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ORIGINAL ARTICLE

Associations between heredity, height, BMI, diabetes mellitus type 1 or 2 and gene variants in the insulin receptor *(INSR)* gene in patients with schizophrenia.

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Abstract

OBJECTIVES: Schizophrenia is a psychotic disorder with high heritability. There are also indications that an autoimmune-mediated process in the brain underlies development of schizophrenia, and that the insulin receptor A may constitute a main antigen target. Therefore, as the insulin receptor gene hitherto has been little studied in schizophrenia, this study was undertaken to investigate this gene in schizophrenia susceptibility.

MATERIALS AND METHODS: To identify gene variants of possible interest, the whole insulin receptor gene was first DNA-sequenced in all or subgroups of patients with schizophrenia and controls, using the Sanger method and the SOLiD technology. Then, association analyses of total 50 identified gene variants were carried out in the whole study population, consisting of 94 patients and 60 controls.

RESULTS: No significant differences in genotype- and allele frequencies for the 50 gene variants were found between all patients and controls. However, in subgroup analyses, rs2229431 and rs747721248 tended to associate with heredity for schizophrenia, rs2229431 associated with height, rs41505247 with body mass index, rs59765738 and rs57476618 with diabetes mellitus (DM) type 1 and/ or heredity for DM type 1, and rs2962, rs2352954, rs2352955 and rs2252673 with DM type 2 and/ or heredity for DM type 2 in patients.

CONCLUSIONS: In this study, we show associations between heredity, height, body mass index, DM type 1, or DM type 2 and gene variants in the insulin receptor gene in patients with schizophrenia. Taken together, these findings clearly point to that the insulin receptor gene is involved in schizophrenia susceptibility.

INTRODUCTION

The literature on the schizophrenia illness provides strong evidence for a role of genetic factors in its aetiology (Craddock *et al.* 2005). A variety of genes, each with small or moderate effect, have been suggested to be involved (Gottesman & Shields, 1967), and to date 287 genetic loci associated with schizophrenia have been reported (Allen *et al.* 2008; Bray & O'Donovan, 2019; Chen *et al.* 2015; Forero *et al.* 2016; Giegling *et al.* 2017; Kang *et al.* 2016, 2018; Lam *et al.* 2019; Li *et al.* 2017; Liu *et al.* 2021; Loureiro *et al.* 2019; Pardiñas *et al.* 2018; Ptacek *et al.* 2011; Rees *et al.* 2020; Ripke *et al.* 2014; Ruderfer *et al.* 2018; Rujescu, 2012; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2022; Schwab & Wildenauer, 2013; Sekar *et al.* 2016; Vacic *et al.* 2011; Wang *et al.* 2022; Yu *et al.* 2017). Of all these genetic loci reported, it is the gene region encompassing the major histocompatibility complex (MHC) on chromosome 6p22.1 playing an important role in the immune system that is the most significant and consistent, followed by genes involved in calcium ion import into cells, those involved in cell membrane depolarization during action potential, and those in synaptic transmission (Hall *et al.* 2020; Pardiñas *et al.* 2018; Ripke *et al.* 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2022; Sekar *et al.* 2016). However, a substantial proportion of the heritability for schizophrenia is still unknown; the current single nucleotide polymorphism (SNP)-based heritability, attributable to all measured SNPs, is estimated to be only 24% (Pardiñas *et al.* 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2022).

Since there also are clear indications that schizophrenia is a systemic disorder and not only a brain disease (Flyckt, 2001; Kirkpatrick *et al.* 2014; Moises *et al.* 2002), I and my colleagues sought for a common molecular basis for schizophrenia abnormalities in brain and body, and formulated a hypothesis (described more in detail in three previous studies: Melkersson & Persson, 2011, 2012; Melkersson *et al.* 2011) that impaired cellular signalling via the insulin receptor (INSR), and probably also via the insulin-like growth factor 1 receptor (IGF1R), may underlie known abnormalities associated with schizophrenia in both the central nervous system (CNS) and in peripheral organs.

Besides this support of clinical studies for our hypothesis, there is also growing evidence from in vitro studies in neuroblastoma- and astrocyte- cell lines and post-mortem brains of patients with schizophrenia that impaired cellular signalling via these two receptors may play a role in the pathogenesis of schizophrenia (Altar *et al.* 2008; Bernstein *et al.* 2009, 2017; Chu *et al.* 2009; Zhao *et al.* 2006). Moreover, one of our most recently-published studies points to that an autoimmune-mediated process in the CNS, and to some extent in peripheral organs, underlies the development of a core group of schizophrenia cases and that the INSR-A and IGF1R and their ligands insulin, connecting peptide (C-peptide) and insulin-like growth factor 1 (IGF1) may constitute antigen targets (Melkersson & Bensing, 2021).

