

## IS MICROVASCULAR ABNORMALITY A NEW ENDOPHENOTYPE IN SCHIZOPHRENIA?

Ladislav Hosak<sup>1</sup>, Khurum Hakeem<sup>2</sup>, Marjan Raad<sup>2</sup> & Jan Studnicka<sup>3</sup>

<sup>1</sup>Department of Psychiatry, Charles University in Prague, School of Medicine in Hradec Kralove  
and University Hospital Hradec Kralove, Czech Republic

<sup>2</sup>Charles University in Prague, School of Medicine in Hradec Kralove, Czech Republic

<sup>3</sup>Department of Ophthalmology, Charles University in Prague, School of Medicine in Hradec Kralove  
and University Hospital Hradec Kralove, Czech Republic

received: 9.7.2015;

revised: 28.8.2015;

accepted: 4.9.2015

### SUMMARY

**Background:** A new method of assessment of microvascular abnormality in living schizophrenic subjects via retinal imaging was described by Meier et al. (2013). The principal aim of this review is to summarise the relevant knowledge and suggest further avenues of research into this topic.

**Subject and methods:** On 20th April 2015, we carried out a search using the computer database system PubMed by using keywords „microvascular AND schizophrenia“.

**Results:** Out of the 17 articles found, only seven were relevant. They are generally consistent with the hypothesis of microvascular pathology and brain inflammation as part of the pathogenesis in schizophrenia. It is important to stress that all studies of brain microvasculature in schizophrenia to date have been post mortem findings, apart from the work by Meier et al. (2013) which is related to retinal imaging in living subjects.

**Conclusions:** Based on the literature, we suggest the following research and clinical avenues:

Firstly, to assess whether microvascular abnormality found via retinal imaging, fulfils the criteria for the schizophrenia endophenotype. Secondly, to examine retinal imaging in high-risk individuals for schizophrenia. Thirdly, to determine whether the fMRI findings and cognitive abilities of schizophrenia patients in both longitudinal as well as cross-sectional studies, is associated with the microvascular abnormalities assessed by the retinal imaging. Fourthly, to determine if there is a correlation between microvascular retinal pathology and the positive or negative schizophrenia symptoms. Furthermore, to determine if childhood maltreatment results in any abnormalities in retinal imaging. Lastly, to analyse the genetic background of schizophrenia retinal microvascular pathology and to apply anti-inflammatory agents in the treatment and prevention of schizophrenia if brain vasculitis is confirmed.

**Key words:** schizophrenia - microvascular abnormality - retinal imaging – endophenotype – inflammation

\* \* \* \* \*

### INTRODUCTION

Schizophrenia is ranked among the top ten diseases causing disability in patients with increased societal cost (Murray 1996). Schizophrenia is found in 0.5-1% of the population. The quality of life of the patients including their ability to think clearly, experience adequate emotions, and participate in a social life are the most affected abilities. Even if a majority of the subjects with schizophrenia are more or less responsive to the treatment, recovery is only achieved by a third of the patients (Weinberger & Harrison 2011).

Many mental disorders are known to have a complex psychopathology and vast array of causative factors. Gottesman & Gould (2003) elaborated the concept of endophenotypes in psychiatry. This concept makes it possible to define the minor anomalies (mainly biological) in which causal etiologic variables are more easily identified, compared to a complex nosological entity.

The basic requirements for an endophenotype are as follows:

- It should be associated with a given disease.
- It should be a trait-marker rather than a state-marker.
- It should be heritable.
- In affected families, an endophenotype should occur concurrently with a given disease.
- It should be more frequent in the patient's healthy relatives rather than in the general population.

These requirements were consecutively specified (e.g. Bearden & Freimer 2006).

The desirability of endophenotypes is high, due to the fact that symptoms of schizophrenia are rather diverse, thus we speak about a group of diseases than one cohesive entity. Endophenotypes of schizophrenia predominantly described in the literature are neurophysiological, neuromotoric, neurocognitive, and personality-related ones (Braff et al. 2007).

