical and clinical depression research (Pryce et al., 2011). With respect to behavioural tests, it is apparent from Section 3.3 that a small number of tests have been used frequently to assess the behavioural effects of genetic manipulations; these are the sucrose preference test, forced swim test, tail suspension test and the elevated plus maze test. From Section 3.2, it will be clear that, with the exception of the sucrose preference test, these are not the tests with high validity with respect to modeling the psychopathology of depression. As noted, the FST and the TST are screening tests for acute effects of previous- and current-generation antidepressants, and this is the justification for including studies with these tests here, but an objective link between immobility in these tests and any neuropsychological state that characterizes depression is lacking. The elevated plus maze is a conflict-anxiety/anxiolytic screening test and studies using it have been included because of the high co-morbidity of anxiety disorders with depression. Clearly, the valid, translational tests of depression-relevant neuropsychological processes given in Table 3 need to be the focal readouts in future mouse models of behavioural effects of genetic and environmental manipulations (Pryce and Seifritz, 2011).

## 5. Mouse models of the genetics of depression: Considerations for future research

The justification for the study of mice as models of depression is to increase understanding of the aetio-pathogenesis of depression. The ultimate aim of this increased understanding is to discover valid novel molecular therapeutic targets and, in a subsequent stage, to utilize mouse models to screen and develop novel, efficacious antidepressant pharmacotherapies or even combined psycho- and pharmacotherapies. It has to be acknowledged that, to-date, a very substantial amount of research has been conducted with mice that is proposed to be depression-relevant but which in reality has yielded limited evidence and little progress. Why is this and are there grounds for optimism for future research efforts?

The major reasons for the lack of progress to-date include:

- (1) As referred to above, the aetio-pathogenesis of depression is complex and the current poor understanding of it means that there has been little knowledge available to inform the design of valid mouse models. The GWAS and CGCC approaches have yielded negligible positive evidence in terms of understanding the causation of depression. Nonetheless, they have yielded extremely important negative evidence; namely, that the study of polymorphisms in single genes in isolation from other factors will not lead to increased understanding of the aetio-pathogenesis of depression. It is important to accept

this negative evidence and not to invest further resources into carrying out even larger, more powerful studies, just to identify polymorphisms that are statistically significant but negligible in terms of their effect size and, therefore, in their relevance to the understanding of depression causality in the majority of patients. It is essential that the next generation of human studies aimed at increased understanding of depression aetio-pathogenesis uses the following three strategies: GWAS for physiological, neural and psychological risk states or endophenotypes for depression in healthy probands; a prospective genome-wide approach to  $G \times E$  association studies for physiological, neural and psychological symptoms and state markers/intermediate phenotypes of depression; genome-wide expression profiling by specific cell type (e.g. neurons, astrocytes, microglia, oligodendrocytes), also taking epigenetic processes into account, in key mediating brain regions in depression.

- (2) Not until they are informed by the accurate and predictive descriptive evidence provided by human studies using the above three strategies, can valid mouse models be developed in order to test cause–effect hypotheses of direct relevance to depression aetio-pathogenesis. When evidence-based mouse models are developed, should they fail to yield depression-relevant physiological, neural and psychological effects, then it must be concluded that the term, mouse models of depression, is an oxymoron. The explanation would be that a certain level of CNS complexity, inherent to primate- or even human-unique brain regions such as the granular frontal cortices (e.g. Murray et al., 2011), is necessary in order that an organism can demonstrate state markers of this medical condition. If, however, mouse models based on accurate and predictive evidence from human studies do yield depression-relevant physiological, neural and psychological effects, then such models can be utilized to increase understanding of the processes via which environmental factors impact on the body-brain connection and, within the brain, on region- and cell type-specific signaling pathways and gene transcription and translation. Furthermore, once validated reverse-genetics models were established, this would provide the justification for forward-genetics models and extrapolation of the evidence obtained therewith to human studies.

