GENETIC ASPECTS OF DIABETES AND COMORBID DEPRESSION

Doctoral Thesis

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I. Introduction

By the end of the 20th century diabetes mellitus, has become so widespread in the general population that it is now viewed as na endemic disease. The number of those over the age of 20 suffering from diabetes has been estimated at 171 million in 2000, and it's estimated to grow to about 366 million by 2030. In Hungary, there is no central register, which precisely records diabetic patients, but the number of known diabetic population is estimated to be about 5 - 5..5% of the total population. It is also noteworthy that the general experience of the screening tests is that for each known diabetic patient an unrecognized diabetic patient stands. Thus, in Hungary, about one million people are estimated to have diagnosed or undiagnosed diabetes.

In addition to being a widespread disease, diabetes mellitus is a so-called civilization disease. Diseases of civilization are called those illnesses, which increase with the development of the society, the pollution of the environment, and they are enhanced by the changes lifestyle in social relations. Diabetes was unknown previously in communities isolated from civilization, until the impact of the "white man" has not appeared on the environment. Today, as a result of the Western civilization mediated "welfare" the frequency of the type of diabetes 2 is large among us.

At the turn of the 20-21st century, depression is one the most important mental illness from the point of the public health as it affects 340 million people worldwide.

It is estimated that the disease is very likely to have a major significant burden on human society. As the World Health Organization (WHO) predicts, by 2020 depression will be the second most common cause of reduced working capacity among all diseases (after cardiovascular diseases).

According to the Hungarostudy, - a national representative survey that was carried out in 2002 in Hungary- , mild depressive symptoms are present in 27.3%, moderate or severe depression symptoms, requiring clinical treatment were found in 12.9% of respondents while 7,3% of the participants were found to be severe cases, demanding immediate treatment. Depression is not a passing change in mood, triggered by distressing events or overwork. Depression is a serious clinical symptom pattern, a serious psychological disorder that is associated with prolonged mental acts that can get worse by months or years passed by. Clinically depressed patients with severe cases may become incapable of executing the simplest things witch might lead them to try to put an end to their life in the feeling of his inability. Some depressed patients are able to maintain their previous lifestyle, but the disease significantly reduces their effectiveness, and deprives them from their gladness.

According to epidemiological investigations of recent years, the average prevalence of depression in the diabetic population is 20%.

Depression is about two-three times more common among diabetic patients than among the age and gender-matched healthy population. **A common feature of these two endemic diseases is, that both are in part caused by environmental factors (such as the social changes of modern life) and by a genetic predisposition in witch certain genetic variants are not properly adapted to the changed environment.**

In recent years many environmental factors and biological mechanisms have been identified as contributing factors to these diseases but the true etiology of these diseases can not be considered to be clarified up till now. Perhaps this might be the reason why, these illnesses have many treatment options, but still lack a definitive cure.

Recognition of genetic risk factors of these disorders, might lead to early screening options, and also could help understand so far unexplained pathogenetic factors that may reveal pharmacological target in the future that could help develop new, and more effective treatments.

II. Objectives

The main objective of the presented work is the examination of genetic risk factors of diabetes mellitus and comorbid depression, to better understand their underlying pathomechanism.

In the first part of the work case-control association studies were performed in diabetic patients and control subjects by examining genetic polymorphisms. Candidate gene polymorphisms previously not studied or examined in different (not Caucasian) subpopulations were chosen for investigation.

1. Our aim was to examine the SNP (rs2821557) polymorphism in the Kv1.3 (KCNA3) gene's promoter region, located at position -1645, for the following reasons: (1) previous examination of KCNA3 knock out mice indicated that this ion channel may play a role in obesity and the development of insulin resistance, (2) previously studied in a healthy population the gene's T-1645C polymorphism has been shown to be associated with insulin resistance and impaired glucose tolerance. The polymorphism has not been studied in diabetic patients.
2. Our aim was to perform a case-control analysis of the missense (Pro582Ser) SNP (rs11549465) located in the exon 12 of the HIF-1 protein α subunit gene (HIF-1α). Previously this SNP has been shown to be associated with T2DM in a Japanese population but has not been investigated in a Caucasian population.

In the second phase of the work, using self report questioners **the incidence of comorbid depression in diabetic patients was assesed**. The validated Hungarian translation of the Hospital Anxiety and Depression Scale (**HADS**) was chosen to assess depressive symptoms as it has been previously shown it be an useful tool in separateing the mild / moderate / high severity cases.

Two serotonergic (serononin transporter promoter polymorphism (5HTTLPR) and tryptophan hydroxylase 2 (TPH2 promoter SNP-703GT-je, SNP rs4570625) and **two non-serotonergic** (BDNF (brain-derived neurotrophic factor) Val66Met polymorphism, rs6265, and P2RX7 Gln460Arg the purinergic ion channel that causes amino acid change SNP, rs2230912) were **selected for futher genetic investigation as candidate polymorphisms**, which have previously been associated with major and/or occurrence of bipolar depression.

