Psychiatria Danubina, 2012; Vol. 24, No. 1, pp 19–30 *Commentaries*

EPIGENETIC AND GENETIC COMPLEXITY OF PSYCHOSIS: INVITED COMMENTARY ON 'WHY SCHIZOPHRENIA GENETICS NEEDS EPIGENETICS'

Most psychiatric disorders are not caused by mutations in a single gene; preferably they involve molecular disturbances in multiple genes and other cellular signals which control their expression. Recent data demonstrated that complex of 'epigenetic' mechanisms, which regulate gene transcription without influencing the DNA code, have long-lasting effects on gene expressions. This review summarizes recent evidence for the existence of sustained epigenetic mechanisms of gene regulation in the patients with psychosis that have been implicated in the regulation of abnormalities in schizophrenia.

GENETICS

The review by Maric and Svrakic in this issue of the Psychiatria Danubina examines the contribution of genetic and epigenetic factors in the aetiology of schizophrenia (SZ). Accumulated evidence presented in the review strongly suggest that even though genetic susceptibility plays important role in aetiology of SZ (McClellan et al. 2007, Marek et al. 2005, Chubb et al. 2007, Mulle 2008), the situation is much more complex and resolving the issues is likely to require further advances in molecular genetics. A large body of the work addressed the association of several candidate genes with SZ and it is likely that some of these genes are true susceptibility factors, as suggested in recently published articles (Owen et al. 2005, Ross et al. 2006, Straub et al. 2006). These genes are implicated in signal transduction, cellular differentiation, cellular communication, neurotransmitter pathways and neuro-modulator synthesis and degradation. Among the genes which are associated with SZ are (Brown & Derkits 2010, Cannon et al. 2002): Neuregulin 1; Disc I (Disrupted in Schizophrenia I); Dysbindin; G72; Deaminoacid oxidase; Regulator of G-protein signaling; Catechol-o-methyl-transferase; Proline dehydrogenase (McClellan et al. 2007, Marek et al. 2005, Chubb et al. 2007, Mulle 2008). However, SZ is likely to be polygenic disease, with multiple genes each contributing with small effect to the predisposition of the individual. Moreover, SZ is a genetic heterogenic dieses which is explained trough the phenomenon of Copy Number Variations (CNV) which refers to the type of genetic variation of DNA strands which are duplicated, deleted or even rearranged (Van Winkel et al. 2010). This CNV variant has increased in frequency and occurs at strategic points within the genome. Functional consequences of these changes affect neural networks and behaviour. Even though the genes affected by CNV are good candidates for research into identifying susceptibility for SZ, they are not always sufficient to cause it.

EPIGENETICS

Besides genetic factors, environmental factors also play a crucial role in the aetiology of SZ. Indeed, the environmental factors that increase the risk of developing SZ include maternal malnutrition (Susser & Lin 1992), infections in the second trimester (Brown & Derkits 2010), prenatal injury and cytokine exposure (Ellman et al. 2010). However, the gene-environmental interactions modify the biological pathways and lead to the development of SZ could have greater effect than either environmental or genetic factors per se (Meyer & Feldon 2010). This appears to be an exciting area of future research, which is not only likely to improve understanding of the aetiology of SZ, but also to provide potential opportunities for therapeutic interventions, since epigenetic processes are potentially reversible which open up new path for the development of novel therapeutic drugs. The field of epigenetics suggests that environmental factors have an impact on gene expression through changes in DNA methylation and chromatin structure and may play a role in the aetiology of SZ. A recent study has highlighted an additional role of epigenetic processes in mediating susceptibility to SZ (Dempster et al. 2011). Since monozygotic (MZ) twins share a common DNA sequence, their study represents an ideal design for investigating the contribution of epigenetic factors to disease aetiology. Numerous loci demonstrated disease-associated DNA methylation differences between twins discordant for SZ. The top psychosis-associated, differentially methylated region, significantly hypomethylated in the peripheral blood DNA of twins, was located in the promoter of ST6GALNAC1 overlapping a previously reported rare genomic duplication observed in SZ (Dempster et al. 2011). This region was subsequently assessed in an independent sample of postmortem brain tissue from affected individuals and controls, finding marked hypomethylation (>25%) in patients with psychosis. These data support a role of DNA methylation differences in mediating phenotypic differences between MZ twins and in the aetiology of SZ.

It's possible that these epigenetic changes may be the missing link in understanding what causes SZ. The DNA sequence of genes for someone with SZ and for someone without it often looks the same; there are no visible changes that explain the cause of a disease. But now, epigentics represent the tool that show us changes in the second code, the epigenetic code, which may give us very important clues for uncovering the mysteries of major psychosis such as SZ.