Heritability of schizophrenia is 0.6-0.8 (Boshes et al. 2012). Genome-wide association studies (GWAS) have recently dominated in the genetic research into schizophrenia. Millions of single nucleotide polymorphisms (SNPs) are usually examined simultaneously in every

subject in a GWAS. A population of several thousands of patients with schizophrenia is commonly compared with a group of several thousands of healthy controls. This attitude is supplemented by examinations of DNA microdeletions/microduplications (copy number variations; CNVs). Such an anomaly usually affects several genes together. This leads to impairments in the brain development and function due to a lack or abundance of a relevant protein. Several genes have already been identified as being associated with schizophrenia based on the CNV studies, e.g. PRKAB2, NRXN1, BDH1, DLG1, PAK2, TFRC, CHRNA7, NTAN1, COMT, GSTT2 or PRODH (Hosak 2013).

The problem remains that even if we identify suspect genes in a GWAS or CNV study, we still do not know their role in the pathogenesis schizophrenia, so our recent knowledge about the genetic background of schizophrenia does not help us in seeking a proper treatment. To overcome this limitation, genetic research into schizophrenia endophenotypes should be applied (Braff et al. 2007). It is supposed that a genetic background of an endophenotype is more simplistic and can be studied better as compared to the whole nosological entity of schizophrenia, which is clinically and etiopathogenetically heterogenous. Pathogenesis of an endophenotype is also assumed to be simpler than the complex neurobiological mechanisms in schizophrenia. This is how schizophrenia pathogenesis can be discovered gradually, step by step (Corvin 2013).

As a new possible schizophrenia endophenotype, microvascular abnormality measured by retinal imaging recently occurred in the literature (Meier et al. 2013). The aim of our review is to accumulate the relevant knowledge and facilitate further research in this field.

## SUBJECTS AND METHODS

We performed a search using PubMed (computer database) (<http://www.ncbi.nlm.nih.gov/pubmed>) using the key words „microvascular AND schizophrenia“ on April the 20th, 2015. Out of the 17 articles found, only seven were relevant (Bachneff 1996, Hanson & Gottesman 2005, Harris et al. 2008, Kiehl et al. 2009, Kreczmanski et al. 2009, Sinka et al. 2012, Meier et al. 2013).

## RESULTS

Meier et al. (2013) assessed retinal arteriolar and venular calibre via retinal imaging in living individuals with schizophrenia (N=27) against controls without schizophrenia (hypertension N=110; persistent tobacco dependence N=210; persistent depression N=188; healthy controls N=412). Members from the study who suffered from schizophrenia had wider retinal venules (P=0.011) that suggest a microvascular abnormality indicating insufficient brain oxygenation. Statistical analysis found that this was not due to confounding

health conditions or antipsychotic medication. Wider venules were also associated with a dimensional measure of adult psychotic symptoms (P<0.001) and psychotic symptoms experienced in the childhood at age 11 years (P=0.015). These findings support the hypothesis that schizophrenia patients have a microvascular abnormality. The authors conclude that retinal imaging may become an important tool to reveal pathogenesis of schizophrenia in living subjects.

Kreczmanski et al. (2009) assessed the integrity of the microvasculature in subcortical brain regions in schizophrenia. The authors investigated the microvessel length density, total microvessel length and microvessel length per neuron through stereological methods in the caudate nucleus, putamen, nucleus accumbens, medio-dorsal nucleus of the thalamus and lateral nucleus of the amygdala. The study used 13 post mortem brains from male patients with schizophrenia and the same number of age-matched male controls. The analysis showed no significant differences between these two study groups. Kreczmanski et al. conclude that the vascular biomarkers stated above cannot be considered characteristic features of schizophrenia.

Sinka et al. (2012) performed a post mortem stereological assessment of capillary diameters in anterior cingulate cortex to evaluate microvascular changes in late-life schizophrenia (N=8) and mood disorders (bipolar disorder N=10; major depressive disorder N=8) as against age- and gender-matched control cases (N=7). All individuals were drug-naïve or had received psychotropic medication for less than 6 months. Mean capillary diameter was significantly decreased in bipolar disorder and unipolar depression cases whereas schizophrenia patients were comparable with controls. As the authors conclude, the limited number of cases may mask significant differences that would be evident in larger cohorts.

