Epigenetics, Depression and Antidepressant Treatment

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Abstract: The heritability of major depression has been documented in a number of epidemiologic studies. Twin studies have estimated the heritability at about 37% and these estimation can rise up to 70% if severity, relapse rate and age of onset are considered. Despite the relative importance of genetic risk factors in the pathogenesis of this disease, molecular genetic studies, including large genome-wide association studies, only a very small number of candidate genes, explaining little of the variance have been identified. This fact has been termed "missing heritability" and could be accounted by a number of factors including that the presumed causal variants are not tagged by the current genetic approaches, that major depression is truly polygenic, with each polymorphism only contributing very small increases in risk, unaccounted environmental influences and complex epigenetic factors. Epigenetics refers to the regulation of DNA transcription without alteration of the original sequence and is controlled by DNA methylation, histone modifications and non-coding RNAs and can be transmitted through generations. A number of clinical and preclinical studies suggest that epigenetic mechanisms could play an important role in the pathogenesis and treatment of major depression. So far, most studies investigated genes within the hypothalamic-pituitary-adrenal (HPA) axis or the neurotrophin system. It is also of interest that current psychopharmacologic drugs including antidepressants, antipsychotics and mood stabilizers may exert some of their effects by inducing epigenetic changes. Most notably, epigenetic alterations are potentially reversible and accessibe for drug treatment, which lead to the development of novel classes of antidepressant drugs.

Keywords: Major depression, gene, epigenetic, GWAS, antidepressant missing heritability, SNP, HPA-axis.

HERITABILITY OF MAJOR DEPRESSION

Genetic epidemiologic studies revealed a substantial genetic influence to most of the psychiatric disorders [1-4]. Family, adoption and twin studies are the most important tools in genetic epidemiology to discern the contribution of familial, environmental or genetic factors to a given disorder. Unipolar depression aggregates in families with a 2-3 fold increased risk for a first degree relative to develop a depressive episode [1]. Family studies can establish that a given disorder "runs in families", but can not distinguish if the familiality is due to genetic or environmental factors. Adoption and twin studies on the other hand can differentiate between genetic and environmental factors by analyzing genes and environment separately. Adoption studies analyze whether an individuals' risk for a psychiatric disorder depends on the mental health status of the biological or the adoptive parents to disentangle genetic (i.e., similarity to biological parents, who have little or no interaction with the adoptee) from the environmental influence (i.e., similarity to adoptive parents, who have provided the adoptee his or her family/social environment) [1, 6, 7]. Practical, ethical and legal limitations make large-scale adoption studies very difficult to conduct. Twin studies are more tractable, and large twin registries are now available across the world [2].

Twin studies have firmly established a substantial genetic contribution for most of the psychiatric disorders, with heritability estimates ranging from 30 to 80%. A meta-analysis derived from five studies including more than 21,000 individuals revealed a genetic contribution or heritability of 37 % (95% CI=31%-42%) for unipolar depression. Common environmental influences had very small effects, while individual environmental factors have a substantial contribution of 63% (95% CI=58%-67%) [1]. In contrast, the heritability of bipolar disorder is estimated at 60% - 85%, 70% - 85% for schizophrenia, 90% for autism, 50% - 60% for alcohol-related disorders, 60% - 70% for obsessive-compulsive disorder and 40% - 50% for anxiety disorders [3]. When clinical samples are

enrolled [4] or depression diagnosis is ascertained longitudinally in repeated measurements [5] the heritability estimates for major depression appear to be higher. In fact, when severity of the illness, relapse and early on-set of depression are considered, the estimated heritability increases to up to 70% [6].

While extremely important, twin studies only assess the overall genetic contribution to a disorder, but they are unable to identify specific susceptibility genes which increase the risk for a given disorder. This can only be resolved by molecular genetic studies, which have, however, been fraught with a number of difficulties. Psychiatric disorders are not single-gene Mendelian disorders. In fact, a large number of susceptibility genes are supposed to be responsible for the development of a psychiatric disorder, while each gene contributes only a small effect [13-15]. Furthermore the phenotypic expression might be different, even when carrying the same genetic risk factors or the genetic risk might only manifest when individuals are exposed to distinct environmental conditions. In addition, variants in different genes could lead to similar or identical disease phenotypes [7]. All these factors have made the quest for specific genetic risk factors for major depression very difficult and classical linkage analyses in families have not been able to identify convincing genetic risk loci [8].

GENETIC ASSOCIATION STUDIES

In contrast to linkage studies, the advantage of association studies is the increased power to detect small gene effects [9]. Association studies are usually performed in case-control studies of unrelated individuals. In such studies, allele frequencies of markers (e.g. single nucleotide polymorphisms, SNPs) are compared between a case and a control population. Considering the strong evidence supporting complex inheritance for major depression, association studies should be the optimal study design to identify and test candidate genes for this disorder. Nonetheless, association studies have been fraught with failures to consistently replicate initially reported associations. Most candidate genes of major depression are involved in the monoaminergic neurotransmission, which corresponds to the properties of the current antidepressant drugs. Other candidate genes are involved in neuroendocrine and neuroimmune

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pathways. We refer to a number of comprehensive reviews on candidate association studies for more details on this topic [17, 19-21].

With the development of high-throughput genotyping chips and the documentation by the HapMap Consortium (http: //www.hapmap.org) of SNPs tagging most of the common (population allele frequency greater than 5%) genetic variation in a number of different populations, genome-wide association studies (GWAS) have become possible [22, 23]. GWAS, compared to candidate gene studies, may yield unbiased, hypothesis-free insights into the genetic underpinnings of psychiatric diseases. To adjust for the large number of statistical tests and to reduce false positive results, correction for multiple testing has to be applied. An established p-value threshold which accounts for all possible variants in the genome has been set at 5×10^{-8} [24-26].

