NON-PHARMACOLOGICAL METHODS IN THE TREATMENT OF RESISTANT HYPERTENSION

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ABSTRACT

INTRODUCTION: Arterial hypertension is the most common chronic cardiovascular disease affecting about 25% of the adult population. Meta-analyses have demonstrated a linear relationship between blood pressure and the risk of cardiovascular events. Resistant hypertension defined as failure to reach blood pressure targets despite treatment with three antihypertensive drugs including a diuretic represents a serious clinical problem. It has been estimated that it affects between 8.9% and 12.8% of all treated hypertensive subjects. In resistant hypertension the optimal blood pressure is illusive despite very well tailored therapy.

OBJECTIVE: Management of resistant hypertension is exactly the field where blood pressure-controlling non-pharmacological methods fit best. The present article aims at throwing light on these methods' principles of action, on who the target patient groups are and the respective results. Two methods are especially reviewed here: the carotid baroreflex stimulation and the transcatheter renal sympathetic denervation.

Current results from the use of renal denervation suggest stable efficiency of the method, the results becoming significant 6 months after the procedure is applied and sustained for two years in the follow-up. As much as 90% of the treated patients respond to the procedure. The transcatheter renal denervation is associated with only 2.61% of procedural complications. The baroreflex carotid stimulation, too, is known to produce a stable effect on blood pressure: the effect become obvious at 12 months in 88% of the treated subjects. The neurologic complications associated with the procedure are reported to occur in 4.4% of cases.

CONCLUSION: The present review article clearly demonstrates that non-pharmacological methods for treatment of resistant hypertension show great promise despite some open questions concerning their long term effects and procedural safety.

Key words: resistant hypertension, renal denervation, baroreflex stimulation

INTRODUCTION

Arterial hypertension (AH) is the most common chronic disease in modern societies, affecting about 25% of the adult population.1 Meta-analyses have demonstrated a linear correlation between the levels of blood pressure (BP) and the risk of cardiovascular events.2 Inadequate control of BP is a leading risk factor for cardiovascular mortality worldwide as it contributes to 62% of the cerebrovascular accidents, 49% of the cases of ischemic heart disease and about 7.1 million deaths annually.3 In the U.S. the incidence of AH continues to increase with age, but recent data suggests improvement in terms of treatment and control.4 In Europe lower frequencies of optimal drug control of AH are reported compared with those in the U.S.1

Several extensive studies, including INVEST, ACCOMPLISH, CONVINCE, ALLHAT, LIFE have registered failure in achieving the BP target levels with the therapeutic regimens set out in the study protocols. In these studies, 20 to 35% of participants did not reach the BP target levels despite receiving three antihypertensive drugs. In Bulgaria, the high incidence of cardiovascular morbidity and mortality...
correlates with the high incidence of hypertension. The incidence of hypertension for 25-to-64-year olds in Bulgaria is 40.1%, while for people > 45 years it is 50.3%. At present there is practically no uniform updated statistics on the incidence of hypertension in Bulgaria as well as on the incidence of resistant hypertension (RH).

**DEFINITION AND INCIDENCE OF RESISTANT HYPERTENSION**

Resistant hypertension as defined by the Joint National Committee 7 is the failure to achieve goal BP in patients: below 140/90 mm Hg for the general population and below 130/90 mm Hg for patients with diabetes and chronic kidney disease, despite their adhering to full doses of an appropriate 3 antihypertensive drug regimen that includes a diuretic. This definition does not apply to patients with newly diagnosed hypertension. Resistant hypertension is not a synonym for uncontrolled hypertension. The latter includes all hypertensive patients lacking optimal antihypertensive control on the background of inadequate dosage of drug therapy, poor adherence to treatment, with secondary AH and those with true therapeutic resistance. According to the current definition, the RH patients could achieve the desired control of BP with full doses of 4 or more antihypertensive drugs. Although the definition of RH could be considered debatable because of the fixed number of drugs on which the diagnosis is based, it is the very concept of resistance that matters. It focuses on identifying high-risk patients and those that have correctable causes of hypertension and/or those who would benefit from specific diagnostic and therapeutic approaches.7

