Was können wir aus personalisierten ADHS-Modellen lernen? Neuronen in der Petrischale

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Attention-deficit hyperactivity disorder (ADHD) is one of the most prevalent psychiatric disorders with several lifetime impact [7]. Despite tremendous research efforts, the exact causative mechanisms of this neurodevelopmental disorder still have to be elucidated. One major challenge is the difficulty to reflect the complexity and simultaneously the individual features of ADHD with the current *in vitro* or *in vivo* model systems. A new strategy to circumvent this problem is to develop a patient specific model of a “small personalized brain” in a dish using the induced pluripotent stem cell technology to identify the malfunctioning mechanism occurring during neurodevelopment. Currently, this approach has been initiated for the first time in Switzerland to elucidate the molecular and cellular pathways causing ADHD in the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of Psychiatry Zurich.

Introduction

Numerous lines of evidence suggest that the genetic component plays a substantial role in the etiopathogenesis of ADHD, showing a heritability higher than 70% [8]. Nevertheless, ADHD is not a monogenetic disorder, but rather found to have a polygenic character, depicting the disorder as a heterogeneous complex phenotype constituted of multiple risk gene variants [9]. In addition, environmental factors have also been implicated in ADHD, in particularly prenatal stress, leading to the neurobiological susceptibility to the disorder [10, 11] (see Figure 1). Nevertheless, the etiology of ADHD is still largely unknown despite extensive research efforts.

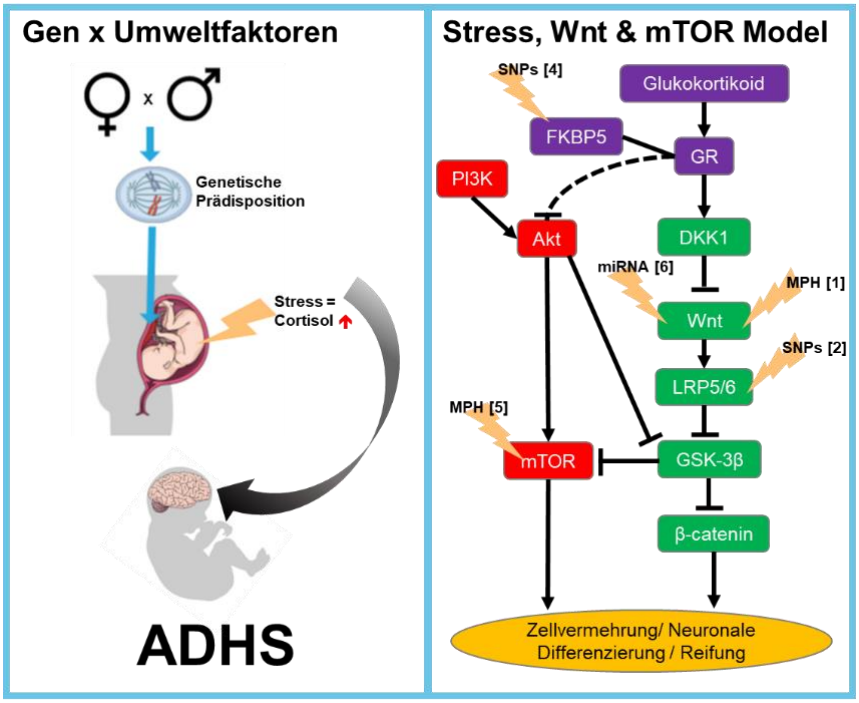

Figure 1: Hypothesis model for Attention Deficit Hyperactivity Disorder (ADHD) combining genetic and environmental risk factors (e.g. prenatal stress) affecting the neurodevelopmental brain development and maturation. Current evidence and hypothesis linking elevated stress (cortisol) with the Wnt- and mTOR-pathways known as key players in cell proliferation, growth, differentiation and maturation. Publications cited in figure [1] [2] [4] [5] [6].

Alterations in brain structure and maturation in children and adolescents with ADHD have been reported [12-16], as for example a delay in cerebral cortex maturation (thickness and surface area), particularly, but not exclusively, in frontal regions important for the control of cognitive

processes. In a recent mega-analysis by ENIGMA consortium subtle differences in cortical surface area were found in children but not in adolescent and adults with ADHD, confirming involvement of the frontal cortex [17]. Structural and functional neuroimaging analysis of ADHD brains suggests that psychostimulants (e.g. methylphenidate, methamphetamine etc.), one of the first line therapies of ADHD, are associated with normalizing the aforementioned structural abnormalities [18-23]. All this supports the observation of brain development and maturation disturbances in ADHD patients, with recent reports describing aberrant structural and functional brain connectivity in these patients that might be reversed/improved following psychostimulant treatment. However, the mechanism of action and the cellular/ neuronal alterations how exactly such process occur is not yet known.