The first GWAS on psychiatric disorders performed by individual research groups have been disappointing, often lacking genome-wide significant association or replications [10, 11]. However, recently large meta-analyses have finally reported consistent loci for schizophrenia [12] as well as bipolar disorder [29] using data from up to 50,000 individuals. This has been made possible by the Psychiatric GWAS Consortium (PGC) which was founded to provide the opportunity to analyze large, combined samples (http: //pgc.unc.edu). The Consortium coordinates meta-analyses and also analyses of phenotypes or syndromes across several different disorders [11]. For major depression, the GWAS Consortium has brought together nine samples with 12,926 cases and 9,618 controls and a large GWAS meta-analysis is currently underway.

Up to now, eight GWAS on major depression have been published (see Table **1**; [10]). Sullivan *et al.* published a GWAS within the Genetic Association Information Network (GAIN) in which 1,738 depressed patients and 1,802 healthy controls were enrolled [30]. No SNP withstood the correction for multiple testing but some of the top associated markers were in the region of the gene Piccolo (PCLO), a gene implicated in neurotransmission. However, replication in several samples provided inconsistent results. Muglia *et al.* compared two European Case-Control studies with patients suffering from a recurrent depression, however, no marker reached genome-wide significance [31]. Lewis *et al.* enrolled more than 3,000 patients and controls in the United Kingdom, but without achieving genome-wide significance [13]. Some of the best associated markers were located within the bicaudal C homologue 1 (BICC1) gene, implicated in neurogenesis. The Genetics of Recurrent Early-Onset Depression (GenRED) project is part of a program of the National Institute of Mental Health to investigate genetics of major depression. Only patients suffering from recurrent depressive episodes with an early age of onset (before the age of 31) and at least one affected family member were enrolled to increase the possibility of the genetic risk [14]. However, no marker withstood the correction for multiple testing, the best association was in a region near the dermatan sulfate epimerase-like (DSEL) genes.

Shyn *et al.* conducted a GWAS with depressed patients from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) [15]. No genome-wide significance was found. Next, the authors conducted a meta-analysis with data derived from STAR*D, the GenRED project and GAIN. Again, there was no marker withstanding the correction, best associated markers lied in ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2 (ATP6V1B2) gene, Sp4 transcription factor (SP4) gene and the metabotropic glutamate receptor 7 (GRM7) gene. All three genes have been previously implicated with bipolar disorder [35-37].

The MDD2000+ project enrolled more than 5,000 patients and controls from different samples [16]. The authors conducted also a meta-analysis including two additional samples, with together over 12,000 individuals [30, 32]. However, no SNP withstood the correction for multiple testing. Evidence for association was found for the adenylate cyclase 3 (ADCY3) gene, galanin (GAL) gene and

the calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C) gene. Interestingly, all three genes have previously been implicated with major depression [39-41]. Rietschel *et al.* conducted a GWAS with approximately 2,000 German individuals [17]. While there was no genome-wide significance, two markers within carboxypeptidase M (CPM) and homer homolog 1 (HOMER1) reached nominal significance in the discovery sample as well as in the replication sample consisting of over 900 individuals.

In the GWAS from our group, we identified a locus on chromosome 12q21.31 which was associated with unipolar depression in six independent samples [18]. A meta-analysis over 6,000 patients and controls provided genome-wide significance for a SNP near the solute carrier family 6 (neutral amino acid transporter), member 15 (SLC6A15) gene, and this could be replicated in an additional sample with over 9,000 individuals. Risk allele carrier status in humans and chronic stress in mice were associated with a down-regulation of the expression of this gene in the hippocampus, a brain region implicated in the pathophysiology of major depression. The same polymorphisms also showed associations with alterations in hippocampal volume and neuronal integrity [18].

MISSING HERITABILITY - DISCREPANCY BETWEEN GENETIC CONTRIBUTION TO MAJOR DEPRESSION AND DETECTED GENES

As already mentioned twin studies revealed a genetic contribution to the development of an unipolar depression of approximately 35-40% [1] and after adjusting for severity, relapse rate and early age of onset, the heritability can increase to over 70% [6]. However, in the large-scale genome-wide association studies as in the more selective candidate gene studies, only few if any genes could be identified which only provide a small increase in risk for major depression. This is in agreement with most hypotheses about the genetics of major depression as risk genes with large relative risks for this disorder would be in contradiction with natural selection [19, 20]. Depression often develops in early age and is linked to a reduced fertility [21] and associated with an increased mortality [22], particularly due to suicide [23], with a large percentage of suicides occurring in adolescence or early adulthood, thus shortening the reproductive period. Genes with substantial effects which reduce the reproductive fitness should therefore be eliminated very quickly by natural selection. Some hypotheses try to solve this apparent paradox. One explanation is the balancing selection [20]. A balance between genetic advantage, for example creative intelligence, and disadvantage, for example symptoms from the psychotic spectrum, is assumed, which would retain the gene variant throughout the generations [24]. The beneficial features could be present in a large proportion of unaffected risk allele carriers who pass on the genetic variant. Susceptibility to major depression has been proposed to be balanced by the ability to elicit care and sympathy [25]. An alternative hypothesis, ancestral neutrality, proposes that genetic variants were adaptive or at least neutral throughout most of the human evolution and have recently become harmful [44, 45].