In his review, Bachneff (1996) emphasizes the important role of local circuit neurons and their microvascular regulatory system in schizophrenia symptoms, especially the cognitive ones. Nitric oxide deficiency is suspected as one of the leading mechanisms in failing to selectively raise regional cerebral blood flow in areas of the frontal and temporal lobes during certain neuropsychological tasks. Nitric oxide is a potent but extremely short-lived vasodilator.

Kiehl et al. (2009) reported postmortem neuropathologic findings in four individuals with a confirmed 22q11.2 deletion and schizophrenia. Cases 2 (male, aged 22 years), 3 (female, 52 years) and 4 (female, 56 years) all had extensive astrocytic gliosis and focal collections of macrophages in the cerebral white matter, suggestive of cerebrovascular pathology. No such changes were found in Case 1 (male, 44 years). The authors concluded that both fetal and early developmental brain abnormalities and later microvascular pathology may play a role in the pathogenesis of the neuropsychiatric phenotype of the 22q11.2 deletion

syndrome and schizophrenia. The 22q11.2 micro-deletion encompasses about 40 genes. The *Tbx1* gene belongs to them, and is known to play a key role in vascular development.

Harris et al. (2008) investigated the cerebral vascular endothelium in the prefrontal cortex of schizophrenic patients (N=9) as against mentally healthy controls (N=7) at the level of transcriptomics using postmortem laser capture microdissection. The RNA analysis showed downregulation of inflammatory gene expression in the patients with schizophrenia. Based on the results, the authors suggest a hypo-inflammatory state in schizophrenia.

Hanson & Gottesman (2005) present a genetic-inflammatory-vascular model to explain at least a part of the symptoms of schizophrenia. Their basic idea is that genetically modulated inflammatory reactions to environmental agents (for example infections, hypoxia or physical trauma) damage the microvascular system of the brain, and thus deteriorate the function of neurons in a given location. The inflammation of vessels can be chronic and mild. Neurons do not get sufficient energy and oxygen, and are also damaged by oxidative stress. This leads to abnormal signal processing therefore evoking the symptoms of schizophrenia. The nature and severity of schizophrenia symptoms would depend on where the brain inflammation takes place. Repeated exposure to triggering agents increases the damage, which may result in exacerbation or deterioration of schizophrenia. On the other hand, if the environmental agents are removed, recovery may occur. Thus inflammation as well as schizophrenia symptoms have a fluctuating course. Inflammatory events early in life (pregnancy, birth complications) are especially deleterious because they damage angiogenesis and organization of neurons in the brain. During life, subsequent CNS insults may be present („accumulation of hits“), and inflammation increases. The greater the number, severity and duration of „hits“, the greater the risk for a deteriorating schizophrenia course. However, the environmental contribution to schizophrenia may be varied, relatively minor, non-specific and ubiquitous. Genetic polymorphisms, for example in the genes for TNF-alpha or interleukin-1, may lead to exaggerated inflammatory responses. The subject's inflammatory response may be more important in schizophrenia etiopathogenesis than the environmental insults themselves.

## DISCUSSION

The findings stated above are consistent with the hypothesis of microvascular pathology in schizophrenia. The work by Meier et al. (2013) is pioneering, the authors were able to examine microvascular abnormality in living schizophrenia patients in a way which is informative about the microvessels in the patient's brains. Their attitude is also relatively easy, available,

non-invasive, quick and cheap. It is important to stress that all studies of brain microvasculature in schizophrenia to date are post mortem.

The work by Hanson & Gottesman (2005) is original, detects numerous associations among genetic, inflammatory, vascular and environmental findings in schizophrenia, and explains them in a logical way. Thus, a part of the underlying pathophysiology of schizophrenia symptoms can be elucidated.

Some of the results stated above conflict with one another. Hanson & Gottesman (2005) suggest the inflammation of microvessels in schizophrenia, but Harris et al. (2008) suppose a hypo-inflammatory state. A potential explanation for these apparently opposing results is overall dysregulation of inflammation, leading to an inappropriate response (either too much or too little inflammation) depending on the stimulus and site (Harris et al. 2008).