We set out to answer the following questionss:

(1) Is the genetic effect detectable in the diabetes **in relation to comorbid depression?**

(2) Is there a **"gene x environment" interaction between genetic factors associated with depression ("gene") and the patients' metabolic status (internal "environment")?**

III. Methods

Experimental individuals, sampling

In our study, 370 2TDM, 50 1TDM and data of 274 control subjects were evaluated. Diabetic patients were recruited randomly from the inpatient and outpatient services of the 2nd and 3rd Department of Internal Medicine at the Semmelweis University. The study was approved by the Local Ethics Committee (TUKEB). Every patient provided written informed consent for their participation.

Genotyping

The 5-HTTLPR short (s) and long (l) variants (14 and 16 repeats, 469 and 512 bp fragments) were determined after the amplification with multicapillary electrophoretic device. SNPs were genotyped using TaqMan probes, and real-time PCR. The method was validated with PCR-RFLP in each case.

Measuring mood questionnaire

The Hospital Anxiety and Depression Scale (HADS) self-report questionnaire was used to measure depressive symptoms.

Genetic association studies

In the case and control groups, the Hardy-Weinberg equilibrium existence was verified by χ^2-test. For statistical analysis the SPSS statistical software package was used.

IV. Results

1. **The -1645CT** polymorphism **of the Kv1.3 (KCNA3) gene** showed **no statistically significant difference between the control and patient groups, in either the allele or genotype frequencies.** As we know this is the only published study in the literature, which studies the KCNA3 T-1645C polymorphism in diabetic patients.

2. **In the case of the αHIF-1 gene our case-control analysis found significant relationship between the CC genotype and type 2 diabetes occurrence.** The rare T allele appears to be protective against the development of the disease. **Our results are confirm the preliminary Japanese study in a Caucasian population.**

3. We **demonstrated, that the HIF-1α polymorphism is** not only associated with type 2 but also with **type 1 diabetes. The** T allele was a protective in this case also. This result is not just another example of the common genetic risk factor of the 1TDM and 2TDM, but in principle it may be important in hypoxia-induced factor in understanding the role of diabetes. It is conceivable, that this rare gene variant has a protective effect in the pancreas during the development stage under hypoxic conditions by a more pronounced activation of target genes.

4. **Investigation of the non serotonergic genes of depression** we found a **significant genetic effect** b**etween the studied P2RX7**

polymorphism and the HADS questionnaire measured depression scores among diabetes patients. However, we did not receive a significant interaction between the genetic effect of and the metabolic status of patients. For the BDNF gene we did not find any significant genetic effects or interactions.

5. Among the **serotonergic candidate genes of depression in the the case of TPH2** a **significant genetic effect was found and in addition we received a significant interaction between the studied polymorphism and the metabolic status of patients and depression scores.** The presence of AT allele is only in patients with good carbohydrate metabolism reduced HADS mood questionnaire measured scores. We have not received similar effects in the case of 5HTTLPR polymorphism.

To our knowledge this is the first reported gene x environment interaction studies in the literature, tested between the emotional state, genetic markers and glycemic metabolism of diabetic patients.

V.Conclusions

Diabetes and depression are both public health concerns. Both are considered multifactorial diseases, caused in part by an accelerated social impacts and adverse environmental factors and in part by certain maladaptive genes incapable of adapting to this changed environment. In recent years, epidemiological studies also shed light on the fact that depression is twice as common in diabetic patients, than in the overall population. Many studies in recent years investigated and identified environmental factors and background biological mechanisms, predisposing to depression, and diabetes, however still the clear etiology of the two diseases and their comorbidity can not be considered to be clarified.

In this work suspected genetic risk factors of the two diseases, diabetes mellitus and comorbid depression were studied for better understanding of their pathomechanism.

In the first part case-control association studies were performed by examining genetic polymorphisms of diabetic patients and control subjects. The aim of our candidate gene investigations, were to evaluate polymorphism which have not been studied previously or have been studied only in another sub-populations so far.

For the SNP (rs2821557) located in the promoter region of the Kv1.3 (KCNA3) gene in the position 1645 there was no statistically significant difference between the control and patient groups, either in

the allele or genotype frequencies. To our knowledge, this is the only study reported in the literature that examines the frequency of the KCNA3 T-1645C polymorphism in diabetic patients.

For the SNP (rs11549465) located in exon 12 of the gene coding the HIF-1 protein α subunit (HIF-1α), our case-control analysis found a significant relationship between the CC genotype and type 2 diabetes. Consistently the rare T allele appears to be protective against the development of the disease. These results are the first to confirm the preliminary Japanese study in a Caucasian population.