Keller and Miller favor the hypothesis of polygenic mutation-selection balance [20]. Psychiatric diseases reflect the inevitable mutational load of many genes underlying human behavior. Generally, harmful mutations are eliminated of the human genetic pool at a rate proportional to their harmful effect on reproductive fitness [26]. For example, a mutation leading to a fitness reduction of 1% remains in the population for approximately 100 generations. Mutations with the most harmful effects are eliminated very fast, thus if such mutations are present they are very rare and occurred lately. Mutations with small effects are eliminated slowly, hence they are more common, older and remain longer in the population. The detection of these genetic effects with small relative risks will require very large samples, possibly as large as the samples that have been required to detect genes associated with height, i.e. about 200,000

Table 1.   Eight GWAS on Unipolar Depression has been Published (Modified after Menke and Binder 2011 [21])

Study	Sample	Cases	Controls	Genome-wide significance	Top Hits
Sullivan *et al*, 2009 [30]	Genetic Association Information Network (GAIN)	1,738	1,802		PCLO
	Netherlands Study of Depression (NESDA)				
	Netherlands Twin Registry (NTR)				
	NEMESIS				
	ARIADNE				
	Replication	6,079	5,893		
Muglia *et al*, 2010 [31]	GSK Munich	1,022	1,000		CCND2
	GSK Lausanne	492	1,052		
Lewis *et al*, 2010 [32]	UK	1,636	1,594		BICC1
	Depression Case Control (DeCC)				
	Depression Network (DeNT)				
	Genome-Based Therapeutic Drugs for Depression (GENDEP)				
Shi *et al*, 2011 [33]	Genetics of Recurrent Early-Onset Depression (GenRED)	1,020	1,636		DSEL
	Molecular Genetics of Schizophrenia (MGS)				
Shyn *et al*, 2011 [34]	Sequenced Treatment Alternatives to Relieve Depression (STAR*D)	1,221	1,636		rs12462886
	Molecular Genetics of Schizophrenia (MGS)				
Wray *et al*, 2010 [38]	MDD2000+ project	2,431	3,673		ADCY3
	Queensland Institute of Medical Research (QIMR)				GAL
	NESDA				
	NTR				
	University of Edinburgh				
	MGS				
Rietschel *et al*, 2010 [42]	University of Bonn	604	1,364		HOMER1
	PopGen				CPM
	Kora				
	Heinz Nixdorf Recall (HNR)				
	Replication	409	541		
Kohli *et al*, 2011 [43]	Munich Antidepressant Response Signature (MARS)	353	366	SLC6A15	
	Replication I	3,389	2,099		
	Replication II	1,636	7,246		

individuals [27] which wil required effort beyond the ones currently undertaken in the PGC.

Other mechanisms which could explain this apparently missing heritability are complex epigenetic factors, i.e. inherited or acquired modifications of DNA and histones that regulate various genomic functions occurring without a direct change in nuclear DNA sequence. These have been proposed to offer new insights in the heritability and pathophysiological understanding of major depression and its psychopharmacological treatment [52-59] and will be the focus of the remainder of this article.

EPIGENETICS

Epigenetics refers to the regulation of DNA transcription without alteration of the original sequence and is controlled by DNA methylation, histone modifications, nucleosome remodelling and non-coding RNAs (see Fig. **1**) [54, 60]. Epigenetic regulation is required for the maintenance of proper genomic function, including the regulation of gene activity or inactivation and of parasitic DNA elements [28]. There is experimental evidence that the erasure of epigenetic marks and subsequent directed rearrangement is essential for the mammalian development [61, 62], however, epigenetic era-

sure is incomplete as demonstrated by a number of meiotically transmitted epigenetic alleles (e.g. MLH1 and Axin [63-65]). When epigenetic marks are not fully erased during meiosis they can be transmitted from generation to generation, although the procedure of the epigenetic inheritance is less stable than the inheritance of the DNA sequence, moreover the probability of passing on the epiallele to the next generation is not 100%, and often the epiallele is reprogrammed during gametogenesis or embryogenesis [66, 67]. Recently, various environmental factors and toxicants have been shown to induce epigenetic transgenerational inheritance of disease states or phenotypic variation, including the fungicide vinclozolin [68], the plastic compound bisphenol A [29], the toxicant dioxin [30], stress responses [31, 32] and nutrition [33]. In the epigenetic transgenerational inheritance the phenotype is transmitted through the germ line in the absence of a direct exposure of an environmental factor, thus these changes must be maintained in at least the F_3 generation [34, 35]. This additional mechanism by which genetic information is passed on transgenerationally may have an impact on approaches to map risk genes and may be one factor explaining why it has been difficult to uncover specific causal gene polymorphisms in diseases with apparently highly heritability such as major depression [35]. A more widespread definition of epigenetics refer to changes in e.g. DNA methylation without evidence for transgenerational transmission. Most of the studies conducted by now refer to this phenomenon including the strong impact of the environment on epigenetic marks and subsequently on gene expression but without evidence for transmission to the next generation.

