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STUDY PROTOCOL

Gene–Environment Interactions in Major Mental Disorders in the Czech Republic

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Background: Mental disorders affect about one-third of the human population, are typically chronic and significantly decrease the quality of life. Presently, the treatment of mental illnesses is far from adequate with a substantial proportion of the patients being pharmacoresistant and suffering from relapses. One of the reasons for this complicated situation is that we do not precisely know about the causes of mental disorders, so their treatment cannot be causal. The etiology of a mental disorder is typically based on a combination of molecular (genetic) and environmental factors.

Aim: The aim of the project is to discover the gene–environment interactions (GxE) in a wide spectrum of mental disorders.

Methods: The design of our study is innovative in the sense that we intend to study large groups of associated mental disorders as a whole instead of in isolation. This would enable us to map out the possible environmental causal factors in detail in relation to their character, magnitude and timing. The project also allows a study of genetics (including epigenetics and microbiomes) as well as the environment simultaneously. We plan on involving three study groups: the first group are patients suffering from schizophrenia or a mood disorder such as major depression, recurrent depressive disorder and bipolar affective disorder; the second group of patients have anxiety disorders; and the third group are healthy volunteers from the general population who are genetically unrelated. All of the study subjects will undergo the following assessments: a psychiatric examination, the identification of stressful life events with the aid of a questionnaire, the examination of their reaction to stress, genetic and epigenetic (microRNA) assessments and the analysis of oral and gut microbiome.

Conclusion: We expect that some of the genetic as well as environmental factors in the studied mental disorders are shared, while some others are specific. We also expect that the GxE (gene–environment interaction) in schizophrenic and affective disorders will be different from the GxE in anxiety disorders and that the GxE in the studied mental disorders will differ generally from the GxE in healthy volunteers. Our results can help in the prevention and individualized treatment of a range of mental disorders.

Keywords: schizophrenia, mood disorders, anxiety disorders, gene, environment, GxE interactions

Plain Language Summary

- Mental disorders affect about one third of the human population and are typically lifelong. They significantly impair the subjects' quality of life and may lead to suicide.
- Their treatment result is not satisfactory, partly because our knowledge of their etiology is limited, so the therapy is more symptomatic than causal.
- The etiology of almost all mental disorders is based on the interaction of genes and the environment.

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- Previous research was mostly aimed at either genetic or environmental factors in isolation and not on their mutual interactions, which led to a bias.
- We propose carrying out a complex study on the interaction between genes and the environment in major mental disorders (psychosis, mood disorders and anxiety disorders).
- We will provide a questionnaire to the patients about stressful life events, we will carry out an examination of the patients´ reaction to stress, do genetic and epigenetic (microRNA) studies and analyse the oral and gut microbiomes of the patients and we will perform the same assessments in healthy subjects who are genetically unrelated.
- We expect that the results of our project may help in the treatment and prevention of major mental disorders.
- The proposed project due to its focus and comprehensiveness represents a priority in the field of psychiatry not only in the Czech Republic but throughout the rest of Central Europe as well.

Background

A lifetime prevalence of mental disorders is estimated to be in the range 12.0–47.4%.[1] Once a mental disorder develops, eg dementia, substance addiction, psychosis, mood or anxiety disorders, it usually continues until the end of the patient ´s life. Other mental disorders, like personality disorders or intellectual disability, are lifelong.[2] Apart from suicide, a mental disorder does not usually lead directly to the death of the patient, but it significantly decreases his or her quality of life, increases the level of disability in the population and places a considerable economic burden on society.[3]

In spite of the attained progress that has been made in treating mental illness, the current treatment regimens of mental disorders are still far from satisfactory. We are able to treat most mental disorders and improve the clinical state of the patient, but a full and continuous remission is rather rare. One of the reasons is that we do not know the exact etiology of mental disorders very well, so the treatment is more likely to be symptomatic rather than causative.[4]