Wnt-signalling and ADHD

In vitro studies, using cell culture (e.g. stem cells, neuronal cell lines etc.) originating from animals or humans are one possible route in the quest to elucidate molecular and cellular alterations linked with ADHD as well as the mechanism of action of its drug therapy. Using several types of cellular models (mouse neuronal stem cells, rat dopaminergic neurons and human neuroblastoma) we could demonstrate that indeed methylphenidate influences cell proliferation and differentiation, promoting maturation of the cells as found in brain imaging studies, in addition to its influence on neurotransmitter levels [24-26]. In particularly, we could demonstrate for the first time that methylphenidate activates Wnt-signalling [1]**,** which was not due to the dopamine transporter inhibition - the main known therapeutic mechanism of this drug - since the selective dopamine transporter inhibitor GBR-12909 treatment demonstrated the opposite effects as methylphenidate [1] (see Figure 1). Interestingly, Wang et al. [6] demonstrated that in ADHD patients a significant correlation between low gray matter volume and three miRNAs (miR-30e-5p, miR-126-5p, and miR-140-3p) was observed, in which these miRNAs were found to be involved with the Wnt-signalling pathway as well (see Figure 1).

The recent Psychiatric Genomic Consortium (PGC) ADHD genome-wide association study (GWAS) results reported for the first time 12 independent loci significantly associated with ADHD. This revealed the involvement of various pathways such as pathways playing a role in synapse formation, neuronal developmental and in Wnt-signalling processes [27]. Of particular interest are the Wnt-signalling pathways with their pivotal role both in the developing and mature brain [28]. Strengthening these findings and hypothesis, we found in a family and case-control study followed by a meta-analysis an association in child and adolescent ADHD with *LRP5* and *LRP6* gene variants (the receptors that activate Wnt-pathway) in four European populations [2]. Moreover, the Wnt-signalling has been reported to be associated with learning and memory [29-31], and some epidemiological evidence pointed to Wnt involvement in behavioural problems including anxiety, working and spatial memory, hyperactivity, and

depression [30, 32], all of which supports the hypothesis that altered Wnt-signalling in ADHD might play a role in brain growth and maturation.

ADHD and Stress

Stress during the prenatal period and early postnatal life puts offspring at risk of developing diseases involving socialization, such as autism spectrum disorder, and attention and cognition, such as ADHD [11]. Indeed prenatal stress may affect embryonic development, which is a sensitive period when plasticity is high and susceptible to environmental factors may play a role. Such prenatal stress may be a consequence of high maternal cortisol levels or indirect through maternal anxiety, depression, trauma or even administration of synthetic glucocorticoids such as dexamethasone or betamethasone. However, the current hypothesis is that the combination of genetic susceptibility, in this case higher genetic load of risk genes for ADHD (high polygenic risk score/PRS), together with stress will exacerbate the risk to develop ADHD (see Figure 1). Indeed, the candidate gene, *FKBP5* (involved in regulating glucocorticoid receptor/GR and its pathway), has been found to associated with various psychiatric disorders including ADHD [33, 34]. In a preliminary study, we found that *FKBP5* associated with ADHD in our case-control sample as well as show a signal in the PGC-ADHD European sample [4]. Additionally, Schmitz et al. [5] demonstrated that treatment of rat dopaminergic pheochromocytoma (PC12) cells with methylphenidate alters AKT-mTOR signalling, which is known to be downstream to the glucocorticoid receptor/GR pathway and to affect, similarly to the Wnt-signalling, cell proliferation, differentiation, maturation and neurogenesis [35] (see Figure 1). Therefore, one may conclude that both pathways may be associated with ADHD at the genetic and stress level. However, to test such hypothesis one would need a model that can both mimic the polygenic risk load of the patients as well as environmental factors. Therefore, a personalized patient specific model is in need.

The iPSC technology

Both neuroimaging studies and human peripheral samples (e.g. blood, saliva, CSF, etc.) have contributed in the research of neurodevelopmental disorders, such as ADHD. However, studying the pathomechanism in patient cells is limited because the relevant tissue, the brain, using biopsy or post-mortem samples are scarce and unlikely available from ADHD patients for research purpose. Besides, animal models are a valuable alternative when studying ADHD, since they provide a window into developmental trajectories within the central nervous system, including some environmental factors. Nevertheless, they also have some pitfalls (e.g. not able to fulfil an entire behavioural phenotype or mimic the polygenic profile of a human patient) [36]. Therefore, there is an urgent need for a personalized neuronal model in a dish originating from individuals with ADHD that maintain their genetic information, and whereby we know their phenotype and

genotype background. Reprogramming human somatic cells into induced pluripotent stem cells (iPSCs) and differentiating them into neuronal stem cells (NSCs) is a powerful technology that provides the means to study living human neuronal cells for modelling complex disorders, as ADHD, *ex vivo* [37]. In recent years, iPSCs have been used increasingly to model neurological and psychiatric disorders [38, 39] using various techniques such as 2D and 3D (organoids) neuronal cultures to investigate physiological, structural, and molecular approaches [40-44]. Specifically, iPSCs derived NSCs are commonly used because they can proliferate extensively and differentiate into various neural lineages (i.e., neurons, astrocytes, and oligodendrocytes) comprising the central nervous system. Thus, iPSCs generated from individual patients (skin biopsies or hair follicles) offer a window into studying processes such as differentiation/maturation, connectivity, molecular alterations (e.g. transcriptomics, protein/enzyme etc.), and electrophysiological activity alterations in an *in vitro* manner.