DNA Methylation

DNA methylation has been one of the most studied epigenetic modification in relationship to major depression. It involves the modification of cytosines in cytosine-guanine (CpG) dinucleotides by adding a methyl group, hydroxymethyl group or other modifications. However, non-CpG cytosines may be also modified to a certain extend. The following paragraph refers to the best studied modification by a single methyl group. By cytosine methylation, access of transcription factors to regulatory elements is reduced and DNA methylation is most often associated with transcriptional repression by decreasing the binding of specific transcriptional enhancers [36]. Thus many genes demonstrate an inverse correlation between the degree of methylation and the level of expression [28]. DNA methylation is read by a family of methyl CpG-binding domain (MBD) proteins (including methyl CpG binding protein 2 (MeCP2) and MBD1-4), which interact with histone deacetylases as well as DNA methyltransferases (DNMTs) to confer gene silencing. The binding of these proteins to methylated DNA also appears to be critical to maintain the DNA methylation status, as the dissociation of this complex and specifically MeCP2 has been associated with site specific de-methylation [77-79]. DNMTs catalyze the transfer of a methyl group from S-adenosyl-L-methionine to the C5 position of the cytosine residues in DNA [37]. Three enzymatically active mammalian DNMTs have been described: DNMT1, DNMT3A and DNMT3B, and one related regulatory protein, DNMT3L, which lacks catalytic activity [38, 39]. DNMT1 is mainly a maintenance methyltransferase preserving methylation patterns during cell divi-
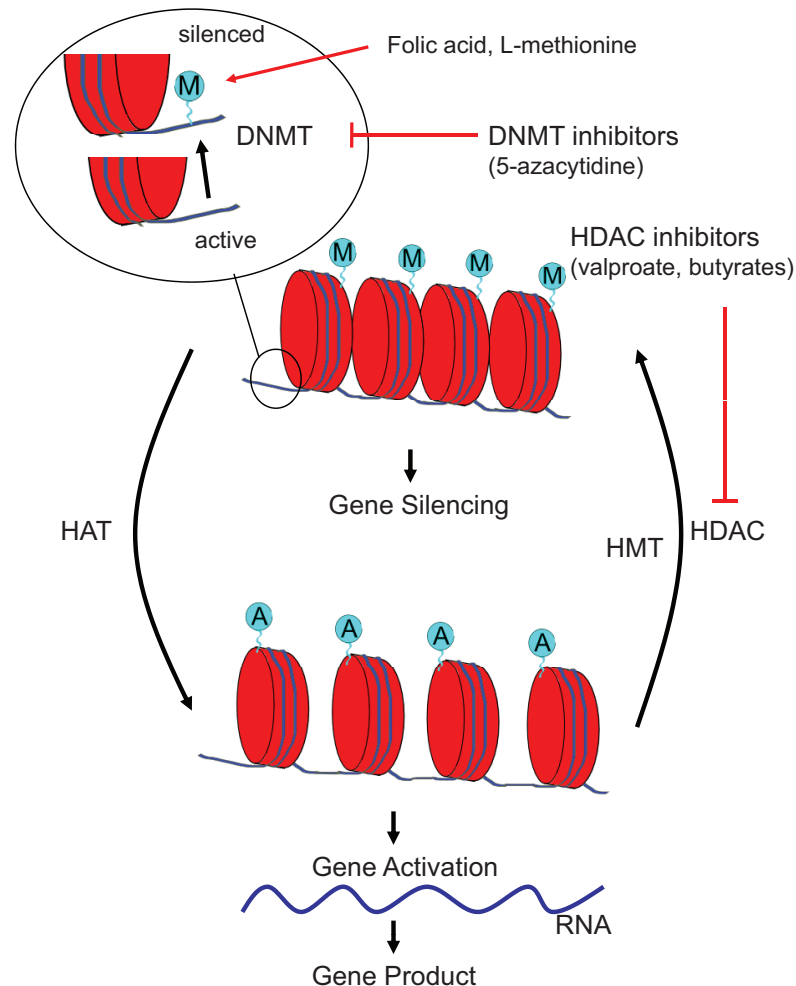
Fig. (1). Gene activation and silencing: Gene silencing is facilitated by adding methyl groups either to DNA (with DNA methyl transferases, DNMTs), or by adding methyl groups to histones with histone methyl transferases (HMTs) or removing acetyl groups by histone deacetylases (HDAC). Gene activation is provided by adding acetyl groups to histones by histone acetyl transferases (HATs). Drugs can alter epigenetic modifications with DNMT inhibitors like 5-azacytidine or with HDAC inhibitors like valproate or butyrates.

sion. DNMT3 enzymes are responsible for de novo methylation during embryonic development [40]. DNMT3A and DNMT3B are closely relate and both contain a PWWP domain (characterized by the presence of a highly conserved proline-tryptophan-tryptophan-proline motif) [41], a PHD-like or ADD domain and a carboxy-terminal catalytic domain [39, 42]. By contrast, DNMT3L has only an ADD domain. DNMT3L exert some of the regulatory activity by interacting with core histones [43]. A novel mechanism of DNA methylation is provided by the enzyme ten-eleven translocation 1 (TET1; one of three enzymes of the TET family). It catalyzes the oxidation of 5-methylcytosine into 5-hydroxymethylcytosine in mammalian DNA [44]. The significance of hydroxymethylation to epigenetic inheritance in disease is unclear so far.

Histone Modifications

Histone modifications are also important mechanisms to alter gene activity. Histones comprise the major protein constituents of the nucleus and form complexes, around which DNA is wrapped, which is the basic building block of chromatin. Chromatin exists in an inactivated condensed state, heterochromatin, which does not permit gene transcription, and in an open, activated state, euchromatin, which allows genes to be transcribed. The switch from hetero- to euchromatin is supported by histone modifications. Histone modifications include acetylation, methylation at lysine or arginine residues, phosphorylation at serine or threonine residues, ubiquitylation or SUMOylation at lysine residues, and ADP-ribosylation at glutamate residues resulting in complex modification patterns with distinct effects on DNA accessibility [45]. The enzymes that mediate histone modifications are the histone acetyltransferases (HATs), which catalyse acetylation and histone deacetylases (HDACs) catalyzing deacetylation and thus regulating the histone acetylation homeostasis [88, 89]. Generally, histone acetylaton opens chromatin structure and faciliate gene expression, while histone deacetalytion closes chromatin structure and silences genes. HATs are divided into two main classes, the type A nuclear HATs and the type B cytoplasmic HATs [46]. Classification of the HDACs relies on biochemical, structural and phylogenetic data [47]. A first differentiation separates group I HDACs, which are zinc-dependent aminohydrolases, from group II HDACs (also known as class III HDACs or SIRTs), which possess a catalytic activity due to nicotinamide adenine dinucleotide (NAD) [48]. Group I HDACs are further separated into classes I (HDAC1, -2, -3 and -8), II (HDAC4, -5, -6, -7, -9, -10) and IV, based on their similarity to yeast proteins [49]. Class II HDACs are divided into two subclasses, class IIa (HDAC4, -5, -7 and -9) and class IIb (HDAC6 and -10) [50]. The specific effects of each of these HDAC genes are not well understood yet, but it is clear that they do also acetylate other molecules than histones [51]. HDAC inhibitors stop the removal of acetyl groups from specific histone residues and thus increase transcriptional activity. Histones can also be modified by histone methyl transferases (HMT), which add methyl groups [52]. Methyl groups can be added to arginine and lysine residues. Arginine methylation in mammals is typically found on residues 2, 8, 17 and 26 of histone H3 (H3R2; H2R8; H3R17 and H3R26) and is catalysed by the protein arginine methyltransferase (PRMT) class of HMTs. Arginine methylation contributes to both activation and repression of chromatin function. Lysine methylation also activates and represses chromatin function, for example, methylation of histone H3K4, H3K36 and H3K79 leads to activation of the chromatin, whereas H3K9, H3K27 and H4K20 methylation is associated with silenced regions. Histone lysine methylation is catalysed by proteins containing an SET domain, a sequence motif named after Su(var)3-9, enhancer of Zeste, Trithorax. Histone methylation can be reversed or antagonized by histone demethylases (HDMs), which include the enzyme families peptidylarginine deiminase (PADI), lysine (K)-specific demethylase 1A (LSD) and Jumonji-C (JMJC) [52].