FUTURE DIRECTIONS

There is much evidence that epigenetic regulation is involved in neurogenesis, neuronal plasticity and learning and memory in SZ. Changes in histone modifications and DNA methylation have been found globally at the promoters of genes that have been implicated in SZ. However, chromatin remodeling is likely to affect more genes, and it is important to investigate this in both animal models of psychiatric conditions and post-mortem human brain tissue. Lasting changes in chromatin modifications in animal models often occur only after chronic behavioral manipulations, which mimic the long-lasting behavioral changes similar with those in psychiatric disorders. A better understanding of the mechanisms by which such stable changes occur in animal models could improve our knowledge not only in basic neurobiology of this illness, but could also provide new therapeutic approach for SZ. Coordinated gene expression arrays and ChIP on chip arrays could be helpful in elucidating the promoter gene targets for histone modifications as well as in the binding sites of transcriptional activators and/or transcriptional repressors in specific brain regions. Such studies will provide a global picture of epigenetic regulation, which is currently lacking. Moreover, advances in mRNA profiling techniques make the possibility to quickly analyze expression across the entire transcriptome and detect changes related to the disease. These molecular signatures could provide biomarkers in clinical testing. However, such abnormalities are usually localized in specific tissues, and these methods are not suitable. This is a major obstacle in CNS illnesses, where brain tissues are less accessible. An alternative approach derives from the finding that pharmacologically induced expressional changes in certain tissues correlate with those obtainable from peripheral-blood samples. The use of these models across the entire transcriptome to detect changes related to disease or treatment response may have a major impact on clinical research in SZ.

This review represents excellent theoretical basis which allows the scientific community to focus on epigenetic mechanisms regarding SZ.

Acknowledgements: None.

Conflict of interest : None to declare.

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Psychiatria Danubina, 2012; Vol. 24, No. 1, pp 19–30

SCHIZOPHRENIA: SEARCHING FOR THE CAUSES THROUGH MANY LAYERS OF COMPLEXITY

The search for environmental causes of schizophrenia yielded very few noteworthy associations, and those that were established fell short of significantly explaining the etiology of this disease in most cases in the population. Once the search of possible environmental causes were nearly exhausted, the attention – and expectations - turned to genetic factors.

For many years, search for genetic causes of schizophrenia was slow and severely restricted by technology available for exploring the DNA. However, in the past 5-10 years genomic research has gone through a true revolution, reflecting the great progress in genotyping technology. Once deemed impossible, today we can genotype billions of DNA markers, and even sequence the whole human genome routinely. Only 10 years ago, the cost of sequencing one human genome was 3 billion US dollars and it took the world 11 years to achieve it. Today, it costs merely 5,000 US dollars (or less, depending of the nature of the order) and it typically only takes a few days to complete.

As a result of this "revolution", researchers were overwhelmed by the amounts of newly available data. New analytical approaches and related software had to be developed, high-capacity secure data storage assured and personal information protection granted; new departments, journals and professions emerged.

On a less pragmatic level, changes were also observed. Once looked down upon, hypothesis-free science became the leading approach to analyzing genome-wide data. While odds ratios below 2 were commonly disregarded, the new and very large studies have taught us that most genetic variants will be truly causal for a studied disease, but still exhibit very small effect size (commonly odds ratios below 1.5). Another important thing emerged through this progress, which has to do more with a social aspects of doing science: while researchers of the 20th century were used to protecting their data, hiding research ideas and competing with others, the genetic revolution of the 21st century has taught us that there is little that we can do on our own.

Large international consortia have been established for numerous diseases and traits. Final sample size of such large collaborations is regularly few tens of thousands of participants, which gives studies power to detect even the smallest true genetic effects. Successes of such large collaborations are published on daily basis. By identifying genes that affect a trait, these findings give us valuable insights into human biology. A recent example is a study comprising >50,000 individuals that has discovered 5 novel loci associated with schizophrenia (Ripke et al. 2011). On one end of the spectrum, there are traits for which we expected to find genetic risk factors, but on the other end of spectrum there are conditions that we didn't think would be affected by genetic variants: for example, a recent study has shown that smoking-related habits, or even coffee-drinking, are also affected by genetic factors (Liu et al. 2010).

Schizophrenia affects approximately 1% of the population and shows a high heritability of 73-90% (Sullivan et al. 2003). In the light of such high heritability and genetic causes of disease strongly supported by family, twin and adoption studies (Harrison & Weinberger 2005), it came somewhat as a disappointment that genome-wide association studies with dense SNP markers yielded very few candidate genes for schizophrenia. Furthermore, the replication of those rare identified genetic variants in an independent sample was often cumbersome. The need for new hypothesis yielded new research directions.

In the early days, microscope was the main diagnostic tool of geneticist and it was only possible to identify major genetic pathologies, such as large visible structural variants - for example, an alteration in the chromosome numbers, such as trisomy 21. Today, we focus our attention to complete zooming-in the DNA and getting the exact sequence of human genome. Conversely, only after zooming-in the DNA did we realize an intermediate source of genetic variation, too small to be detected by microscopes: submicroscopic structural variants, this is most often copy-number variation (CNV) (Redon et al. 2006). Copy-number variant (CNV) is a segment of DNA, 1 kb or larger, that is present at a variable number when compared to the reference genome; they can be simple in structure like tandem duplication, or they can comprise insertions or deletions of a sequence at multiple sites in the genome. A common "copy-number polymorphism" (CNP) is a copy-number variant that is present in more than 1% of the population.