We showed that the HIF-1 α gene polymorphism studied is not only associated with type 2, but with also with type 1 diabetes and that the presence of T allele had a significant protective effect. This result is not only another example of a common genetic risk factor of T1DM and the T2DM, but in principle it may be important in understanding the role of hypoxia-induced factor in diabetes. It is conceivable that the rare gene variant exerts it's protective effect during hypoxic conditions during the developmental stage of the pancreas by a more pronounced activation of target genes.

In the second phase of our work, the mood questionnaire aimed to assess the incidence of comorbid depression in our diabetic patient group.

For genetic experiments we selected 2 serotonergic (5HTTLPR and TPH2) and two non-serotonergic (and P2RX7 BDNF) candidate genes, which previously have been associated with major and / or the occurrence of bipolar depression. Investigation of the non-serotonerg

genes of depression showed the significant association between the P2RX7 polymorphism and the HADS questionnaire measured depression levels among patients with diabetes. However, we did not receive a significant interaction of the genetic effect and metabolic status of the patient. For the BDNF gene we did not find any significant genetic effects or interaction.

In case of TPH2, one of the studied serotonergic depression candidate genes, in addition to a significant genetic effect we also found a significant interaction between the polymorphism and the measured metabolic status of the patients in relation with the level of depression. The presence of the T allele, lowered mood questionnaire measured HADS scores only in patients with good carbohydrate metabolism. In case of the 5HTTLPR we have not received similar changes. To our knowledge, this is the first reported gene environment interaction studies in the literature, between the emotional state, genetic markers and glycemic status of people with diabetes.

VI. Publications

The thesis is based on the following publications:

English-language publications:

1. Nagy G, Ronai Z, Somogyi A, Sasvari-Szekely M, Rahman AO, Mate A, Varga T, Nemoda Z: P2RX7Gln460Arg polymorphismis associated with depression among diabetic patients. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32 (8), 1884-1888, 2008, IF: 2.638

2. Nagy G, Kovacs-Nagy R, Kereszturi E, Somogyi A, Szekely A, Nemeth N, Hosszufalusi N, Panczel P, Ronai Z, Sasvari-Szekely M. Association of hypoxia inducible factor-1 alpha gene polymorphism with both type 1 and type 2 diabetes in a Caucasian (Hungarian) sample. BMC Med Genet. 10(1):79, 2009. IF: 2.762

Hungarian-language publications:

1. Dr. Nagy Géza, Dr. Rosta Klára, Dr. Ruzicska Éva, Dr. Somogyi Anikó, Dr. Sasvári-Székely Mária: Újabb eredmények a 2-es típusú diabetes mellitus genetikai tényezőiről. Magyar Belorvosi Archivum 2007 62. 115-122.

2. Dr. Nagy Géza, Dr. Nagy Réka, Dr. Székely Anna, Dr. Sasvári-Székely Mária, Dr. Somogyi Anikó: A KCNA3 gén T1645C polimorfizmusának vizsgálata diabetesben. Magyar Belorvosi Archivum 2008; 61. 129-133.

Publications not closely related to the thesis:

English-language publications:

1. Anikó Somogyi, Klára Rosta, Péter Pusztai, Zsolt Tulassay Géza Nagy: Antioxidant measurements. Phisiological Measurements 2007 28 (2007) R41-R55. IF:1.47

Hungarian-language publications:

1. Somogyi A, Ruzicska E, Varga T, Rácz K, Nagy G.: Development of silent gastric carcinoid in a type 1 diabetic patient with primer hypothyreosis. Orv Hetil. 2007 Sep 2;148(35):1667-71 (Markusovszky Award)

2. Dr. Somogyi Anikó, dr. Nagy Géza: Zsíranyagcsere zavarok, diagnózis és terápia. Medicus Anonymus 2005/6 29-36.

3. Somogyi Anikó dr., Herold Magdolna, Kocsis Ibolya dr., Nagy Géza dr., Somfai Gábor dr., Studinger Péter dr.,: Az E-vitamin-pótlás hatása fiatal férfiak és nők lipoproteinjének vitamintartalmára. Orvosi Hetilap 2005 35. szám, 1813-1818.

4. Somogyi Anikó dr., Nagy Géza dr., Rosta Klára dr., Pusztai Péter dr.: Inzulinkezelés 2-es típusú diabetes mellitusban Granum 2006. 2. szám, 39-42.

5. Somogyi Anikó dr. Nagy Géza dr. Rosta Klára dr.:Az oxidatív stressz szerepe a diabétesz microvascularis szövődményeinek kialakulásában. Diabetologia Hungarica 2006. március 27-32.