Most mental disorders require interactions between the genes and the environment to develop.[5–10] If the genetic or environmental factors of a mental disorder are studied separately, the research results would inevitably be biased. The solution to this issue is to study both the genetic and the environmental factors simultaneously, in order to find connections between the two. Some of these findings were already published and replicated, such as the influence of the expression of the MAOA (monoamine oxidase A) gene and the degree of violence experienced in childhood on antisocial behavior in adulthood or the influence of stress on the development of depression depending on the BDNF (brain-derived neurotrophic factor) Val66Met polymorphism.[11,12]

A recent approach on testing the statistical interactions between the genetic and environmental factors in the complex etiology of mental disorders is the genome-wide environment interaction study (GWEIS).[5] This way, the genome of study subjects as well as the significant environmental factors able to influence the development of a mental disorder (exposome) are assessed in a comprehensive way. Similarly to the evaluation of genetic risk factors (polygenic risk scores), the environmental risk factors are also appraised via the usage of an omnibus test (poly-environmental risk scores). These studies where a lot of genetic and environmental factors are both assessed together would have more validity than research aimed only at genes or the environment. It is useful to study psychiatric diagnoses in a cumulative way, as groups of diseases rather than isolated clinical entities, because: (a) the diagnosis of a mentally ill subject frequently changes throughout his or her life; (b) comorbidities within mental disorders are pervasive, and the risk persists over time;[13] (c) not all recent psychiatric diagnoses have a sufficient validity (eg schizoaffective disorder or mixed anxiety-depressive disorder); (d) many detected genetic polymorphisms represent a shared heritability in common disorders of the brain;[14] and (e) many environmental factors are non-specific: eg either seriously stressful life events may induce major depression in some subjects but episodes of schizophrenia in other ones. In addition to this, different environmental factors may contribute to the development of the same disease in predisposed individuals; eg in schizophrenia there were found to be correlations with the season of the patient´s birth, the occurrence of obstetric complications, a history of abuse or trauma during childhood, the use of cannabis as well as exposure to stressful events.[15] Even if the specificities of the individual environmental factors as related to one psychiatric diagnosis are low, more specificity may be attained by a unique combination of these E (environmental) factors. An excellent review of how to "embrace" the reality of the development of mental illness due to the complex interplay of genetic and environmental factors was presented by Uher et Zwicker.[16] According to the authors, the GxE interactions should be assessed in a prospective, longitudinal way throughout the whole life of the individual (developmental context), with a specific focus on the first two decades of life. This type of

research can be performed without any a priori hypotheses (hypothesis-free design), which was already common and fully accepted in the GWAS (genome-wide association studies) in psychiatry in the past. In most mental disorders, the consecutive and complex interactions of GxGxExE (a complex interplay) are present rather than a simple causation by GxE. The timing of these interactions is significant, eg because while cannabis abuse may easily induce schizophrenia in adolescence in a predisposed boy or girl, it rarely leads to the same disease in older subjects. The genetic background of the patient, usually polygenic, sensitizes humans to the influence of extrinsic factors (gene–environment interaction; GxE). Childhood trauma in many cases, results in an increased sensitivity to stress thus leading to anxiety and depression in adulthood.[17] It is also necessary to assess the pregnancy of the patient´s mother, because many deleterious factors already start working in utero. The occurrence of seriously stressful life events should also be looked into concerning the patient´s ancestors, because of the intergenerational transmission of trauma via epigenetic mechanisms.[18] Protective environmental factors which have for the most part been neglected in research; eg a happy childhood, should also be evaluated in a GWEIS. Eventually, statistically significant GxEs ought to be independently replicated and be able to serve as a foundation for future research into their underlying causal biological mechanisms.[16]

MicroRNAs (miRNAs) are small, highly conserved non-coding RNA (ribonucleic acid) molecules, containing about 22 nucleotides that are involved in the regulation of gene expression. Recent studies show that miRNAs are significant biomarkers of schizophrenia, bipolar disorder, major depression and anxiety disorders.[19–22] That is why we consider the miRNome analysis in our study subjects as an integral and complementary part of genetic research.