Studies of CNVs brought in a new dimension and revived the genetic research in psychiatric illnesses, offering a new direction in search for the cause. The boost of extra optimism came from the studies of autism that have reported an increased frequency of de novo CNVs in affected children (Sebat et al. 2007). The potential of the copy-number variants to affect the disease susceptibility need not surprise us since they outnumber variation brought by SNPs and cover as much as 12% of the genome (Feuk et al. 2006). For schizophrenia, first Walsh et al. (2008) have shown that multiple rare CNVs are associated with schizophrenia; later, Xu et al. (2008) have shown an increase in de novo CNVs in the sporadic schizophrenia cases.

Assessment of the function of genes disrupted by CNVs, which tried to establish whether their function

might be related to schizophrenia, discovered groups of genes overrepresented in individuals with schizophrenia: Walsh et al. (2008) found it was genes important for brain development, including cell signalling, synaptic potentiation and axonal guidance. Later study of Xu et al. (2008) found genes associated with neural development, small GTPase activity and RNA processing to be overrepresented in these cases (Xu et al. 2008).

But this great new knowledge that we have rapidly gained was still not enough to really understand genetic basis of schizopherenia and explain disease aetiology. It was recently shown that common single nucleotide polymorphisms explain about 30% of genetic causes of schizophrenia, and that further 30% is explained by rare, large copy-number variants – which still leaves a large proportion of genetic causes of schizophrenia unexplained.

In their comprehensive review, Maric & Svrakic set out to introduce yet another dimension in genetic variation, associated with schizophrenia: epigenetic modifications. The authors thoroughly describe evidence in support of the role of epigenetic factors in pathophysiology of schizophrenia and firmly establish epigenetics as another dimension relevant for the development and progression of schizophrenia, a new dimension that has the potential to further explain genetic causes of schizophrenia.

The existence of epigenetic modifications that effectively change DNA calls for the reassessment of one of the central genetic paradigms – of life-long sameness of DNA. Furthermore, a wide range of environmental factors - from chemical and biological, to psychosocial exposures - have been shown to change epigenetic profile and functioning of DNA. In a way, this is not surprising: there is a mechanism that enables the effective change in DNA. After all, it seems unlikely that what is optimal in youth remains optimal throughout 80 years of human life and modifications of the DNA could increase adaptation to the environment. The complexity is even greater: the most recent study has established the important role of DNA sequence itself that determines methylation (Lienert et al. 2011).

In concluding remarks, it is worth noting that epigenetics has brought about a new concept, by showing that environmental factors effectively modify DNA; in other words, environmental factors affect genetic make-up. While we always rendered it likely that both environment and genetics contribute to disease development, it was expected that the end sum of their effects determines disease development. The new epigenetic concept described in the Maric & Svrakic review describes the intertwining effects of one on another, emphasising the continuous interactions of genetic and environmental factors, where each has the ability to change the other. We believe even further levels of complexity to be revealed in the future and interactions between different dimensions, such as the interaction of environment and DNA, will become recognised.

Acknowledgements: None.

Conflict of interest : None to declare.

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EPIGENETICS IN SCHIZOPHRENIA: MORE THAN AN EPIPHENOMENON

In their article "Why Schizophrenia Genetics Needs Epigenetics: A Review", dr. Maric and dr. Svrakic review a large proportion of the current literature on the contribution of genetics, gene-environment interactions and epigenetics in the neurobiological cascades that are thought to underlie the etiology of the psychiatric diagnostic category of schizophrenia. The authors provide a useful theoretical framework by which clinicians and scholars can attempt to understand the complex etiology of this clinically heterogeneous, neurodevelopmental disorder, and they furthermore formulate valuable proposals for future directions in research. Although the review by Maric & Svrakic is quite extensive in summarizing the evidence on epigenetics in schizophrenia, I'd like to add a few important findings.

While the review by Maric & Svrakic provides a good overview on the evidence on DNA methylation and histone alterations in schizophrenia, findings from two recent genome wide association studies (comparing schizophrenia cases versus controls) and one recent whole-genome wide DNA methylation study (comparing individuals from monozygotic twins discordant for schizophrenia and bipolar disorder) have further strengthened the evidence suggesting a crucial role for the epigenetic machinery in schizophrenia. First, a recent GWAS analysis in combination with a large meta-analysis on schizophrenia has suggested that common variants in a cluster of the histone genes HIST1H2BJ, HIST1H2AG, HIST1H2BK, HIST1H4I and HIST1H2AH (at chromosome 6p22.1) are among the variants with the most significantly increased risk of schizophrenia (Shi et al. 2009). Second, the largest GWAS analysis on schizophrenia in the literature to date has furthermore provided evidence for a strong association between a single nucleotide polymorphism in the gene encoding microRNA137 and schizophrenia (Ripke et al. 2011). By being post-transcriptional regulators that bind to target mRNA transcripts (usually resulting in repression of gene expression, i.e. gene silencing), microRNA molecules are considered a component of the epigenetic machinery. Interestingly, and in line with a role of microRNA molecules in schizophrenia, elevated levels of microRNAs were found in the dorsolateral prefrontal cortex of schizophrenia patients (Santarelli, Beveridge, Tooney & Cairns 2011). Third, a very recent study that interrogated the methylation profile of the whole genome of DNA extracted from whole blood samples of monozygotic twins pairs discordant for schizophrenia and bipolar disorder, showed distinct genetic loci with differential methylation profiles in affected twins (Dempster et al. 2011), thus providing further evidence for a role for epigenetics in schizophrenia.