microRNA / Small Interfering RNA

MicroRNAs (miRNAs) which are small RNA molecules with only 19-22 nucleotides are able to control gene expression at a post-transcriptional level and are directly connected to the epigenetic machinery through a regulatory loop [53]. DNA methylation or histone acetylation can affect miRNA expression, while miRNAs can control the epigenetic machinery by directly targeting the enzymatic components. MiRNAs are transcribed by RNA polymerase II as long primary transcripts with hairpin structures and processed in the nucleus by RNAse III Drosha into pre-microRNAs. These precursor molecules are exported to the cytoplasm by an Exportin 5-mediated mechanism, where RNAse III Dicer mediates the generation of miRNA [53]. MiRNAs regulate gene expression at the posttranscriptional level amongst others by binding to the 3'-UTR of target mRNAs and increasing mRNA degradation.

It has been shown, that miRNAs can directly target the DNA methyltransferases (DNMTs) DNMT-3A and -3B [54], as well as the maintenance DNA methyltransferase DNMT1 [55]. Additional epigenetic mechanisms involve the inhibition of HDAC4 [56] and HDAC1 [57].

Methodological and Technological Limitations

Two main questions need to be considered when investigating epigenetic changes in disease: The technique and its pros and con especially redarding molecular resolution and the tissue or cell type investigated. Exemplarily we will discuss this for DNA methylation. The current gold standard technique for fine mapping of methylated cytosines is based on the treatment of genomic DNA with sodium bisulfite. Unmethylated cytosines are converted into uracil, whereas methylated cytosines are resistant to bisulfite and remain unchanged [58]. Bisulfite treatment is not able to distinguish between different cytosine modifications as a major drawback for future investigation. In the past, numerous other techniques were established to shed light on DNA methylation including PCR approaches, affinity based methods, methods based on restriction enzymes and more recently array platforms and high throughput sequencing. These techniques have their unique pattern of advantages and disadvantages regarding resolution, genome coverage and accuracy which limits the conclusions one can draw from the resulting data sets.

Further confounding factors in epigenetic studies are tissue heterogeneity and cellular heterogeneity. Different cell types exhibit distinct epigenetic profiles across different genomic regions as a result of cellular differentiation. Even within specific tissue types like brain there is a regional and cell type specific methylation profile. There is considerable cellular heterogeneity. A possible solution might be the laser capture microdissection technology. This approach enables the isolation of specific cell types or single cell from whole tissue [59]. The examination of more accessible tissue sources like leukocytes may provide the possibility to perform repeated measurements for monitoring issues and longitudinal observations in larger patient cohorts. While gene expression profiles in peripheral blood cells are not likely to reflect profiles in neuronal cell populations, there is an overlap between gene activity measures in brain and peripheral blood [60]. Though DNA methylation is cell type or tissue specific, there are overlapping profiles across tissues as well. By analyzing well-accessible tissue like peripheral blood, one has to keep in mind that these tissues can be dynamically modified and systemic diseases including psychiatric disorders and their treatment can change tissue composition resulting in alteration of epigenetic and expression profiles. Future studies are required to determine the overlap and possible co-regulation of DNA methylation in brain and peripheral or better accessible tissue. Peripheral tissue might not directly reflect the pathophysiology in the brain but might serve as surrogate marker for the course of the disease or treatment progress.

MAJOR DEPRESSION AND EPIGENETICS

DNA Methylation

The research groups around Michael Meaney and Moshe Szyf were the first to demonstrate that early psychosocial environment can have longterm consequences on gene expression via epigenetic mechanisms using a rodent model of maternal care [104-106]. They showed that postnatal maternal care in rats, measured by increased pup licking, grooming (LG) and arched-back nursing (ABN), leads to epigenetic modification of an NGF1-A transcription factor binding site in the promoter region of the glucocorticoid receptor (GR) gene (NR3C1) in DNA from hippocampus [61, 62]. The GR promoter sequence was always methylated in the offspring of the low-LG-ABN mothers, while it was rarely methylated in the offspring of high-LG-ABN mothers. Cross-fostering produced a methylation pattern of the GR promoter region which was associated with the rearing mother, supporting the importance of the early environment. In line with a decrease in DNA accessibility with increasing DNA methylation, binding of the NGF1-A transcription factor to the GR promoter was greater in offspring of high- compared with low-LG-ABN mothers, and consequently GR gene expression in the hippocampus was greater in the offspring of high- compared to low-LG-ABN mothers.

Murgatroyd *et al.* investigated the impact of early life stress on DNA methylation in the arginine vasopressin (AVP) gene enhancer region. Using early maternal separation in mice as an early stressor, they found a persistent upregulation of the AVP expression due to hypomethylation of the AVP gene enhancer region. This decrease in DNA methylation was mediated by decreased binding of the methyl CpG-binding protein 2. DNA binding of this molecule and thus protection from de-methylation has been shown to be linked to specific neuronal activation [78, 79]. This longterm increase in AVP expression was also associated with differences in stress hormone measures as well as behavior in adult animals. In fact, mice who were subjected to maternal separation showed deficits in the forced swim test and in avoiding learning tasks which was partly reversible by treatment with AVP receptor antagonists [63].