The prevalence of resistant hypertension in the general population is still unknown due to the small study sample of the studies published to date. Currently there is no large prospective trial that can answer this question. According to some small studies, prevalence of RH ranges from around 5% in general practice, with little selection of patients, to 50% in clinic of nephrology.8 According to data provided by the National Health And Nutrition Examination Survey, for 2005 and 2006, only 68% of hypertensive patients in the U.S. reach BP levels less than 140/90 mm Hg, while the prevalence in patients with diabetes or chronic kidney disease is 40%.5,9 For 2002-2008, NHANES reports of 8.9% prevalence of resistant hypertension in hypertensive patients and 12.8% in antihypertensive drug-treated hypertensive patients.10 In Europe the prevalence of cases with optimal control in treated hypertensive patients is between 19% and 40% according to data from five European countries.1 The provided data suggest that resistant hypertension is much more common than it is thought to be; unfortunately there is no accurate statistics since the degree of therapeutic control is influenced by many factors.

**PROGNOSIS OF RESISTANT HYPERTENSION**

So far there have been conducted no studies exploring specifically the prognosis of patients with resistant hypertension. On the other hand, according to data from population studies, hypertension-related target organ damage, risk of myocardial infarction, stroke, heart and renal failure directly correlate with the levels of BP.6,11 These observations, particularly in the context of high comorbidity in patients with resistant hypertension (obesity, diabetes, dyslipidemia, chronic renal failure) lead to an adverse outcome if BP is not brought to normal. The degree of reduction of cardiovascular morbidity and mortality while seeking to get the target BP have not been analyzed specifically but the benefits can be seen in the results of several studies.2,6

**NON-PHARMACOLOGICAL ALTERNATIVES IN THE TREATMENT OF RESISTANT HYPERTENSION – ARE THEY NEEDED?**

Despite the availability of a large arsenal of medications, achieving the target control in many patients with RH is impossible, even with well tailored therapy because of side effects, inadequate compliance and adherence and last but not least, for financial reasons. It is in these patients that non-pharmaceutical methods for control of resistant hypertension are tested. The purpose of this review is to shed light on their methodology, principles of action, suitable patient populations and results available up to now. Two principal methods are currently in clinical testing - carotid baroreflex stimulation and endovascular (trans-catheter) renal sympathetic denervation (RSD).

**TRANSCATHETER RENAL SYMPATHETIC DENERVATION**

**PATHOPHYSIOLOGICAL RATIONALE**

Over the last decades the focus of research on treatment of AH has been on the renin-angiotensin system. The proven efficacy of drugs blocking this axis resulted in inadequate attention afforded to methods of influencing another major system implicated in causing arterial hypertension, namely
the sympathetic system. There is general consensus at present that excess activity of the sympathetic nervous system leads to onset and sustenance of elevated BP in patients with AH.12,13

Measurement of organ-specific catecholamine spillover is of fundamental interest for the quantification of catecholamine excess. Today, determination of norepinephrine spillover in plasma by isotope dilution is considered the golden standard. Using this technique the usual levels of organ-specific catecholamine spillover in the plasma of healthy individuals have been found to be as follows: 5-25 ng/min from the heart, 40-110 ng/min from the kidneys, 30-120 ng/min from the lungs and 50-130 ng/min from the skeletal muscles.14

There is compelling evidence that the renal afferent nerves linked to the hypothalamus can stimulate sympathetic spillover.15 Bilateral renal denervation results in significant reduction of both total and the local renal catecholamine spillover of 48-75%.16 This response of the central nervous system to afferent renal nerves is the basis of sympathetic activation and hypertension in patients with end stage renal disease.17 This is the rationale for the hypothesis that patients with resistant hypertension can benefit from denervation of the afferent sympathetic fibres of their kidneys. This hypothesis suggests that the renin secretion from the kidney would decrease together with the catecholamine spillover because of the sympathetic trigger for its release.

CATHETER METHOD OF RENAL DENERVATION – CHARACTERISTICS
Currently one system for trans-catheter renal sympathetic denervation has been well studied in randomized clinical trials - Symplicity® of Medtronic/Ardian (Mountain View, California, USA). The system consists of two components - ablation catheter and an automatic portable radio-frequency generator with low power and built-in safety algorithms.