As a result of i) the promise of epigenetics as a bridge between nature and nurture (Gottesman & Hanson 2005), ii) breakthrough findings in the field of epigenetics, and iii) the currently available methodologies to detect epigenetic marks (Laird 2010), the field of epigenetic research in neuroscience and psychiatry is developing very rapidly. A current threat for epigenetic research is overinterpretation of the findings to date, for example by interpreting findings as indicative of methylation being the cause and solution to virtually any psychopathological disorder (Miller 2010). It is thus important to view the current state of the literature as preliminary and to be very cautious, especially because most of the findings of epigenetic changes associated with certain behavioral or psychiatric phenotypes in animals (let alone in humans) have not been replicated consistently. For example, the breakthrough findings in animal studies by the group of Michael Meaney showing that maternal behavior during very early life of their offspring determined behavior, epigenetic profiles and gene expression in adulthood of the offspring (Weaver et al. 2004), and that these changes were reversible with methyl supplementation (Weaver et al. 2005), have not yet been consistently replicated by other animal laboratories.

The first wave of epigenetic studies in neuroscience and psychiatry have thus increased awareness of the possibilities but also of the numerous technical and analytical challenges that require detailed exploration (Petronis 2010). For example, the limited accessibility to high-quality human brain tissue from wellphenotyped patients, in combination with the cell-typespecific and temporal-specific nature of the epigenetic machinery poses significant challenges for epigenetic studies in psychiatry (Rutten & Mill 2009, Pidsley & Mill 2011). Despite the problems of tissue specificity, one could propose to use other, more accessible tissue sources like leukocytes or bucchal epithelial cells for epigenetic analyses in the hope that the patterns observed will reflect those of the tissue of interest, i.e. the central nervous system. Interestingly, there is evidence that many epimutations are not limited to the central nervous system, but can also be detected in other tissues (Wong et al. 2010, Uddin et al. 2010) – this may be especially the case for environmentally-induced changes where exposures may induce changes throughout the body.

Another limitation of current epigenetic research is that the characterization of the 'normal' epigenome, especially for tissues such as the brain has only recently started, and correlations across tissue types have not been rigorously performed (Callinan & Feinberg 2006).

As discussed by Maric & Svrakic, findings of carefully employed epigenetic studies will be of high

relevance for a better understanding of the complex etiology of schizophrenia, and of the contribution of GxE (Van Winkel 2010) and the heritability of epigenetic marks in filling the 'missing heritability' in schizophrenia (Crow 2011, Maher 2008). Future studies should involve multiple research disciplines (epidemiology, molecular genetics, neuroscience, psychiatry and psychology) and make use of genetically-sensitive epidemiological designs such as family and twin designs, preferentially using prospective data collection while employing statistical analysis approaches for studying GxE interactions and epigenetic modifications in a hypothesis-driven as well as an agnostic manner (Thomas 2010, van Os & Rutten 2009). Epigenetic studies may furthermore benefit greatly from using translational perspective, i.e. combining observational human investigations and experimental animal investigations. Such studies will likely provide very valuable insights in the dynamics of the biological mechanisms underlying the development towards, as well the onset and course of major psychotic disorders.

CONCLUSION

To conclude, it is very likely that epigenetic alterations in schizophrenia are more than an epiphenomenon, and we have now reached the exciting stage where it is feasible to start investigating the ways in which environmental factors interact with the genome to bring about epigenetic changes in gene expression and risk for disorders such as schizophrenia.

Acknowledgements: None.

Conflict of interest : None to declare.

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WHY SCHIZOPHRENIA GENETICS NEEDS EPIGENETICS: A REVIEW

Schizophrenia is a complex multifactorial and polygenic disease. Although family, twin and adoption studies have consistently suggested that genetic factors play an important aetiologic role in schizophrenia, until now, very little is known about the nature and number of these genetic factors. The overall heritability of the disease is 63-85%. The genetic basis may involve at least several genes of only mild influence with interactive effects but the relationship between observed genetic risk factors and specific DNA variants or protein alterations has not so far been identified.