Microbiota are one of the key regulators of gut-brain function and have led to the appreciation of the importance of the microbiota-gut-brain axis. This axis starts to be significant in investigations of the biological background of neurodevelopmental and neurodegenerative mental disorders.[23] In a mouse model, it appears that the manipulation of the intestinal microbiome alters the expression of genes in the brain.[24] Some studies that were done on psychiatric patients suggest that the use of antibiotics is associated with an increased risk of psychotic episodes. Oro-pharyngeal microbiota in schizophrenia were found to be different between the cases and the controls.[25] Mental disorders are generally associated with a reduced microbial diversity and show differences in the human microbiome compared to the controls. In some reports, specific microbial taxa have been associated with physical health as well as with symptoms of depression, psychosis, etc.[26] It has been shown that changes in the gut microbiome following the administration of quetiapine in patients with bipolar disorder, could be seen as a marker of prediction of the outcome of treatment.[27] Studies also suggest increased intestinal inflammation and permeability, which may be among the principal mechanisms by which microbial dysbiosis influences the overall physiological function of the patient.[26] In addition to that, microbiome may correlate with some miRNA levels, so miRNAs may be one of the possible links between the brain and the intestine.[28]

Study Aims

The aim of our GWEIS study is to conduct a survey of the gene–environment interactions in the etiopathogenesis of selected mental disorders that are serious in nature and to compare them with unrelated healthy volunteers. The basic design of the proposed study is cross-sectional, but we plan to follow-up the available study subjects prospectively. We intend to measure the exposome from a life-long perspective. Because stress is one of the most important factors that can trigger an episode of a mental disorder, the study is also centred on the assessment of the subject´s reactivity to stress.

We plan to compare the miRNA profiles among our study groups using unsupervised hierarchical clustering analysis. We expect that the differences in the miRNAs´ expression will be related not only to the psychiatric disorders themselves, but also to the lifestyle of the patient, their microbiome and their reactivity to stress. We will analyse the microbiome from samples taken from oral and stool swabs and compare the data among the study groups as well as to assess the microbiomes in relation to the miRNAs levels, the lifestyle of the studied subjects as well as their diet.

Theoretical Hypotheses

Even if our attitude is skeptical and theory-free, it is logical to assume that

- the study groups will be different from each other in the presence of individual environmental risk factors and environmental protective factors as well as the polyenviromic risk score and the polyenviromic protective score;

- the study groups will differ from each other in the presence of individual genetic polymorphisms as well as the polygenic risk score;
- the study groups will be different from each other regarding the GxE (product of the polyenviromic risk score and polygenic risk score);
- the study groups will be different from each other in terms of their miRNAs expression and their microbiomes.

Methods

The Participants and Study Setting

Three groups of study subjects will be evaluated:

- Patients suffering from schizophrenia or a mood disorder (major depression, recurrent depressive disorder, bipolar affective disorder)
- Patients with anxiety disorders
- Unrelated volunteers from the general population who are mentally healthy.

The study subjects, who are all volunteers, will be recruited among the patients and staff (healthy volunteers) at the departments of psychiatry and at the university hospitals (healthy volunteers) in Hradec Kralove and Olomouc. The psychiatric patients who visited the hospital for the first time as well as the patients who are undergoing continuous psychiatric treatment will be enrolled.

The patients who will be recruited at the psychiatry department will already have been diagnosed with a mental disorder. There is no way to know if the healthy volunteers will develop a mental disorder later in life, which could be a limitation of the study. Mentally healthy volunteers who have a history of psychiatric illness will not be included in our study.

Evaluations

Psychiatric Examination

Each volunteer will be examined by a licensed psychiatrist (K.L., L.H.) as of the possible recent development of psychopathology and it´s diagnosis according to the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) classification.[29]

The Questionnaire of Stressful Life Events

We adapted the original questionnaire of stressful life events applied in the EU-GEI (European network of National Schizophrenia Networks Studying Gene–Environment Interactions) study on psychosis.[30,31] This questionnaire covers the subject´s whole life, including possible adverse agents and situations in utero and also if there were any major stressful life events in the subject´s parents and grandparents.