Similar results could be observed in humans. McGowan *et al.* analysed the extent of DNA methylation of the neuron-specific glucocorticoid receptor (NR3C1) promoter in hippocampi from suicide victims with and without history of child abuse compared to control subjects who had died from causes other than suicide and had not been abused as children. They found that the DNA at the NR3C1 promoter was more methylated in abused suicide victims than in non abused suicide victims or control subjects. Glucocorticoid receptor mRNA was also reduced in abused suicide victims compared to the other two groups [107] in agreement with findings from previous gene expression studies [64]. Increased DNA methylation of the glucocorticoid receptor gene could also be detected in the blood of newborns, whose mothers suffered from a depression in the third trimester of pregnancy. The same newborns exhibited increased salivary cortisol levels after a visual stimulation test at three months of age [65]. These findings support the possibility that the dysregulations in hypothalamic-pituitary-adrenal (HPA) axis function observed with major depression [66] could be mediated by DNA methylation of relevant regulatory regions.

Early environment has also been shown to affect DNA methylation of other genes that have been associated with the pathogenesis of depression, such as the neurotrophic system which contains the gene encoding brain-derived neurotrophic factor (BDNF) [67, 68] and its receptors the tyrosin receptor kinase (Trk) family, including TrkB encoded by the NTRK2 gene, which are major mediators of neurogenesis and synaptic plasticity [69]. This system has been linked to response to antidepressant as well as the pathophysiology of depression and suicide [114-116] and is also involved in stress hormone system regulation [117, 118]. Roth *et al.* reported BDNF gene silencing in the prefrontal cortex of early maltreated rat pups due to DNA methylation. Rat pups were exposed to stressed caretakers that behaved abusively. The offspring of the female rats exhibited the same BDNF DNA methylation pattern in the prefrontal cortex and hippocampus as theirs maltreated mothers, which suggests a generation-to-generation transmission of previously acquired DNA methylation patterns [119]. Ernst *et al.* investigated the promoter region of a BDNF receptor (TrkB) isoform (TrkB.T1) in post-mortem brain tissue in suicide victims with depression [120] and found a hypermethylation of the TrkB.T1 DNA with consecutively reduced mRNA and protein expression [70]. The TrkB.T1 variant is known to mediate BDNF-induced calcium signaling through astrocyte networks [71].

A number of other candidate genes have been investigated in postmortem brain tissue for differences in DNA methylation with major depression and suicide. Poulter *et al.* found an increased DNA methyltransferase 3b (DNMT3B) mRNA expression in postmortem brain tissue of depressed suicide victims in comparison to controls without depression which was accompanied by a hypermethylation of the gamma-aminobutyric acid (GABA) A1-receptor (GABRA1) gene promoter [72]. Differences in DNA methylations of 5-hydroxytryptamine (serotonin) receptor 2A (5HTR2A) receptor [123] and catechol-O-methyltransferase (COMT) genes [73], both regulating the monoamine system have also been also associated with depression.

Histone Modifications

There is some evidence for histone deacetylase (HDAC) dysfunction in the pathophysiology of major depression. A decreased HDAC2 protein expression was identified in the nucleus accumbens (NAc) of patients with major depression [74]. Acetylated histone H3, which decreases HDAC2 levels in the NAc were also increased in a chronic social defeat stress model [74]. In contrast, a study evaluating 11 HDACs in peripheral leukocytes of subjects with major depression and bipolar disorder during euthymia or depressive episodes found increased expression of HDAC2 and HDAC5 mRNA during depressive episodes compared to controls and patients in remission, which suggested a state-dependent alteration [75].

MicroRNAs

Effect of stress and stress hormone activation have also been linked to effects of microRNAs (miRNAs or miR). In an animal model of repeated stress, Uchida *et al.* could demonstrate a decreased GR mRNA expression in the paraventricular nucleus, as well as an enhanced miR-18a expression. The miR-18a inhibited translation of GR mRNA suggesting that a decrease in GR may be, at least in part, the result of the increased miR-18a expression [76]. Additionally, Vreugdenhil *et al.* found two miRs (miR-124a and miR-18a), which were able to reduce GR protein levels [77]. More recently, Turner *et al.* predicted miRNA binding sites within the first GR exon [78]. Further investigation of these binding sites may lead to more information about the regulation of GR via miRNAs.

Restraint stress is one of the most commonly used animal models of stress linked to depressive behaviour. Rinaldi *et al.* demonstrated several miRNAs to be transiently increased in the frontal cortex after acute stress. For example, the expression of miR-9, let-7a, miR-26a/b were only altered after acute stress, but not after repeated stress or 5 days after stress exposure, which suggests that acute stress modulates miRNA expression quickly to allow neurons to respond to external stimuli [79]. In contrast, Meerson *et al.* could show, that miRNAs in the central amygdala and in the hippocampus were differently regulated after acute and chronic restraint stress, with chronic stress causing larger changes than acute stress [80]. Next, a knowdown of miR-183, which was altered after acute and chronic stress, resulted in an increase of SC35 protein levels. SC35 promotes the alternative splicing of acetylcholinsterase from the

synapse-associated isoform AChE-S to soluble AChE-R protein and the expression of SC35 is increased during stress [80].

Another model of stress and depression is the learned helplessness paradigm [81]. Smalheiser *et al*. determined miRNA expression changes in the frontal cortex of rats following repeated footshocks. They found robust adaptive miRNA responses to inescapable shocks in rats which did not develop the learned helplessness phenotype, whereas rats which developed learned helplessness showed only a blunted miRNA response [82].

A study investigating microRNAs previously implicated in circadian rhythm found a polymorphism in the pre-microRNA-182, which was associated with insomnia in patients suffering from major depression [83]. Alterations in the pre-mi-R-182 resulted in a significant overexpression of miR-182 in cells transfected with the mutated form of the pre-miR-182 and increases downregulation in some of its target genes which include adenylate cyclase 6 (ADCY6), clock homolog (CLOCK) and TSC22 domain family, member 3 (DSIP) which have been implicated in the regulation of ciradian rhythms [83]. Another genetic association study which analyzed polymorphisms in the mi-R-30e recently implicated with schizophrenia demonstrated an association between a polymorphism in the mi-R-30e precursor and major depression [84].