Linkage and association studies showed at least 12 chromosomal regions containing more than two thousands known genes and specific genes as being involved in the etiology of schizophrenia. Polymorphisms are associated with schizophrenia for neuregulin 1 (NRG1 located at Ch 8p13), dystrobrevin-binding protein 1 (DTNBP1 located at Ch 6p22), G72/G30 (D-amino acid oxidase activator gene region in Ch 13q34), proline dehydrogenase (PRODH located at Ch 22q11), regulator of G-protein signaling 4 (RGS4 located at Ch 1q21-22), catechol-O-methyltransferase (COMT located at Ch 22q11), disrupted in schizophrenia (DISC1 and DISC2 located at Ch 1q42), serotonin 2A receptor (5HTR2A located at Ch13q14) and dopamine receptor D3 (DRD3 located at Ch3q13) genes. However, replication studies have led to contradictory data for all these genes. The first four genes may be involved in glutamatergic signalling, mainly through NMDA receptors. More recently, the International Schizophrenia Consortium found no genes with genomewide significance in their large European sample (>3,000 cases and controls). A similar result was obtained by the SGENE consortium and by the Molecular Genetics of Schizophrenia (MGS) study using large samples of respectively European (both) and African American groups. When the three European samples were combined (>8,000 cases and 19,000 controls), a significant association with several markers spanning the major histocompatibility complex (MHC) region on chromosome 6p21.3-22.1 was reported. In addition, the study conducted by the SGENE consortium found significant association near the neurogranin gene (NRGN) on 11q24.2 (coding for a synaptic protein) and for an intronic SNP in transcription factor 4 (TCF4) on 18q21.2. These findings implicating the MHC region are consistent with an immune component to schizophrenia risk, whereas the association with NRGN and TCF4 points to perturbation of pathways involved in brain development, memory and cognition (review by Thibaut 2010).

Indeed, there is increasing evidence for an overlap in genetic susceptibility across the traditional classification categories. For example, the NRG1 gene might contribute to a range of psychosis-related phenotypes (schizophrenia, bipolar disorders with manic or mood incongruent psychotic features, schizotypal personality traits or even psychosis that occurs in patients with Alzheimer's disease (Harrison and Law 2006)).

Maric & Svrakic have conducted an excellent review about epigenetics in schizophrenia. They argued that epigenetic model of SZ provides a framework to integrate a variety of diverse empirical data into a powerful etiopathogenetic synthesis. The promising future of this model is the possibility to develop truly specific prevention and treatment strategies for SZ. Environmental factors are rarely sufficient to cause SZ independently, but act in parallel or in synergy with the underlying genetic liability. According to these authors, epigenetic misregulation of the genome and direct CNS injury are probably the main mechanism to mediate prenatal environmental effects (e.g., viruses, ethanol, or nutritional deficiency) whereas postnatal risk factors (e.g., stress, urbanicity, cannabis use) may also affect risk via use-based potentiation of vulnerable CNS pathways implicated in SZ.

Maric & Svrakic pointed out that there is accumulating evidence that both common genetic variants with small effects and rare genetic variations with large effects determine risk of SZ. They also reported that thousands of common single nucleotide polymorphisms (SNPs), each with small effect, cumulatively could explain about 30% of the underlying genetic risk of SZ; on the other hand, rare (1-3 SZ cases per 1000) and large copy number variants (CNVs) including several genes with high but incomplete penetrance, variable in different individuals explain about additional 30% of SZ cases. In a given subject they constitute a strong risk factor with a relative risk of 7 to 20. For example, the 1q21.1 region, which contains 27 known genes, has been previously linked to schizophrenia. A schizophrenia candidate gene lies also in the 15q region: CHRNA7 encodes the alpha7 subunit of the nicotinic acetylcholine receptor, and has been linked to the schizophrenia-associated phenotype of auditory evoked potential deficits. But the relevant genes in these regions and the neurobiological mechanisms are poorly understood. These rare CNVs have a high phenotypic variability (Merikangas, Trends Genet 2009) (from SZ to autism, BPD, or mental retardation (1q21.1 and 15q13.3) even within the same families (16p11) and the same CNVs may even be associated with changes in head circumference (1q21 and 16p11).

According to Maric & Svrakic, although these rare CNVs frequently develop de novo, it is not clear whether they affect risk independently or via interaction with a polygenic liability in the background.

Maric & Svrakic have also briefly mentioned endophenotypes or intermediate phenotypes which are closer to the underlying genetic or neurophysiological mechanisms respectively and thus likely to provide a

more valid classification of phenotypes. There are numerous candidate intermediate phenotypes and endophenotypes in the literature, ranging from neurophysiology (e.g., prepulse inhibition, mismatch negativity, abnormal phase synchronicity in beta- an gamma-oscillations, etc), molecular biology (e.g., COMT polymorphism, etc), to neuropsychological tests (e.g., working memory tests, etc).

To meet endophenotype criteria, candidate markers have to be: (1) heritable; (2) primarily state-independent; (3) associated with illness in the population; (4) to co-segregate with the disease within families; (5) and to be found in affected, as well as unaffected family members, at a higher rate than in the general population (review by Gottesman & Gould 2003). In addition, these endophenotypes may be used for classification or diagnosis (Louchart-de la Chapelle et al. 2005) and in the development of animal models.