Modelling ADHD with iPSC

For the study of neurodevelopmental disorders, the reprogramming of cells extracted from a plucked hair follicle represents an ideal choice, since hair samples can be harvested with minimal distress to the affected child and thus avoiding invasive procedures such as skin biopsy. Recently, we created a pipeline with several technical improvements generating iPSC lines from hair follicle keratinocytes of children and adolescents with ADHD and healthy controls (Figure 2) [45] (see also review [3]).

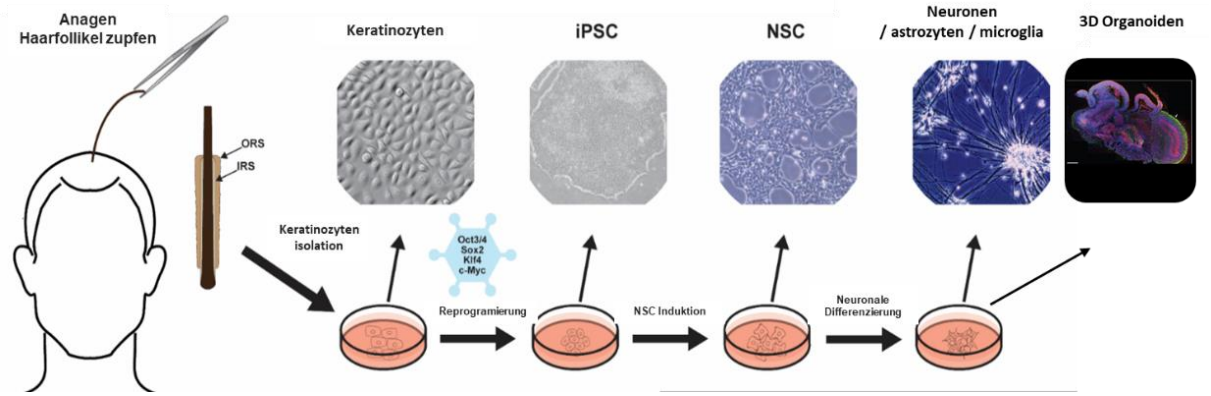

Figure 2: Schematic outline of the workflow for the generation of personalized iNCs and organoids existing in the laboratory, including quality controls (e.g. pluripotency markers using qPCR & immunostaining, mycoplasma, GWAS for genetic aberrations, etc.). Modified from [3].

As it is postulated that the molecular dysfunctions underlying ADHD occur during early development of the nervous system, it is particularly interesting to compare the dynamics of the differentiation and maturation into neuronal cells from patient specific stem cells and control lines, but, also test how stress may exacerbate the genetic predisposition in ADHD lines compared to heathy controls. A multitude of experiments, such as proteomics, transcriptomics, metabolomic analyses [46] and electrophysiology, can be performed to assess molecular abnormalities in ADHD specific lines. Ultimately, therapy approaches to address such

abnormalities can be developed, which may open the door to the discovery of new therapeutic options (see Figure 2).

Conclusions

The research on ADHD benefits from a range of approaches that aim to uncover the cause of the disorder and to find new therapeutic options. Nevertheless, there is still a huge lack of knowledge of the molecular mechanisms underlying this neurodevelopmental disorder. Since it is not possible to study cells from the human brain directly, an alternative approach has to be identified. iPSCs can be generated from easily accessible cells, such has hair follicle derived keratinocytes or blood cells, and have the potential to differentiate in every cell type of the body. The differentiation of iPSC into neuronal cells recapitulates key points of neurodevelopment under controlled conditions, exposing a specific time frame of early central nervous system development. This technology is particularly suitable for disease modelling since the generated cells maintain the original genetic background of the donor and thus might expose disease specific deficits at the molecular level. The establishment of the here described personalized system, using *in vitro* neuronal culture or complex brain organoid, could contribute to fill the gap of knowledge behind ADHD research and eventually be used for the discovery of new pharmaceutical drugs.

Call for support

The Translational Molecular Psychiatry research group will be more than grateful for any type of support for the “modelling ADHD using iPSC” study. For participation (involving sample collection and questionnaires) as healthy control or ADHD patient, please contact Prof. Edna Grünblatt (edna.gruenblatt@kjpd.uzh.ch; +41-(0)43-556 4039). Any other donation will be more than welcome.

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