CATHETER RENAL DENERVATION – METHODOLOGY
The endovascular trans-catheter renal sympathetic denervation is performed via femoral artery access with 6Fr introducer. Intra-procedural anesthesia is necessary, usually by using midazolam and morphine hydrochloride. Short guiding catheters (GC) - 47-55 cm are used to place the ablation catheter in position; RDC (Renal double curve) or IMA (Internal mammary artery) are preferred and these are introduced by a 0.035-inch guidewire. Heparin is used to achieve the intra-procedural anticoagulation, at the usual dose of 5000 IU with activated clotting time (ACT) control at 250 seconds. When performing a control angiography of the vessel the use of diluted contrast agent with isotonic saline in 1:1 proportion is recommended. The ablation catheter is introduced after cannulation of the renal artery with GC. It has a flexible tip (controlled by the handle of the catheter) that allows its positioning along the artery and application of pinpoint ablations in a spiral fashion distally to proximally. Ablation cycles are pre-programmed and have duration of 120 seconds. An initial impedance of the catheter tip above 250Ω ± 15-20Ω (in respiratory excursions) is sought, which indicates good contact. Local temperature during ablation reaches 52 °C. The aim of renal denervation is to conduct 4 to 6 ablations along both renal arteries in a spiral fashion at a distance of at least 5 mm one from the other treating the lower section in the distal segments of the artery, and the upper sections in the proximal segments. Areas of the vessel with apparent stenosis and calcification should be avoided.

There are several contraindications for carrying out renal denervation, including:
• Significant renal stenosis;
• Aneurysms;
• Lack of a common stem of the renal artery;
• Extreme tortuosity;
• Vessel diameter less than 4 mm;
• Previous stent in the renal artery.

RENAL DENERVATION - CLINICAL TRIAL RESULTS
Given the available definitive pathophysiological rationale for renal denervation benefits, two major studies have been carried out using the Symplicity® system - Symplicity HTN-1 and 2.

Symplicity HTN-1 study includes an initial non-randomized cohort of 45 patients with resistant hypertension (systolic arterial pressure (SAP) ≥ 160 mm Hg on ≥ 3 antihypertensive drugs including a diuretic) and glomerular filtration rate (eGFR) ≥ 45 mL/min. A follow-up to the 12th month was sought. Subsequently, the cohort was expanded to 153 patients followed to the 24th month.

Procedure characteristics – the duration of the procedure was reported to be 38 minutes with an average of 4 ablations per artery. Minor complications were registered in 4 out of 153 patients - 1 case of dissection of the renal artery during placement of ablation catheter and before performing ablation - without consequences; 3 cases of complications at puncture site treated without sequel. With regard to safety 81 patients were followed at 6 months by imaging methods (computerized...
tomography, magnetic resonance imaging (MRI) or duplex sonography), showing no evidence of vascular abnormalities at ablation spots; in one patient progression of underlying renal stenosis was detected which was not related to the denervation procedure. No significant change in renal function and electrolyte homeostasis was reported during follow-up.

The results in terms of blood pressure control are present from the first month after the procedure - decrease of SAP by 20 mm Hg and diastolic arterial pressure (DAP) by 10 mm Hg. The results are sustained and reinforced over time; at first-year follow up the decrease in SAP and DAP was by 23 and 11 mm Hg; and at the second year by 32 and 14 mm Hg respectively compared to baseline values.

Symplcity HTN-2 is a prospective, randomized multicenter study designed to demonstrate the relative effectiveness of catheter-based renal denervation to treat resistant hypertension in 106 patients randomized in a 1:1 ratio to controls. Patients with resistant AH aged 18-85 years with standard treatment for this condition were included. Main exclusion criteria were hemodynamically and anatomically significant stenosis of the renal artery before intervention; eGFR < 45 mL/min/1.73m²; diabetes mellitus type I; contraindications for MRI; valvular stenosis, where the reduction in BP would be risky; myocardial infarction, unstable angina pectoris or cerebrovascular accident in the previous six months. The average baseline values in the studied groups are as follows: SAP (systolic arterial pressure) - 178 ± 18 mm Hg; DAP (diastolic arterial pressure) - 97 ± 16 mm Hg – (p > 0.05). The studied patients received an average of 5.2 ± 1.5 antihypertensive drugs in the renal denervation group and 5.3 ± 1.8 in the control group (p > 0.05). The primary endpoint - effect on office BP at 6 months was achieved with a significant result in favour of the renal denervation group. The reported decrease of SAP/DAP was 33/11 mm Hg compared to controls (p < 0.0001). The fall in blood pressure in denervated patients led to marked reduction in the dose of medications (-20%) compared to controls (p = 0.04). A more than 10 mm Hg fall in SAP was recorded in 84% of the patients who underwent renal denervation. Dynamic changes in BP between investigated groups during follow-up are presented in Fig. 1.