For the purpose of our GxE study, we will look into the presence of environmental factors from the questionnaire in every subject, whether they are present or not.

Of interest to us are the environmental risk factors for schizophrenia in which their effect size is known:[32,33]

- Whether the subject was born in the winter or spring.
- Upbringing in an urban area.
- History of cannabis abuse.
- Advanced age of the father when the subject was conceived.
- Presence of obstetric or perinatal complications.
- History of physical abuse, sexual abuse or neglect.
- Death of a parent when the subject was a child.
- Being a member of an ethnic minority.

For mood and anxiety disorders, we are interested in the following environmental risk factors in which their effect size is known:[34–41]

- Alcohol abuse.
- Smoking.
- Serious chronic somatic disease.
- Traumatic brain injury.
- Obesity.
- A low level of physical activity.
- The presence of any job related stress.
- Shift work.
- Lack of sleep.
- Seriously stressful events in adulthood.
- Migration.
- Ethnic minority status.
- Living a solitary way of life.

(Remark: Some of the risk factors for mood and anxiety disorders are the same as the risk factors for schizophrenia, eg stressful life events in childhood or cannabis abuse, and were already mentioned in the Padmanabhan´s list of risk factors.[32])

Protective factors for mood and anxiety disorders in which their effect size is known:[42–44]

- A Mediterranean diet.
- Physical activity.
- Social support.

The Examination of Reactivity to Stress

We will apply the Virtual Reality Version of the Trier Social Stress Test (TSST-VR).[45] Cortisol in the subject´s saliva will be measured shortly before the test, immediately after the test, and 10, 20, 30 and 40 minutes after the test. Within the same time period, the heart rate will be continuously assessed using pulse oximetry. This will be supplemented by the subject´s statement on how much stress is being perceived by him/herself. Within one week before the TSST-VR, we will measure the circadian patterns of salivary cortisol, because these patterns are altered in major mental disorders, eg schizophrenia.[46] We will collect a sample of saliva 3 times during a 24 hour period. The first sample will be collected immediately after waking up, the second one 30 minutes later and the third one hour before the patients sleeps. It is found that the salivary cortisol values follow the same pattern as serum cortisol itself, and have a high correlation with some parameters showing disturbances in the hypothalamus-pituitary-adrenal axis.[47]

Genetic Assessments

Blood samples of all study subjects will be genotyped at the Department of Biochemistry, at the Faculty of Science of Masaryk University in Brno, in the Czech Republic. For the analysis of DNA (deoxyribonucleic acid) polymorphisms, the most up-to-date method will be used – the targeted re-sequencing using the NGS (next-generation sequencing) system NextSeq from Illumina. For a targeted re-sequencing of highly polymorphic and/or gene function sites of selected genes, the NimbleGen SeqCap Target Enrichment method enabling the specific enrichment of gene regions of up to 96 samples will be used. Individual samples for sequencing analysis will be multiplexed in order to obtain about 100x depth of coverage, which is generally accepted as adequate in the detection of SNPs (single-nucleotide polymorphisms). The data will be analysed according to the workflow provided by the manufacturer for the evaluation of the NimbleGen SeqCap EZ Target Enrichment Data consisting of the assessment of the quality of sequencing reads, read filtering, mapping against the human genome, duplicate removals, the assessment of coverage statistics, variant calling and variant filtering. The final data will be exported to a file for statistical analyses. The technology described above is routinely used in our laboratories.[48,49]