There are grounds for optimism, with respect to human depression research, mouse-model depression research, and therefore the essential bi-directional interaction between these:

- (3) The importance of improved definition and understanding of the psychopathologies of depression and other mental disorders has been recognized, even to the extent of improving the definitions and descriptions of the affected emotional-cognitive processes in diagnostic classification (Insel et al., 2010; Soskin et al., 2012). This development will facilitate the study of psychological endophenotypes and psychopathological state markers/intermediate phenotypes, as well as the development of behavioural readouts in mice with objective face validity (e.g. Pryce and Seifritz, 2011). Intimately related to this point is the need to take account of the heterogeneity of depression, both in terms of the diagnostic symptoms exhibited by individual patients and the differences in diagnostic symptoms between patients. The focus on individual symptoms will be commensurate with identification of underlying neuropsychopathology and, in turn, underlying genetic-environmental aetiology.
- (4) The need for rigorous and replicated prospective genome-wide  $G \times E$  studies and  $cG \times E$  studies, before specific genes become the focus of subsequent human research and indeed mouse model research, is being recognized (e.g. Duncan and Keller, 2011). Genomic and proteomic technology is already advanced



to an extent that allows for identification of polymorphisms at a deep-molecular level. The study of environmental life events is becoming better defined in terms of what characteristics of the life event are of physiological and/or psychological relevance (Kendler et al., 2003), and in terms of the importance of the stage at which the environmental life event is experienced (Danese et al., 2007, 2010). There can be reasonable grounds for optimism that this approach will lead to the discovery of accurate and predictive evidence for the  $G \times E$  aetiology of depression. This descriptive evidence can then be investigated in informed mouse  $G \times E$  model experiments.

- (5) Magnetic resonance imaging (MRI) studies are allowing for improved understanding of the brain regions and their inter-connectivity that underlie depression (Drevets et al., 2008; Mayberg, 2003). This knowledge is allowing for increased understanding of the neuro-psychopathology of depression (Disner et al., 2011). This, in turn, will inform research into the brain regions which should be focused on in *postmortem* gene expression studies. Evidence on whether and how those genes that are up- or down-regulated in depression-relevant brain regions are related to the genes that are identified in genome-wide  $G \times E$  studies will lead to the discovery of accurate and predictive evidence for the aetio-pathology of depression. This evidence can then be investigated in informed mouse model experiments, including the use of high-resolution MRI technology for the detailed functional study of the mouse brain (Rudin, 2009).
- (6) The physiological systems that are being considered as body-brain mediators of the environmental aetiology are expanding beyond the HPA axis. Most interesting in this respect is the attention being given to the dynamic stress reactivity of the immune system (Dantzer et al., 2008). As noted above, of those few genes for which association with depression has been reported with some level of replication, several are directly or indirectly involved in the functioning of the immune system. The high homology of the mouse and human immune systems and the processes via which peripheral immune events can impact on the brain, means that mouse model studies here should be highly informative (Miller et al., 2009).
- (7) Although based on serendipity rather than bi-directional human and animal model research, actual or potential new anti-depressant mechanisms of action are being identified that provide potential insights into the aetio-pathology of depression. Important examples here are the dual-mechanism melatonin receptor agonist and serotonin 2C antagonist, agomelatine (de Bodinat et al., 2010), and the NMDA receptor antagonist, ketamine (Aan Het Rot et al., 2012). Of course, when a functioning bi-directional translational research strategy for novel anti-depressant therapies is put in place, then target discovery and validation and lead compound development will be hypothesis driven rather than based on serendipity, and genetic and genetic-environmental mouse models will be essential to this process. In these models, it is essential that the behavioural readout tests exhibit face and construct validity, of course. It is also important that they are commensurate with repeated testing and thereby allow for the assessment of gradual responsiveness to chronic treatment. Readouts such as the learned helplessness effect and fear conditioned freezing are commensurate whereas screening tests such as the forced swim test are not.
- (8) Applying these methodological and conceptual advances, mice with their inherent advantages in terms of short generation interval, genomic mapping, molecular-genetic tractability, CNS and physiological complexity, environmental responsiveness and complex learning-motivational-emotional systems, can make a significant, iterative contribution to increasing the

inter-disciplinary scientific understanding of depression and its treatment.

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