The levels of SAP / DAP measured by ambulatory blood pressure monitoring (ABPM) at six months show significant difference between the groups: -20/-12 mm Hg for the group with RSD

![Figure 1. Distribution of office SAP between groups at baseline and at six months.](image-url)
The decrease in SAP and DAP after renal sympathetic denervation is progressive and sustainable over time. Follow-up of BP at one, three and six months shows decrease from baseline levels for SAP (-20/-24/-32 mm Hg) and for DAP (-7/-8/-12 mm Hg) – p < 0.001.

With regard to procedural safety no serious adverse events were observed throughout the study. Magnetic resonance follow-up of the renal arteries at six months, carried out in 43 patients, showed no abnormalities at any of the ablation sites. Progression of previously present renal artery stenosis, unrelated to RSD, was observed in one patient. No inter-group and time/temporal dynamics in the levels of eGFR, creatinine and cystatin-C was found during follow-up.

The results from the Symplicity HTN - 1 and 2 convey several key messages. Practically 90% of patients with RH undergoing RSD respond clinically to the procedure; in 84% of all treated a drop in SAP with more than 10 mm Hg is observed. The decrease in SAP and DAP is statistically significant and progressive over time and it was preserved until the end of follow-up period. The lack of procedural damage to the renal vessels, the low incidence of vascular complications and the high efficiency raise hope for widespread clinical use.

In Europe and Australia there are ongoing and planned clinical trials for other clinical conditions accompanied by sympathetic hyperactivity - insulin resistance, heart failure, obstructive sleep apnea.

**BARORECEPTOR STIMULATION**

The carotid baroreflex represents an essential element in the homeostasis of arterial pressure. Carotid baro-receptors are sensitive to pressure in the artery and modulate sympathetic tone in a direction opposite to the signal – i.e. high blood pressure leads to a decrease in sympathetic tone and low – to an increase. The change of efferent signals as a result of baro-receptor stimulation produces a reciprocal increase in parasympathetic activity, decrease in heart rate, vasodilation, decreased vascular resistance, increased sodium-uresis and reduced production of renin by the kidneys. The concept of interference in the function of carotid baro-receptors is not new. Various devices for exogenous electrical stimulation of baro-receptors were invented in the 50s and 60s of the last century. The principal idea behind these is to have an external generator creating electromagnetic impulses that are to be transmitted to the implanted receiver with a coil antenna.

These impulses are then transferred to electrodes that come into contact with the carotid sinus. This concept is based on the assumption that the signals will be identified by the central nervous system as a continuous increase of BP and this will result in inhibition of the sympathetic tone and decrease in BP. Some animal studies from that period show not only temporary but long standing reduction of BP within one year of follow-up.

In 1967 Braunwald et al. published data on the use of baroreflex stimulation in patients with stable angina pectoris aimed at alleviating chest pain. It turned out that the device of the described type (with an external transmitter) that was used, effectively reduced myocardial oxygen consumption and prolonged time to onset of angina attacks and ST depression. The initial enthusiasm for the method, however, rapidly waned because of the high incidence of adverse events and serious technical shortcomings those devices.

Modern technologies appear to be able to overcome many of these shortcomings which results in a restoration of the interest in baroreflex stimulation (BS). At present there is one device for carotid baroreflex stimulation approved for human use - Rheos™ (CVRx Inc, Minneapolis, Minn). The first generation Rheos is a pulse generator similar to a pacemaker that is implanted into the right subclavian area. It is connected to two lead electrodes which are positioned in the perivascular area of both carotid sinuses. The generator can be programmed non-invasively. An experienced team consisting of a surgeon, anesthesiologist, hypertension specialist and a technician is needed for the implantation procedure.