We will focus on the genes relevant to the mental disorders under study: ADRA2A, AKT1, BDNF, CCKAR, CHI3L1, CHRNA7, CLDN5, CNR1, COMT, CRFR1, CTXN3, DISC1, DPYSL2, DRD3, DTNBP1, EGF, GABRA1, GABRB2, GAD1, GRIK3, GRM3, HTR2A, HTR3A, IL10, MAOA, MAOB, MCHR1, MLC1, NEUROG1, NOS1, NOS1AP, NOTCH4, NPY, NRG1, NR3C1, OLIG2, OPMR1, PDE4B, PLA2G4A, PLXNA2, PPP3CC, PRODH, RELN, RGS4, RTN4, SLC6A4, SNAP25, SRR, SYN2, TNF, TP53, UFD1L, XBP1, YWHAE, ACE, TNF alpha, TGF beta, MTHFR, IL1, IL2, IL6, CRP, GSK3 and others according to the lists published by Schmidt-Kastner et al, Roussos et Haroutunian, Hosak and Jiang et al and according to our previous studies.[50–57]

Epigenetic (miRNA) Assessment

We will use next-generation sequencing to analyse the miRNAs in all samples.

The serum sample will be stored in a stabilizing solution. The collected samples will be used for miRNA isolation and the RNA will be stored at −70°C until its use. For the preparation of miRNA sequencing libraries, we will use the NEBNext® Small RNA Library Prep Set for Illumina (New England Biolabs, MA, USA). The sequencing libraries will be subsequently loaded in the NextSeq 500 sequencing device (Illumina, CA, USA). The online databases such as the TargetScan, microT-CDS and Tarbase will be used to identify the target genes of the differentially expressed miRNAs.

Microbiome Analysis

Oral and gut microbiota will be collected from the study subjects by obtaining oral smears and faecal specimens. The determination of bacteria will be performed by the metagenomic sequencing method by using 16s rRNA. Samples of stool will be elaborated according to the instructions for the QIAamp PowerFecal DNA Kit (Qiagen, Germany). The collected samples of buccal swabs will be used for gDNA/total RNA isolation using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen) according to the manufacturer's instructions and the DNA/RNA will be stored at −20°C/-70°C until use. Isolated DNA will be used as a template in PCRs (polymerase chain reactions) with the 16S modified 515F-806R primer pair flanking the V4 hypervariable region, including the linker, pad, barcode and the P5 and P7 Illumina adapters.[58] Amplification will be done according to an already introduced protocol using the Platinum SuperFi Mastermix (Thermo) in a volume of 25 ul when amplification products will be purified by using the Agencourt® AM

Pure XP beads. Consequently, the purified PCR samples will be checked and quantified by using the Qubit, Fragment Analyser (Advanced Analytical) and the Kapa Library Quantification Kit (Roche). The final library will be subjected to NGS using a MiniSeq® High Output Kit (2 x 150 paired end sequencing) on a MiniSeq® sequencer (Illumina, USA). The Mothur software package will be used for sequence analysis, OTU detection, taxonomic assignment, and phylogenetic analysis.[59] We will calculate the species richness (alpha diversity) by using the Chao, Shannon and Simpson indexes. We will analyse the statistically significant changes in beta diversity by utilizing the weighted variant of UniFrac. The analysis of the principal components, using the Jaccard index, will be carried out with a Phyloseq package, to compare the bacterial populations among different samples and to define the core microbiome in our samples.[60,61] All of the data analysis will be done according to the Human Microbiome Project (NIH; National Institutes of Health) pipeline to allow data deposition and comparison with the already analysed samples. Real-time PCR with primers flanking the V3/V4 hypervariable region will be carried out to quantify total bacteria.

Data Monitoring

The data will be continuously collected and entered into an Excel table by the researchers. All databases will be organized anonymously, according to the unique ID numbers which will be assigned to the individual participants at the beginning of the study. The data will not be accessible on the internet. The access to the data will be restricted only to the research team.

Statistical Analysis

All of the statistical analyses will be carried out using the R software (R Core Team).[62]

The polyenviromic risk score (PERS) will be computed for each participant as recommended by Padmanabhan et al, similarly to the calculations of the polygenic risk scores.[32] We will binarize environmental risk factors, and apply an additive model. The log of the odds ratio for each environmental risk factor will be multiplied by either "1" (risk factor present in the individual) or "0" (risk factor absent in the individual). These products will be added together and the sum will be divided by the total number of assessed risk factors. The association of selected environmental risk factors with the relevant mental disorder will be tested using the Fisher's exact test.