A number of studies thus indicate that epigenetic mechanisms are affected by exposure to stressful environment and in depression. Several studies investigated epigenetic modifications in genes of the HPA-axis. DNA methylation changes of the glucocorticoid receptor and the arginine vasopressin gene due to influences of early psychosocial environment have been shown in animal models. Additionally, DNA methylation changes within the GR gene were also observed in human postmortem brain tissue following early child abuse and in blood cells in newborns of mothers affected by major depression. Several other candidate gene approaches revealed DNA methylation alterations in genes encoding BDNF, GABA-A receptors, 5-HT$_{2a}$ receptor and COMT. Histone modifications could be observed in animal models of depression-like behavior, and also in human postmortem brain tissue and in peripheral leukocytes of depressed patients. Recent studies provide insight in epigenetic alterations due to microRNAs with distinct microRNA responses in various stress models in rodents.

ANTIDEPRESSANTS AND EPIGENETICS

While many patients benefit from current psychopharmacologic treatment with antidepressants, only half of the depressed patients show a complete remission, which underscores the need for more effective agents [85]. The delayed response to antidepressant treatment even though the monoaminergic targets are occupied within hours, still represent an unresolved puzzle. This delay could be mediated by more longterm adaptations, like epigenetic regulations [45]. The following paragraphs dicuss two avenues – first the epigenetic effects of currently used antidepressants and second the potential use of drugs interfering with epigenetic mechanisms as antidepressant drugs (see also Fig. **1**).

Epigenetic Effects of Currently Used Psychopharmacologic Drugs

Interestingly, current psychopharmacologic drugs have been shown to modify epigenetic regulation, particularly by decreasing methylation levels of DNA. The mood stabilizer and anticonvulsant valproate causes a global reduction in DNA methylation levels, which was observed in rat hepatic cells and in human embryonal kidney cells [86, 87]. The tricyclic antidepressant amitriptyline also reduced DNA methylation in rat primary astrocytes [139]. Additionally, the antipsychotics clozapine and sulpiride but not haloperidol or olanzapine may activate DNA de-methylation in brain [88]. The stimulant metamphetamine alters the DNA methylation profile of genes expressed in the brain and it could be shown that

acute metamphetamine treatment significantly decreases DNA methyltransferase 2 mRNA in the rat brain [89].

Drugs may not only exert their action by activating demethylation, but also by blocking histone deacetylases (HDACs), effectively using two mechanisms to activate genes [90]. The mood stabilizer and anticonvulsant valproic acid is one example [139, 143]. Another mood stabilizer, topiramate is also a potent HDAC inhibitor [91]. Valproic acid and topiramate both impact neuronal differentiation [145, 146]. The above-described down-regulation of BDNF due to histone methylation in a defeat stress model [92] could also be reversed by chronic imipramine treatment, which leads to histone acetylation. This histone acetylation was long-lasting and mediated by a selective HDAC down-regulation.

While the antidepressants imipramine, amitripyline or the mood stabilizers valproat and topiramate predominantly showed gene activating epigenetic modifications, Cassel *et al*. showed a reduced acetylation of histone H3 and an increased histone deacetylase 2 activity following treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine [93]. Similar to this observation, monoamine oxidase inhibitors can increase global levels of histone H3 lysine 4 (K4) methylation by inhibiting the demethylation of H3-K4 and thus causing transcriptional derepression of specific genes *in vitro* [94]. Demethylation of histone H3-K4 was catalysed by BHC110/ lysine specific demethylase 1 (LSD1), an enzyme with close structural homology to monoamine oxidases [95]. In fact, the most potent inhibitor of demethylation by BHC110/LSD1 was tranylcypromine [94]. It has been shown that tranylcypromine is a time-dependent, mechanism-based irreversible inhibitor of LSD1 which exhibited limited selectivity for human MAOs versus LSD1 [151-153]. On the basis of tranylcypromine, new compounds were developed to inhibit LSD1 as well as MAO A and MAO B [154]. Next, dysregulation of histone acetylation and methylation was observed to result in silencing of tumor suppressor genes and cancer progression, inhibitors of enzymes that catalyze the these epigenetic marks thus have therapeutic potential for treating cancer. The pharmacologically inhibiting LSD1 with the tranylcypromine, in combination with HDAC inhibitors, led to the synergistic apoptotic cell death in Glioblastoma multiforme (GBM) cells [96].

Antidepressants like the SSRIs may also influence microRNAs. As neuronal upregulation of the BDNF gene may be a critical factor for the efficacy of antidepressants, a study analyzed BDNF mRNA and protein expression in a human glioblastoma-astrocytoma cell line exposed to the antidepressant paroxetine [97]. The authors found that paroxetine treatment rapidly increased BDNF mRNA and protein expression and at the same time miR-30a-5p expression, which is a posttranscriptional inhibitor of BDNF synthesis [98], was increased as well [156]. Thus the simultaneous increase in miR-30a-5p potentially limited the BDNF protein expression. Baudry et at. could show that the microRNA-16 (miR-16) inhibits the serotonin transporter (SERT) which is the pharmacological target of the SSRI antidepressants [99]. In mice, chronic treatment with the SSRI fluoxetine increased miR-16 levels in serotonergic raphe nuclei, which reduced SERT expression.Thus miR-16 may contribute to the therapeutic action of SSRI antidepressants in monoaminergic neurons [99].