Rheos Hypertension Pivotal Trial is a prospective randomized double-blind multicenter study including 265 patients with resistant hypertension. Participants were randomized in a 2:1 ratio in two groups (A and B). In the first group the implanted device was active throughout the whole 12-month period, whereas in the second (placebo group) it was activated after the first 6 months. The main criteria for inclusion were values of the SAP > 160 mmHg on the background of at least 3 anti-hypertensive drugs, diuretic including. Both groups showed a significant fall in SAP - in Group A SAP was reduced to target levels in 41% of patients after 6 months and in 54% - after 12 months. A surprisingly strong reduction in SAP was observed in group B during the control period which is explained by the monthly monitoring and adjustment of drug therapy within the study. At 6 months 21%
of patients in group B had reached the target SAP and 46% after 12 months. The reduction in BP at 12 months was at least 50% of that at the sixth month which demonstrates a sustained response. Response to therapy reaching target BP values of < 140 mm Hg is reported in 88% of the patients at one year follow up with an average decrease of SAP by 35 mmHg as well as reduction of left ventricular hypertrophy (LV mass index).25 Despite these good results, the study did not achieve two of its five initial endpoints - acute short-term efficacy and procedural adverse events. The latter raise the most serious concern - although 76% of all the procedural complications are permanently resolved, permanent neurological complications (dysphonia, dysphagia, muscle rigidity) and general surgical complications were observed in 4.4% of the patients. Despite these concerns the study showed the long-term efficacy of baro-receptor activation to treat resistant hypertension. Its results provided impetus for further research and ultimately led to the development of the second generation device for baro-stimulation - Barostimneo ™, which is focused primarily on improving procedural safety. Compared to the first generation the Neo ™ device has a considerably reduced size of the electrode and the device itself. Implantation is unilateral and is possible without the use of general anesthesis.

The results from the initial follow up of 40 patients show a similar effect on blood pressure compared to first generation devices. A multicenter randomized trial is under way with 120 patients included up to present. Results of a trial in patients with heart failure are also expected.

CONCLUSIONS

The non-pharmacological methods in the treatment of resistant hypertension - renal sympathetic denervation as well as baro-receptor stimulation provide unambiguous results on efficacy, though not occurring fast. Clinical trials’ results send the clear message that around the sixth month after the procedures there is a significant decline in BP, which is stable and sustained over time. There are still many open questions. Regarding RSD - whether after the second year of the procedure its effect would decrease and whether other groups of patients would benefit even at lower levels of BP. As for the BS the risk benefit ratio of procedural safety versus clinical outcome and, last but not least, the pharmaco-economic justification of the procedure, still remain unclear. Despite the questions regarding each of the methods, the results cited in this review can be considered promising for the future of renal denervation and carotid baro-receptor stimulation for the treatment of resistant hypertension.

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Нефармакологические методы лечения резистентной гипертонии

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Резюме

Введение: Артериальная гипертония представляет собой самое частое хроническое заболевание настоящего времени, затрагивающее приблизительно 25% взрослого населения. Метаанализы свидетельствуют о наличии линейной зависимости между уровнями артериального давления (АД) и риском сердечно-сосудистых заболеваний. Резистентная гипертония, определяемая как отсутствие достижения целевых уровней АД, независимо от приема трех и более антигипертензивных медикаментов, в том числе и диуретика, является серьезной клинической проблемой. Ее частота, по данным больших исследований, варьирует от 8.9% до 12.8% среди всех гипертоников, подвергнутых нефармакологическим методам лечения. Несмотря на наличие арсенала медикаментов, в ряде резистентных гипертоников антигипертензивный контроль невозможен даже в случаях хорошо индивидуализированной медикаментозной терапии. Цель: Существует немалая ниша нефармакологических средств контроля АД при резистентной гипертонии. Работа ставит себе целью показать методологию, принцип действия, подходящие группы пациентов и результаты. Рассматриваются два метода — каротидная барорефлексная стимуляция и транскатетерная рениновая денервация симпатической нервной системы. Полученные до сих пор результаты относительно рениновой денервации указывают на прочный эффект на АД, значимый с 6-го месяца после процедуры и удерживающийся до второго года прослеживания. Респондеры процедуры представляют 90% пациентов, на которых оказано соответствующее воздействие. Транскатетерная рениновая денервация представляет метод низкой частоты процедурных осложнений — до 2.61%. Барорефлексная стимуляция также дает устойчивый эффект на АД, при чем на 12 мес. эффект наблюдается в 88% случаев. Частота прочих неврологических осложнений в границах 4.4%.