We will calculate the polygenic risk score (PRS) as suggested by Dudbridge.[63] Each of the single nucleotide polymorphisms (SNPs) will be tested for an association with the relevant mental disorder (based on the case/control status of an individual) using the Fisher´s exact test. Only the SNPs with a frequency higher than 5% will be included in the analysis. SNPs with a frequency lower than 5% will be analysed as being potential mutations. We will determine whether a mutation can affect gene function (eg a change of the amino acid of a protein). In the event that a mutation can affect the gene function, we will proceed by analysing the occurrence of this mutation in relation to the mental disorders in the families being studied, in the same manner as we have done in the work on the genetics of oligodontia.[48] Then, all of the SNPs with P values exceeding the selected threshold and being approximately in linkage equilibrium with the others, will be used to calculate the PRS for each participant using the logistic regression model. The polygenic risk score uses an additive model to quantify an individual´s genetic loading for a disorder, as influenced by the presence of multiple risk alleles. To calculate an individual´s polygenic risk score, the log of the odds ratio for each risk allele is multiplied by the number of risk alleles (0, 1 or 2) in the subject. This is done for all SNPs that are included in the score. These products are added together and divided by the total number of single nucleotide polymorphisms included in the score.[32]

To test the joint effects of the selected environmental risk factors and polygenic risks, the PRS will be binarized according to the procedure suggested by Guloksuz et al.[64] According to this procedure, a participant is considered to have significant polygenic risk when exceeding the third quartile of the values of PRS in the control group. Based on this, four groups will be delimited for each environmental factor: (1) participants with the polygenic risk of a relevant mental disorder, but without exposition to the selected environmental factor (eg cannabis abuse); (2) participants exposed to the environmental factors, but without polygenic risk; (3) participants exposed both to the environmental factors and polygenic risk; (4) reference exposed neither to the environmental factors nor polygenic risk.

The interactions between each environmental factor and the polygenic risk of the relevant mental disorder will be assessed by comparing the values or the frequency variations of the environmental factors between these four groups. Multivariate logistic regression will be used to test

the differences that control the influence of the patient's sex and age as well as the environmental factors that will be assessed. By using this method, the information that will be obtained will indicate whether the environmental factors act alone or through the interaction with polygenic risks. Similarly, the differences in the expression of miRNA between these groups and the diversity in the microbial species (Chao index) will be tested by using the non-parametric Kruskal–Wallis test to explore their relationship with the environmental and genetic risk factors.

We will gain additional information by running the analysis for specific haplotypes using the PERS method.

According to the literature, study groups > 1000 individuals in GxE psychiatric research are sufficiently large enough to ensure a sufficient statistical power to detect an effect of a given size with a sufficient degree of confidence (the power level of 0.80; the significance criterion with a value of 0.05).[64]

Ethical Issues

The study will be performed in agreement with the Declaration of Helsinki as amended in 2018.[65] The study was approved by the Ethics Committee of the University Hospital of Hradec Kralove (Reference number: 201903 S10P) and the Ethics Committee at the University Hospital of Olomouc (Reference number: 25/19). All participants will voluntarily sign the written informed consent form.

Timetable

Preparation phase: November 2019 – December 2019. Data collection phase: January 2020 - December 2022, Data analysis and publishing: January 2023 – December 2023.