In a review about endophenotypes in schizophrenia comparing healthy controls to schizophrenic subjects and/or relatives to healthy controls, Allen et al. (2009) have reported the largest effect sizes (Cohen's d) ($\geq$1.5) for the following endophenotypes: neurological deficits, auditory-evoked P50 paradigm, fMRI-activation during 2-back task, Continuous Performance Test and oculomotor-delayed response. The auditory-evoked P50 paradigm was described by Freedman et al. to measure deficit in sensory-motor gating in schizophrenic patients. Animal and human studies have suggested a role for septohippocampal cholinergic activity (involving the alpha 7 subunit of nicotinic receptors) in sensory gating. Several studies have shown that promoter variants (in the alpha 7 gene) or variants located in the alpha 7-like gene are associated with P50 inhibition deficits (Raux et al. 2002, review by Leonard & Freedman 2006).

In a given proband, when a cytogenetic event is necessary and sufficient to predispose to a psychiatric phenotype (e.g. psychosis), molecular cytogenetics may constitute a powerful alternative strategy to nominate candidate genes. Thus, the DISC 1 gene (Ch 1q42) has its roots in a balanced translocation (1:11) that segregated with schizophrenia, bipolar disorder and recurrent major depression; the Velo-Cardio-Facial Syndrome (VCFS) has been an important source of promising candidate genes with broader clinical relevance (e.g., PRODH, COMT). DNA sequence variations within the 22q11 VCSF deleted chromosomal region are likely to confer susceptibility to psychotic disorders (prevalence of SZ 25-30%). Two genes believed to contribute to risk of both schizophrenia and bipolar disorder, GRIK4 (located on chromosome 11q23) and NPAS3 (on chromosome 14q), were also identified at breakpoint regions of translocations in patients with co-morbid psychosis and mental retardation (Pickard et al. 2005, 2006). GRIK4 is expressed in the amygdala, hippocampus and entorhinal cortex, kainate receptors are involved in hippocampal synapses responsible for neuronal plasticity. In mice lacking NPAS3, there is virtually no neurogenesis in the adult hippocampus (Pieper et al. 2005).

The PRODH gene, which encodes the mitochondrial enzyme proline dehydrogenase, is located in this 22q11 region. Homozygous deletion and/or missense mutations (variants affecting highly conserved amino acids and causing drastic reduction in enzyme activity) of this gene were associated with high plasma proline levels, severe mental retardation and epilepsy. The same molecular alterations of the PRODH gene, but at the heterozygous state, were associated with moderate hyperprolinemia in certain forms of psychosis (Jacquet et al. 2005). Interestingly, Liu et al. (2002) have reported an association between several of these PRODH variants and schizophrenia. In addition, high levels of prolinemia observed in a subset of 22q11 deletion syndrome patients were associated with lower IQ, and, in some cases, epilepsy (Raux et al. 2007). Lastly, homozygous truncating mutation of the PRODH gene in mice results in hyperprolinemia, in a dysregulation of glutamate release associated with a cortical dopaminergic hypersensitivity to amphetamine, in a deficit in prepulse inhibition (a sensory gating impairment) and in deficits in associative learning, that are associated with schizophrenia (review by Paterlini et al. 2005). In the same way, COMT polymorphisms interact also with other genes such as the PRODH gene for developing psychotic symptoms in the 22q11 deletion syndrome (Raux et al. 2007).

One question that arises is whether the CNVs in such cases act simply by influencing IQ, which in turn has a non-specific effect on increasing risk of schizophrenia, or whether there are specific CNVs for MR plus schizophrenia, and some which may indeed increase risk of schizophrenia independent of IQ.

Interestingly, Maric & Svrakic, using analogy with cancer, proposed a "two hit" scenario in which a genetic defect (e.g., recessive mutation) would not result in illness unless accompanied by some other genetic or environmental variables (e.g., somatic mutation or epigenetic silencing of the normal allele, respectively) leading to the expression of the mutation. Maric & Svrakic made the following assumption, which is relevant in complex diseases such as psychiatric disorders, phenotypic differences arise when multiple and variable genetic factors interact nonlinearly among themselves and with multiple and variable environmental factors, all with variable timing, duration, and severity. Non linearity of the process also indicates that genetic liability for SZ does not ipso facto mean phenotypic expression of the illness, but rather implies a graded presentation that includes a spectrum from mild to most severe cases. They reported compelling evidence that SZ may be the most severe outcome of a familial polygenic liability for aberrant CNS architecture and function, also called "SZ spectrum" disorders. The SZ spectrum disorders may include mental retardation, autism, SZ, bipolar disorders with psychotic features and even fronto temporal dementia. For example, an increased morbid risk of schizophrenia

was observed within families with frontotemporal dementia and the same mutations may be observed in both diseases (Valosin Containing Protein gene located on Ch 9p and involved in protein degradation and ProGRranuline gene located on Ch 17q, a growth factor) (Schoeder et al., in press). Within 3 families, patients with the same mutations may present either with frontotemporal dementia or with SZ.

Finally, according to Maric & Svrakic, the existing data point to late adolescence as the critical development period during which temporal patterning of brain activity is expected to reach adult levels. A failure of this process could fully expose and even accentuate underlying brain abnormalities in SZ, usually first manifested during late adolescence. In case of greater severity of genetic liability the onset of the disease may even be earlier.