Заключение: Несмотря на все еще имеющиеся вопросы, результаты, представленные в обзоре, обещающие наступление будущего нефармакологических методов при лечении резистентной гипертонии.
EPIGENETIC ASPECTS IN SCHIZOPHRENIA ETIOLOGY AND PATHOGENESIS

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ABSTRACT

Epidemiological evidence suggests that etiology of schizophrenia may involve both the influence of genetic factors specific for the individual and the impact of the environment. It is quite likely that a crucial role in the disease development is played by molecular mechanisms mediating the interaction between genes and environment. Modern research have shown that epigenetic mechanisms or chemical modifications of deoxyribonucleic acids (DNA) and histone proteins remain unstable throughout life and can be changed by environmental factors. Thus the epigenetic mechanisms outline an attractive molecular hypothesis of the environment modelling role and the environmental contribution to schizophrenia progression. We give in the present study a general outline of schizophrenia as a pathological entity and discuss the role and involvement of environment versus genetic determinant (nature versus nurture) in the pathophysiological processes. Additionally, we focus on DNA methylation discussing the evidence for the role of that process in schizophrenia. Thirdly, we review the post-translational histone modifications and their role in schizophrenia. These investigations might surely lead further to the development of epigenetic therapy that looks promising in regard to symptom alleviation and the disease-associated cognitive deficit.

Key words: epigenetics, schizophrenia, microRNA molecules, histone modifications

INTRODUCTION

Schizophrenia is a severe disabling disturbance affecting approximately 1% of the population worldwide. The pathologic alterations comprise three main abnormalities: 1) positive symptoms, including hallucinations, delusions and disorganized thinking; 2) negative symptoms, consisting of apathy, anhedonia, abulia and poverty of speech, and 3) cognitive dysfunction, including impaired functioning of memory and overall disorganization. Altogether, these symptoms convert patients into socially isolated and poorly work adaptable persons. Patients with schizophrenia are among the most wronged and maligned members of society. Treatment with antipsychotic drugs only alleviates schizophrenia symptoms and therefore elucidation of etiological mechanisms causing schizophrenic disturbance would probably help the development of novel therapeutical interventions and more effective treatment.

microRNA AND PSYCHIATRIC DISORDERS

Psychiatric disorders are characterized with global dysregulation of multiple signal pathways. Although these conditions may incorporate a genetic component, the variability of patients with seemingly the same type of pathology makes it hard to identify key genes engaged in the development of psychiatric disorders.1 Probably it also makes the effectiveness of various medications different. These observations suppose engagement of still poorly investigated regulation molecules. Studies on microRNA are focused mainly on schizophrenia and less on bipolar affective disorder. The mechanism of action of the class of small regulatory RNA molecules makes them appropriate for investigation as a potential therapeutical tool in patients suffering from such disturbances.2

There is a growing body of evidence that DNA methylation profile can be linked with specific alleles associated with affective disorders. For example

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T polymorphism of C677T in methylhydrofolat reductase gene (MTHFR), recently associated with depressive illness.\(^3\)

**MicroRNA and Schizophrenia**

Differences in the expression levels of certain microRNA molecules have been found in the brain of schizophrenic patients compared to samples from healthy subjects. Another possible link between microRNA biogenesis and schizophrenia as a neurodegenerative disease has been proposed, investigating the function of DGCR8, which is a key protein in microRNA biogenesis, and is encoded by a segment of chromosome 22, prone to deletion in schizophrenia patients. Thus the impaired biogenesis can be associated with the onset and progress of schizophrenia. Currently, the genes encoding microRNAs, located on the X-chromosome are cloned in order to identify alterations that may be consistent with X-related schizophrenia.\(^4\) The results confirm these rare variants in the sequence of mature precursors of microRNA molecules (mRNA-18b, mRNA-502, and mRNA-505) or mature microRNA (let-7f-2, mRNA-188, mRNA-325, mRNA-509-3, mRNA-510 and mRNA-660) are more frequent in male patients, compared to healthy individuals.\(^5\) These studies presume that altered microRNA function may contribute to the disease development, but further studies proving the association are needed. The identification of messenger RNA (mRNA) molecules that are targets of these microRNAs is a key step in the formation of an overall idea of the pathways, affected by the proven abnormal expression of protein coding iRNA.