Discussion

The Czech Republic is a Central European country. Its area of 78,866 km^2 is comparable in size to Hungary, Austria or Ireland. The number of inhabitants is about 10.6 million and the unemployment rate is equal to 2.8%. The proportion of people living below the poverty threshold is very low as compared to other European countries because of the long tradition of egalitarian policies of the Czech Republic. About ten per cent of the population is represented by immigrants, mostly coming from Slovakia, Ukraine, Vietnam or Russia. Only one third of the population declare themselves to be religious, with the prevailing religion being that of the Roman Catholic faith (83.4%). The average life expectancy in the Czech Republic is 78.3 years and the fertility-rate runs on average at 1.57 deliveries per woman of child bearing age. The most recent gross domestic product per capita totals to 35.010 USD. The Czech Republic is an industrially-developed country with a long tradition of strength in the sectors of machinery production, automotive manufacturing and chemical technologies. Some things that make the Czech Republic stand out in a positive light is the high employment rate among women and a quality healthcare system that is highly affordable and easily accessible. It is also worth noting that the Czech Republic has one of the lowest perinatal mortality-rates in the world, a tolerant attitude to sexual minorities and a high degree of vaccinations in the general population against contagious diseases.[66] On the other hand, the Czech Republic has one of the higher rates of alcohol consumption among European countries.[67] As far as we know, the suggested study will be the first GWEIS in the Czech Republic as well as in Central Europe.

The advantage of our project is that it is a comprehensive assessment of gene–environment interactions in major mental disorders. Standard GxE evaluations are supplemented with a virtual test of a reactivity to stress with the detection of miRNAs and microbiomes, which demonstrates recent knowledge on the important factors in gene–environment issues.[68,69] Our study is innovative in the sense that we intend to look at the combinations of various environmental factors while also considering their timing, so as not to study them in isolation from each other.

In order to obtain results that are both robust and meaningful, we will make use of the findings of our cross-sectional study to formulate hypotheses which we plan to test in a consecutive study on GxE with a longitudinal design.

With the extension of the study, we intend to perform an analysis of the endophenotypes that are relevant to schizophrenia (eg prepulse inhibition, mismatch negativity, oculomotor antisaccade, letter-number sequencing and continuous performance tests), major depression (eg neuroticism, morning cortisol deviation, frontal asymmetry of cortical electrical activity and biases of attention and memory), bipolar disorder (eg deficits in verbal memory, sustained attention and executive function or abnormal structural and resting state brain neuroimaging findings) and anxiety disorders (eg aberrant amygdala responsiveness to anxiety-related stimuli).[70–73]

The proposed project is limited by the fact that we will only look for the statistically significant gene–environment interactions and their neurobiological explanation may be

sought after in further studies. The suggested examination of the reactivity to stress only reflects psychosocial stress, but this type of stress is prominent in the induction of an episode of a mental disorder.[4] We are aware of the fact that the TSST-VR only measures responses to acute stressors and not chronic psychosocial stressors, which would be more relevant to the longitudinal aspect of the environmental factors we intend to monitor. That is the reason why we will interpret the TSST-VR results with caution, strictly in association with the data obtained from the questionnaire of stressful life events in every subject. As far as we know, there is currently no test for assessing chronic psychosocial stress in humans. Another limitation of our study is that the population being surveyed, in comparison to other countries like the United States for example, is not as genetically and culturally diverse because of the relative homogeneity of the Czech population as a whole. Thus the GxE interactions highlighted by the results may only be relevant to the Caucasian populations of Central European descent, but not necessarily to other populations.

Conclusion

The aim of our project is to initiate pioneering GxE research in the field of psychiatry in the Czech Republic. After we collect the pilot results and publish them, we intend to apply for a research grant. The results of the project may become applicable in the study of neurobiology of major mental disorders, and possibly in their treatment and prevention. We would welcome and greatly appreciate any comments from the readers that could serve in improving our work.

Abbreviations

BDNF, brain-derived neurotrophic factor; DNA, deoxyribonucleic acid; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; E, environmental; EU-GEI, European network of National Schizophrenia Networks Studying Gene–Environment Interactions; GxE, gene–environment interaction; GWAS, genome-wide association study; GWEIS, genome-wide environment interaction study; MAOA, monoamine oxidase A; miRNA, microRNA; NGS, next-generation sequencing; NIH, National Institutes of Health; PCR, polymerase chain reaction; PERS, polyenviromic risk score; PRS, polygenic risk score; RNA, ribonucleic acid; SNP, single-nucleotide polymorphism; TTST-VR, the Virtual Reality Version of the Trier Social Stress Test.

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Disclosure

The authors report no conflicts of interest in this work.

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