In the past, microvascular changes that could affect brain perfusion in schizophrenia have rarely been studied. Nowadays, there is increasing evidence of the involvement of microvascular abnormalities in schizophrenia.

Hudson et al. (1997) detected the abnormal vascular response to niacin in schizophrenia (N=28) against bipolar disorder subjects (N=18) and controls (N=28) ( $p < 0.0001$ ). This supports the hypothesis of the microvasculature dysfunction in schizophrenia.

Curtis et al. (1999) found abnormalities of the nail fold capillary beds in some schizophrenia patients (N=139) as compared to unipolar, bipolar (unipolar + bipolar N=66) and nonpsychiatric (N=119) controls.

Disturbances of cerebral blood flow have repeatedly been observed in subjects with schizophrenia via brain imaging – positron emission tomography (PET), single-photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI). A consistent body of evidence shows reduced cerebral blood flow especially to anterior regions (Schultz & Andreasen 1999).

Several replicated candidate genes for schizophrenia, e.g. the regulator of G-protein signalling 4 gene, are regulated by hypoxia (Olechnowicz et al. 2012). This is also important from the microvascular aspect.

Nevertheless, conclusions cannot be firmly drawn without further in vivo study of the microvasculature in schizophrenia patients (Harris et al. 2008). Retinal and cerebral microvessels are structurally and functionally homologous because they share similar embryological origins, unlike cerebral microvessels, retinal microvessels can be noninvasively measured in vivo via retinal imaging (Meier et al. 2013).

The limitation of retinal imaging in schizophrenia is the reason that nobody knows whether microvascular abnormalities are already present before the onset of schizophrenia, or occur during the course of the disease.

In the research into microvascular pathology in schizophrenia, polymorphisms and epigenetic changes in the genes related to immune reactivity should be further investigated. It is also useful to record the number and character of environmental „hits“ triggering inflammation in schizophrenia patients (Hanson & Gottesman 2005).

To assess whether microvascular abnormality is an endophenotype in schizophrenia, the following steps are necessary:

- To examine a sufficient number (which is advocated by the power analysis of the applied statistical test) of patients with schizophrenia, their first-degree relatives, patients with another serious mental disorder (e.g. bipolar disorder) and healthy volunteers by retinal imaging.
- To examine groups of schizophrenic patients in longitudinal studies, i.e. during the course of the disease while the symptoms are changing. Besides other demands, criteria for endophenotypes include the state independence (Gur et al. 2007).

Additional avenues for schizophrenia exploration include:

- Retinal imaging in individuals at high risk for schizophrenia. This includes examination of individuals with early „psychotic experiences“ (sub-syndromal psychosis).
- Concurrent brain fMRI and retinal imaging to determine if the vascular abnormalities in the retina and the brain are related.
- Looking for the relationship between cognitive performance and retinal venule calibre in cross-sectional and longitudinal studies.
- Cross-sectional studies exploring relationships between specific symptoms or syndromes (for example positive and negative schizophrenia symptoms) and the calibre of the retinal venules.
- Inclusion of the history of childhood maltreatment in future studies of relationship between retinal microvascular pathology and schizophrenia. This is because childhood maltreatment is an important environmental „hit“ which may be associated with the subsequent local brain micro inflammatory reaction (via stress mechanisms) and have a powerful effect on mental and physical health in the adulthood.

## CONCLUSIONS

If microvascular abnormality were proved as the endophenotype in schizophrenia, the research into its genetic background would help elucidate pathogenesis of schizophrenia and possibly find new treatment and preventative options. If brain vasculitis is definitely confirmed in schizophrenia, anti-inflammatory agents could be prescribed in the patients as well as high-risk individuals (Hanson & Gottesman 2005).

## Acknowledgements:

The article was supported by the Research Grant of the Ministry of Health of the Czech Republic No. NT/14504. The authors thank Professor Jan Volavka, MD, PhD, from the New York University School of Medicine for his valuable contribution to the content of this manuscript.