**Role of microRNAs as Biomarkers**

Biomarkers are objectively measurable biologic characteristics which can be used as indicators of normal or pathologic processes. Thanks to recent advances in molecular biology, the range of potential biomarkers has expanded to include genomic profiling, transcriptomic and proteomic analysis. In this regard, microRNA molecules are emerging as a novel class of biomarkers in several diseases, including CNS disorders. In fact microRNA expression profiling has been used to characterize CNS embryonal and differentiated tissues to discriminate cancer from normal tissues, to identify tissue of origin of metastatic cancer. Thus epigenetic modifications and mechanisms constitute a common set of factors called endophenotype.\(^6\)

**A Model of MicroRNA Associated Dysregulation of the Synaptic Structure and Function in Schizophrenia**

The activity of the microprocessor complex (ribonucleoprotein complex associated with the processing of microRNA molecules precursors) is increased in the cortical regions as a consequence of the disease-related expression of DGCR8 gene (a gene encoding a protein involved as a subunit in the construction of the microprocessor complex). The acceleration of the processing level results in an increase of the precursors of microRNA molecules, which are exported from the nucleus and processed by Exportin-5 (XPO5 gene encoding exportin-5) and Dicer (RNAase III similar endoribonuclease) enzyme activity. The already exported microRNA molecules are incorporated into RNA-inducing slicing complex (RISC) binding to 3’ non-translationable regions of their target (target transcripts) messenger RNA molecules, encoding synaptic components as neurotrophins/ligands (BDNF, Reelin), neurotransmitter receptors (GRM7, GRIN3A, HTR2A, DRD1) and structural components defining the receptor density of the post-synaptic membrane (DLG4). This interaction reduces the stability of the transcripts and their translational capability. The abnormal levels of microRNA result in a reduction of synaptic protein levels, which in turn leads to alterations in synaptic structure and function.\(^7\)

**Epigenetics and Schizophrenia**

Epigenetic regulators of gene expression, including DNA methylation and posttranslational histone modification might take part in some of the molecular alterations, associated with schizophrenia. For example, in the prefrontal cortex of schizophrenia patients, unusual DNA or histone methylation at site of certain genes or promoters is related to changes in gene expression. These observations are of interest from neurodevelopmental perspective, since increasing amount of evidence for the role of epigenetic modifications of a significant part of the genes and loci, highly regulated in the first years of the development, appear. Additionally, there is indirect evidence that a subset of the antipsychotic drugs, including atypical drugs such as clozapin, interfere with chromatin-remodelling mechanisms.

The challenges in the field include: 1) lack of clear association between the disease and the epigenetic alterations, 2) the lack of cell-specific chromatin analysis, impeding the description of epigenetic alterations in specific cell populations and
3) insufficient knowledge on the stability or turn over of epigenetic “markings” on certain chromatin loci. Despite these disadvantages, studies on DNA changes and histone modifications in chromatin isolated from patients’ and controls’ brain tissue samples, may present precious knowledge on the genetic-epigenetic risk of schizophrenia, especially in the majority of cases with unclear genetic cause.

**HISTONE MODIFICATIONS**

Four main histone proteins (H2A, H2B, H3, H4) constructing the octamer, wrapped by 146 bp. of the genome DNA are known. This unit of the chromatin fibre is called nucleosome. In addition, linking histones such as H1 construct the protein skeleton of the internucleosomal DNA.8-10 A number of post-translational modifications of specific histone residuals are known, located predominantly in the N-terminal tails of the main histones, including lysine methylation and acetylation, arginine methylation, serine phosphorylation, as well as ubiquination and binding of SUMO protein to lysine residuals.11,12

It is considered that a part of these modifications can be regulated by the transcriptional activity or are related to the epigenetic control of gene expression.13 For example, lysine residues acetylation determines chromatin regions with potentially high levels of gene expression, while the modifying molecule, SUMO protein, is usually being associated with inhibition of transcription.11 Currently, insufficient information of only part of the histone modifications, associated with schizophrenia is present. Investigations on the quantitative characterization of histone modification level in prefrontal cortex samples from schizophrenia patients and controls show increased methylation of histone H3-arginine 17 (H3-methyl-R17) residuals in majority of the cases, showing unambiguous deficit in the process of gene expression.14 Since H3-methyl-R17 modification is found predominantly in the neuron nucleus,14 it might be assumed that some of the schizophrenia cases are characterized with more generalized alterations in some of the types of chromatin modifications in neurons and other cells. The latter hypothesis is additionally supported by other investigations, describing altered histone acetylation in experiments with immunoblot of peripheral blood lymphocytes from schizophrenic patients.15,16 While these basic molecular chromatin alterations in schizophrenia remain of interest, investigating peripheral blood cells is convenient in the light of their use as a substrate in the search of potential diagnostic biomarkers.15