CONCLUSION

In conclusion, armed with GWAS data in these heterogeneous populations, additional risk genes can be identified through strategies aimed at refining the phenotype that are not constrained by the current dichotomous view of the functional psychoses.

However, if the goal of GWAS is to find genetic variants that are predictive of risk or that shed light on the pathogenic mechanisms of the disease, then clearly, even if such variants can be found by largely increasing sample sizes, their identification alone would not achieve either of these goals. If we want to understand the biology of the disease, it might be easier to concentrate our efforts on the identification of rare inherited and non-inherited variants with large effect on the phenotype. Such rare variants are also easier to model in animals (relative to common variants with very small functional effect).

Acknowledgements: None.

Conflict of interest: None to declare.

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Psychiatria Danubina, 2012; Vol. 24, No. 1, pp 19–30

FROM GENES TO MENTAL REPRESENTATIONS: A COMMENT TO "WHY SCHIZOPHRENIA GENETICS NEEDS EPIGENETICS"

The review by Svrakic and Maric deals with an important issue in schizophrenia research, the role of genetic factors in the pathophysiology of schizophrenia. While numerous now classical family and twin studies paved the way for this field of research, a plethora of information has been gathered by numerous research groups on a wide variety of genes that may play a role. Svrakic and Maric clearly summarize this evidence by concluding that common genetic variants with small effects and rare genetic lesions with large effects need to be considered. Several critical issues follow from this conclusion: if we have many potential genetic factors, which of these is the culprit in individual cases? And will the search for the pathophysiological mechanism initiated or sustained by genetic factors ever lead to the discovery of final common pathways of pathophysiology allowing for a limited number of clinical phenoltypes to emerge from the multitude of genes and their products?

Svrakic and Maric then focus on the role of epigenetic factors in the pathophysiology of schizophrenia. The role of epigenetics has been a focus of recent interest not only in schizophrenia research, but also for other mental disorders like major depressive disorder, post-traumatic stress disorder and anorexia nervosa (Toyokawa et al. 2012). In schizophrenia research, this research field has caused considerable interest following the observation that with classical genetic techniques only associations between genetic alterations on the one hand and the development of schizophrenia on the other hand could be elucidated. Also, the contribution of individual genes was shown to be very small leading Crow (2011) to the conclusion that less than 2% of the 80-90% heritability of major psychiatric disorders like schizophrenia was attributable to genes identified by linkage and association. The disappointment is apparent and was put into a succinct form by Gershon and coworkers asking "Where is the missing heritability?" (Gershon et al. 2011) and proposing larger study cohorts and novel sequencing techniques as the next steps of identifying any putative missed genetic associations. Especially for genome-wide association studies, a large number of participants is needed to lead to more informative results (Bergen & Petryshen 2012). Given these outlooks, the public plead to funding bodies for sustaining funds for genome-wide association studies in spite of the current disappointment should be supported (Sullivan et al. 2012a). In neurophysiology, alterations of the default mode brain network and connectivity have gained considerable attention (Whitfield-Gabrieli & Ford 2012). An important research approach is therefore to develop new ways of classifying psychopathology based on dimensions of observable behavior and neurobiological measures an approach currently fostered by a research initiative of the U.S. National Institutes of Mental Health (Cuthbert & Insel 2010). A solid foundation in a rigid clinical diagnosis of schizophrenia is an essential requisite for any such studies, as even minor degrees of diagnostic misclassification can impact on the rate of false-positive estimates for example of genetic correlations (Wray et al. 2012).

A suggestion has been put forward to consider epigenetic effects of meiotic differentiation as a major pathogenic and human-species specific aspect of the pathogenesis of schizophrenia (Crow 2012). Although there is still a considerable lack of understanding what the pathophysiological consequences of any genetic findings may be on the function of the brain, epigenetic regulation could also help to connect the known linkages and associations with the known environmental factors like virus infections, urbanicity or cannabis use. Epigenetic dysregulation came into play here because it is the central pathway of how environmental factors influence gene expression, which also applies to the regulation of genes expressed in the human brain (Toyokawa et al. 2012). Svrakic and Maric scholarly summarize the evidence for the epigenetic "misregulation" of a large number of genes in schizophrenia. An important issue, however, would also be to distinguish those epigenetic mechanisms involved in the pathophysiology from those secondary to the disorder itself or caused by drugs used in schizophrenia or even the lifestyles of persons affected by schizophrenia (Abdolmaleky & Thiagalingam 2011). Grayson critically discusses a lack of evidence linking genetic findings with the expected results of protein expression in the human brain (Grayson 2010). Clearly, the epigenomic approach needs now to be complemented by a "proteomic" approach (Plazas-Mayorca & Vrana 2011). Another caveat is that some epigenetic "misregulation" may actually not be pathogenic but an attempt at repair (Dudley et al. 2011).Thus, while the epigenetic hypothesis of schizophrenia has much evidence for it, the exact role of epigenetic misregulation in schizophrenia is far from clarified. Svrakic and Maric show how known environmental risk factors for schizophrenia may explain such interactions. Interestingly, microRNAs have recently been shown to be able to mediate such interactions between the immune system and neurodegenerative disorders (Ha 2011), and one of the more recent additions to the plethora of schizophrenia genes was a microRNA (The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium 2011).