**Conflict of interest:** None.

## References

1. *Bachneff SA: Regional Cerebral Blood Flow in Schizophrenia and the Local Circuit Neurons Hypothesis. Schizophr Bulletin* 1996; 22:163-82.
2. *Bearden CE & Freimer NB: Endophenotypes for psychiatric disorders: ready for primetime? Trends Genet* 2006; 22:306-13.
3. *Boshes RA, Manschreck TC, Konigsberg W: Genetics of the schizophrenias: a model accounting for their persistence and myriad phenotypes. Harv Rev Psychiatry* 2012; 20:119-29.
4. *Braff DL, Freedman R, Schork NJ, Gottesman II: Deconstructing Schizophrenia. An Overview of the Use of Endophenotypes in Order to Understand a Complex Disorder. Schizophr Bulletin* 2007; 33:21-32.
5. *Corvin A: Schizophrenia at a genetics crossroads: where to now? Schizophr Bulletin* 2013; 39:490-5.
6. *Curtis CE, Iacono WG, Beiser M: Relationships between nailfold plexus visibility and clinical, neuropsychological, and brain structural measures in schizophrenia. Biol Psychiatry* 1999; 46:102-9.
7. *Gottesman II & Gould TD: The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry* 2003; 160: 636-45.
8. *Gur RE, Calkins ME, Gur RC, Horan WP, Nuechterlein KH, Seidman LJ et al.: The Consortium on the Genetics of Schizophrenia: Neurocognitive Endophenotypes. Schizophr Bulletin* 2007; 33:49-68.
9. *Hanson DR & Gottesman II: Theories of schizophrenia: a genetic-inflammatory-vascular synthesis. BMC Med Genetics* 2005; 6:7.
10. *Harris LW, Wayland M, Lan M, Ryan M, Giger T, Lockstone H et al.: The Cerebral Microvasculature in Schizophrenia: A Laser Capture Microdissection Study. PLoS ONE* 2008; 3:e3964.
11. *Hosak L: New findings in the genetics of schizophrenia. World J Psychiatry* 2013; 3: 57-61.
12. *Hudson CJ, Lin A, Cogan S, Cashman F, Warsh JJ: The niacin challenge test: clinical manifestation of altered transmembrane signal transduction in schizophrenia? Biol Psychiatry* 1997; 41: 507-13.
13. *Kiehl TR, Chow EWC, Mikulis DJ, George SR, Bassett AS: Neuropathologic Features in Adults with 22q11.2 Deletion Syndrome. Cerebral Cortex* 2009; 19: 153-64.
14. *Kreczmanski P, Heinsen H, Mantua V, Woltersdorf F, Masson T, Ulfing N et al.: Microvessel length density, total length, and length per neuron in five subcortical regions in schizophrenia. Acta Neuropathol* 2009; 117: 409-21.

15. Meier MH, Shalev I, Moffitt T, Kapur S, Keefe RSE, Wong T et al.: Microvascular Abnormality in Schizophrenia as Shown by Retinal Imaging. *Am J Psychiatry* 2013; 170:1451-9.
16. Murray CJL: *The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020*. Harvard School of Public Health on behalf of the World Health Organization and the World Bank, Cambridge, 1996.
17. Olechnowicz SW, Fedele AO, Peet DJ: Hypoxic induction of the regulator of G-protein signalling 4 gene is mediated by the hypoxia-inducible factor pathway. *PLoS ONE* 2012; 7: e44564.
18. Schultz SK & Andreasen NC: Schizophrenia. *Lancet* 1999; 353:1425-30.
19. Sinka L, Kovari E, Santos M, Herrmann FR, Gold G, Hof PR et al.: Microvascular changes in late-life schizophrenia and mood disorders: stereological assessment of capillary diameters in anterior cingulate cortex. *Neuropathol Appl Neurobiology* 2012; 38:696-709.
20. Weinberger DR & Harrison PJ: *Schizophrenia*. Wiley-Blackwell, Chichester, 2011.

Correspondence:

Professor Ladislav Hosak, MD, PhD  
Department of Psychiatry, University Hospital Hradec Kralove  
Sokolska 581, 500 05 Hradec Kralove, Czech Republic  
E-mail: hosak@lfhk.cuni.cz