**STUDIES ON SCHIZOPHRENIA INHERITANCE**

The evidence for disease aggregation in certain families shows the presence of inheritance component. In fact, there are serious studies supporting the family component in schizophrenia. The risk of schizophrenia and related disorders in first-degree relatives is approximately 9 times greater than the risk of schizophrenia in the general population.17,18 The degree of genetic identity to the proband is strongly related to the degree of risk, and consequently the first-degree relatives are at higher risk of psychosis compared to more distant relatives of the affected individuals.14

Twin studies are ordinarily used in the assessment of a disease inheritance. Extensive investigations on twins with schizophrenia have shown that the schizophrenia risk in a sibling (co-twin) of the proband is significantly higher in identical (monozygotic, 53%) than in dizygotic (15%) twins, so that the genetic predisposition to schizophrenia has been assessed at 68%.19,20 The monozygotic discordance may be explained by the reduced penetrance of the schizophrenic genotype, which is supported by the observation that a higher risk of schizophrenia is present in the offspring of the unaffected twins of discordant monozygotic pairs (nephews).19,20 A possible explanation of these observations may be attributed to environmental factors, altering gene function, which on its turn creates vulnerability to schizophrenia.21

**CONCLUSION AND FUTURE DIRECTIONS**

Currently, it is becoming clear that the epigenetic modifications not only present molecular mechanisms, responsible for significant changes in the behaviour and the brain function, but also play a significant role in the dynamic character of the adult CNS in response to the environment. In that manner, the epigenetic basis of schizophrenia turned into an attractive molecular hypothesis, supported by numbers of evidence up to this moment. Unfortunately, post-mortem studies are not able to show if the epigenetic mechanisms are causative to schizophrenia. Therefore, model species like rodents would be probably introduced in the future studies of neuropsychiatric disorders. One of the most important questions to be addressed by future studies is the capability of epigenetic treatment of alleviating the cognitive deficit caused by schizophrenia. The fact that histone methylation may be important for the early diagnosis of some schizophrenia cases, shown by post-mortem studies on gene expression
alterations, is encouraging. Additionaly, the pharmacogenetic models for schizophrenia treatment are of great importance. Up to the present, limited clinical and scientific data on the role of DNMT (DNA methyltransferase) and HDAC (histone deacetylase) inhibitors in the alleviation of some molecular and behavioural manifestations of rodent cognitive dysfunction, is present. Nevertheless, beneficial effects of the inhibition of DNMT or HDAC have been observed in rodents in terms of relaxation of some molecular and behavioural manifestations of cognition dysfunction. Such observations are promising for the potential of the drugs, able to change chromatin characteristics, as an applicable therapy in schizophrenia treatment. The studies on epigenetic modifications in the process of normal brain development, regulation of normal cognition, and in post-mortem tissue samples, bring hope to the future, when the molecular mystery underlying the disease will be solved. Elucidating the pathophysiological mechanisms would certainly facilitate the development of more effective therapeutic strategies.

REFERENCES

ЭПИГЕНОТИЧЕСКИЕ АСПЕКТЫ ЭТИОЛОГИИ И ПАТОГЕНЕЗА ШИЗОФРЕНИИ

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РЕЗЮМЕ

Эпидемиологические исследования демонстрируют одновременно влияние специфического генетического фактора индивида и воздействие окружающей среды на этиологию шизофрении. Молекулярные механизмы, определяющие взаимодействие между генами и окружающей средой, по всей вероятности играют значительную роль в процессах развития заболевания. Современные исследования показывают, что эпигенетические механизмы или химические модификации дезоксирибонуклеиновых кислот (ДНК) и гистоновые протеины остаются лабильными во время жизни и могут изменяться под влиянием факторов окружающей среды. Таким образом эпигенетические механизмы очерчивают привлекательную молекулярную гипотезу относительно моделирующего участия окружающей среды и ее вклада в развитие шизофрении.

В настоящей работе: 1. Представляется общий обзор шизофрении как заболевания; обсуждаются роль и участие среды в отношении генетической детерминанты (nature versus nurture) в процессе патологии. 2. Авторы сосредоточивают свое внимание на метилирование ДНК (обсуждаются доказательства роли этого процесса при шизофрении). 3. Рассматриваются посттрансляционные гистоновые модификации и их роль при шизофрении.

В будущем эти исследования могут привести к развитию эпигенетической терапии с обещающими результатами для облегчения симптоматики и когнитивного дефицита, ассоциированного с заболеванием.