A study by Zhou *et al*. demonstrated changes in hippocampal miRNA levels following chronic treatment with the mood stabilizers lithium and valproate [100]. The predicted targets of these miRNAs were genes involved in neurite outgrowth and neurogenesis. Additionally, the authors could show that treatment with lithium or valproate increased the expression of these potential target genes *in vivo* which included dipeptidyl-peptidase 10, metabotropic glutamate receptor 7 (GRM7), and thyroid hormone receptor, beta. For example, treatment of primary neuronal cultures with lithium or valproate lowered the levels of miR-34a and elevated the levels of GRM7, a predicted target of miR-34a [100]. Consistent expression changes of miR-34a, miR-152, miR-155, and miR-221 were found

following chronic lithium treatment in lymphoblastoid cell lines [101].

Drugs Interfering with DNA Methylation and Histone Modifications

As detailed above, both stress and depression as well as antidepressants may have longterm epigenetic impact that could mediate neurophysiological and behavioral effects. One possibility to directly interfere with these mechanisms would be drugs which alter epigenetic modifications.

DNA Methylation

DNA methyl transferases (DNMTs) catalyse the transfer of methyl groups from S-adenosyl methionine to cytosine residues within CpG-rich regions of the genome. DNA methylation generally leads to transcriptional silencing, thus DNMT inhibitors can prevent DNA methylation and potentially reactivate silenced genes (see Fig. **1**) [102].

DNA methylation can be affected by dietary levels of methyl-donor components such as folic acid or homocystein. Dietary supplementation of folic acid during pregnancy has been shown to increase DNA methylation and alter methylation-dependent phenotypes in offspring [103]. Individuals displaying folate deficiency are more likely to develop a depressive episode [104], and are less likely to respond to antidepressant drugs [105]. Folate treatment has been shown to improve depressive symptomatology [165, 166]. Folate depletion was also associated with decreased leukocyte DNA methylation in women and an increased plasma homocysteine [106, 107]. Homocysteine is metabolized to S-adenosyl-methionine, a methyldonor, which might influence DNA methylation. Homocysteine was shown to affect global gene promoter DNA methylation, and the acute administration of homocysteine leads to demethylation of promoter DNA with a subsequent increase of gene expression in human neuroblastoma and human embryonic kidney cells [169]. This mechanism might be relevant for DNA methylation of the BDNF locus. Hippocampal BDNF levels were reduced in an animal model after administration of homocysteine, which was accompanied by an impairment of memory consolidation [108]. In contrast, pre-treatment of folic acid, which is associated with DNA hypermethylation [109], could prevent reduction of BDNF levels by homocysteine [108]. Interestingly, increased levels of homocysteine have been associated with major depression [172, 173].

MeCP2 is believed to restore stress-related changes in DNA methylation by recruiting DNMTs. Thereby MeCP2 might help to protect DNA methylation patterns over time [36]. In a cocaine-induced behavioral sensitization model in mice, cocaine treatment resulted in a DNA hypermethylation and increased binding of MeCP2 at the protein phosphatase-1 catalytic subunit (PP1c) promoter. Pharmacological inhibition of DNA methylation by zebularine treatment, which proved to have anticancer properties [110, 111], decreased cocaine-induced DNA hypermethylation at the PP1c promoter. Additionally, zebularine could restore the BDNF expression in an early maltreatment animal model by decreasing promoter DNA methylation [112].

Histone Modifications

Another epigenetic target for antidepressant drugs is chromatin remodelling. Currently, HDAC inhibitors are most often used for experimental manipulation of epigenetic features [113]. HDAC inhibitors stop the removal of acetyl groups from specific histone residues and thus increasing transcriptional activity (see Fig. **1**).

The currently availabe HDAC inhibitors usually block a range of HDACs, which could affect many cellular mechanisms including cytotoxicity, cell cycle control and immune modulation [114]. The HDAC inhibitor sodium butyrate exerted antidepressant effects in a depression mouse model [115], and it could improve memory function in an Alzheimer's disease mouse model [116]. First used in cancer therapy, Vorinostat (suberoylanilide hydroxamic acid, SAHA) is the first clinically approved HDAC inhibitor to induce tumor suppressor and metastasis inhibitory genes by shifting the acetylation-deacetylation balance towards acetylation [117, 118]. In a defeat stress model the HDAC inhibitors MS-275, which is a selective HDAC1 inhibitor and can cross the blood-brain-barrier [119], and SAHA were injected into the nucleus accumbens of mice and elicited antidepressive effects comparable to fluoxetine treatment [74].

Beyond their well-known effects on monoamine-pathways, current antidepressants may also exert their effects by activating epigenetic mechanisms. Many psychopharmacologic drugs, including tricylic antidepressants, mood stabilizers such as valproate and topiramate, and also the antipsychotic drug clozapine, may induce gene activation by either decreasing methylation levels of DNA or by blocking HDACs. On the other hand, epigenetic effects leading to transcriptional silencing have been described for the SSRI fluoxetine and for monoamine oxidase inhibitors like tranylcypromine. In addition, drugs specifically targeting epigenetic mechanisms, such as HDAC inhibitors have shown some promising effects in animal models of depression and are already clinically approved in cancer therapy. However, issues of specificity, tolerability and clinical application still need to be clarified before initiating the first clinical studies.

CONCLUSION

Epigenetic mechanisms may be one factor explaining the missing heritability in major depression, as it is a mechanism for environmental exposure to have longlasting and sometimes even transgenerational marks on gene transcription [35]. Maternal care as well as childhood trauma may lead to epigenetic marks which could later influence the stress-hormone-system and either trigger the occurrence of a depressive episode or provide stress resilience. These epigenetic changes have been shown to be transmitted to the next generation in some cases which can lead to an increased vulnerability to stress and depression in the offspring. In contrast to genetic variations, however, these epigenetic changes are potentially reversible and accessibe for drug treatment. Current psychopharmacologic treatment with antidepressants, antipsychotics, mood stabilizers and stimulants may already exert some of their effects by epigenetic modifications. Drugs specifically targeting epigenetic mechanisms show some promise as antidepressant drugs in animal models, but issues related to their selectivity, mode of action, toxicity and brain permeability have to be addressed [114] before initiating clinical trials.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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