In an interesting chapter, Svrakic and Maric then discuss constraints like the timing of the insults and the chronicity of the exposure to damaging agents that may be decisive as to whether a certain factor becomes pathogenic or not. The critical periods in the ontogenetic origins of schizophrenia would obviously be an important issue (Perrin et al. 2010). Svrakic and Maric refer to the complexity of the issues at stake as the "complex nonlinear development of schizophrenia", which is an intellectually appealing terminology which would need more thorough work-up in order not to be confused with the use of the term "nonlinearity" in other contexts in schizophrenia research, namely the nonlinear dynamics of neurophysiological factors as measured by neuroimaging or electroencephalography.

On this ground of evidence, Svrakic and Maric briefly touch on a hypothesis that neurobiology of schizophrenia does not match its clinical classification. The most interesting idea in this chapter is to distinguish between equifinality and multifinality, an idea which in its basic assumptions was also covered by the recent approach towards schizophrenia as a "pathway disorder" (Sullivan, 2012b). It is, however, not clear how this should be transferred into any new classification system. Subtyping leading to a vast number of individual pathways does not seem to be a practical solution. For the time being and until a final verdict is out on such complex and multifaceted issues, it may be necessary to keep the concept of "schizophrenia" for classification purposes but supplement it with optional information on pathophysiological factors as they become available. This approach may later be changed once differential pathophysiologies of schizophrenia are discovered and lead to different treatments. This would be the case, for example, if we knew which brain circuits or "modules" are disturbed in individual cases, and if the malfunctions of such modules could be treated individually (Zielasek & Gaebel 2008). Evidence indicates that such disturbed modularity can be detected in several mental disorders including schizophrenia (Seitz et al. 2011).

Describing schizophrenia as a complex multifactorial disorder with highly variable course and clinical expression does not make research into the pathophysiology of schizophrenia easier, but it is probably the best approach towards hypothesis formulation for research projects addressing the pathophysiology of this disorder. The evidence for subtyping schizophrenia is rather weak, as shown also by the fact that the workgroup on the classification of psychotic disorders for the development of DSM-5 has decided to omit the classical subtyping into paranoid, hebephrenic/disorganized and catatonic subtypes (http://www.dsm5.org). A new subtyping appears to make sense only on the grounds of disturbed neurocircuits and the CNTRICS initiative is one important step in this direction ircuits (Carter et al. 2012). The evidence is mounting that disturbances of brain modularity can be detected in patients with schizophrenia but also other mental disorders like dementias. In the hopefully not too far away feature, such findings may become the cornerstones of pathophysiologically based classifications of mental disorders. The recent renewed interest in research about gene-environment interactions or other integrative concepts of schizophrenia shows that several approaches are possible in order to reconcile neurobiological and clinical observations in schizophrenia (Gaebel & Zielasek 2011). Given the complexity and inter-individual multitude pathophysiologies, clinical presentations, longitudinal courses and prognoses of schizophrenia, such integrative approaches are definitely needed. A central challenge here is to unify the influences of all these elements in a coherent framework. How do external environmental stimuli and internal neurobiological and psychological pathways interact to form an individual representation of reality in the human brain, which is the basis of the subjective experience of a person of herself/himself as a unique, self-conscious and an acting human being? How can we then explain in this framework the experiences of delusions and hallucinations so characteristic of schizophrenia? This is no easy task but experimental paradigms using, for example, functional magnetic resonance imaging are now beginning to tackle this clinical question, which is, however, strongly related to the l question of the representation of the external world and our subjective experiences in the human brain.

The introduction of pathophysiological factors into the classification of schizophrenia would be a Kuhn-ian paradigm shift in the conceptualization of schizophrenia (Gaebel & Zielasek 2009). We are currently investigating such paradigm shifts in schizophrenia concepts in the framework of a joint research project between psychiatrists, linguists and philosophers, also assessing how to define and detect such paradigm shifts (http://www.sfb991.uni-duesseldorf.de/en/summary/). The results may even become interesting for those looking for criteria for changes in classification systems of mental disorders, which will have to respond to a number of needs and criteria for assessing diagnostic classifications including ease of use, utility, reliability, and both predictive and criterion validity (Jablensky 2011). Until the time has come to take paradigmatic steps in the classification of schizophrenia, it probably remains best evidence-based practice to keep the construct of schizophrenia as it is, maybe with some improvements in selected aspects. The WHO Work Group on psychotic disorders, chaired by Wolfgang Gaebel and consisting of a panel of international psychiatric experts from all around the world, is currently discussing such issues with a view to identify those areas of schizophrenia research that may lend themselves as the basis of novel classification criteria in schizophrenia. In such a broader context, progress in epigenomics research along the concept proposed by Svrakic and Maric is eagerly awaited.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation, Collaborative Research Centre 991, Project B06) and institutional funds of the Department of Psychiatry and Psychotherapy, Medical Faculty, Heinrich Heine University Düsseldorf.

Conflict of interest: None to declare.

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