The INSR is a heterotetrameric protein composed of two extracellular α-subunits which contain the ligand-binding site and two membrane-spanning β-subunits which possess intrinsic tyrosine kinase activity and are responsible for transducing the ligand-binding signal to the interior of the cell (Olefsky, 1990). The INSR is initially coded from the *INSR* gene as a single-chain polypeptide precursor, which undergoes post-translational cleavage into a separate α- and β-subunit, followed by export to the plasma membrane and formation of a mature INSR (Ebina *et al.* 1985; Ullrich *et al.* 1985). The INSR is expressed as two alternatively spliced isoforms, which differ by the absence (isoform A) or presence (isoform B) of a 12-amino-acid insert (coded from the 36-nucleotide-long exon 11 of the *INSR* gene) at the carboxy terminus of the α-subunits of the receptor (Seino & Bell, 1989; Seino *et al.* 1989). The regulation of the alternative splicing of the INSR is not fully known, but in vitro, two sequences in intron 10 of the *INSR* gene have been shown to modulate the alternative splicing: a 48 nucleotide sequence at the 5'-end that functions as a splicing enhancer leading to an increase in exon 11 inclusion; and an inhibitory 43 nucleotide sequence at the 3'-end upstream from the branch point sequence which favours the skipping of exon 11 (Kosaki *et al.* 1998). It has also been demonstrated in vitro that nucleotides within exon 11 itself and within the promoter region of the *INSR* gene may influence the alternative splicing (Kosaki *et al.* 1998; McKeon *et al.* 1990). Furthermore, the relative expression in cells of the two isoforms of the INSR is regulated both in a tissue-specific manner and developmentally (Seino & Bell, 1989). The INSR isoform A (INSR-A) is predominantly expressed in the CNS, peripheral nervous system, blood cells and spleen, while the INSR isoform B (INSR-B) is predominantly expressed in the liver; and in other kinds of cells both isoforms are expressed in various proportions (Moller *et al.* 1989; Mosthaf *et al.* 1990; Sesti *et al.* 1994). Preferential expression of INSR-A occurs also in fetal tissues and in many cancers, which correlates well with the observation that INSR-A is expressed in dedifferentiated cells (Denley *et al.* 2003; Frasca *et al.* 1999; Kosaki & Webster, 1993). Studies regarding functional differences between the two receptor isoforms have revealed different ligand-properties including an approximately two-fold higher affinity to insulin for INSR-A, compared with INSR-B (Mosthaf *et al.* 1990; Yamaguchi *et al.* 1991, 1993). Similarly, INSR-A, in contrast to INSR-B, binds insulin-like growth factor 2 (IGF2) with high affinity

Tab. 1. Sanger sequenced regions in the insulin receptor gene in patients with schizophrenia and control subjects in part I of the study

Sanger sequenced *INSR* gene regions	Gene positions[a]	Sequences[a]	Numbers of P and C (n/ n)
INSR gene regions possibly involved in the alternative splicing of the INSR[b]			
part of 5′ near gene, whole exon 1, part of intron 1	19:7294103 – 7293674; 19:7294092 – 7293663	TGTAGCTGGC.....GCCAGGACGG	86/ 60
part of intron 9, whole exon 10, part of intron 10	19:7152967 – 7152479; 19:7152956 – 7152468	GAGCCCCCCT.....TTACCTCTCC	86/ 60
part of intron 10, whole exon 11, part of intron 11	19:7150834 – 7150360; 19:7150823 – 7150349	GCTGAGTCAG…..CCAATTCAGA	86/ 60
part of intron 11, whole exon 12, part of intron 12	19:7143305 – 7142764; 19:7143294 – 7142753	ATCCTTCAGA…..CAAGGACAGA	86/ 60
Remaining 8 exons in the *INSR* gene coding the α-subunit of the INSR, together with parts of their surrounding introns			
part of intron 1, whole exon 2, part of intron 2	19:7267932 – 7267336; 19:7267921 – 7267325	GCTTTCTGTT…..GATACAGGGT	94/ 56
part of intron 2, whole exon 3	19:7184669 – 7184327; 19:7184658 – 7184316	TCTCTCTCTC…..ATTCCAGCAA	93/ 54
part of intron 3, whole exon 4, part of intron 4	19:7174787 – 7174456; 19:7174776 – 7174445	CCCCCCTCCC…..TGGCTCCCCC	94/ 55
part of intron 4, whole exon 5, part of intron 5	19:7172540 – 7172177; 19:7172529 – 7172166	TTTGCAGCCT…..TTTTAGGAGG	94/ 56
part of intron 5, whole exon 6, part of intron 6	19:7170876 – 7170465; 19:7170865 – 7170454	GGAGAGGATT…..GGTGGGTGGG	94/ 55
part of intron 6, whole exon 7, part of intron 7	19:7168295 – 7167760; 19:7168284 – 7167749	AAAACTGGCT…..GCAGACAGCT	94/ 55
part of intron 7, whole exon 8, part of intron 8	19:7166463 – 7166109; 19:7166452 – 7166098	GTGACGGTCT…..TGCTTTTAAT	94/ 56
part of intron 8, whole exon 9, part of intron 9	19:7163366 – 7163000; 19:7163355 – 7162989	ATCTCCTGAC…..GTTTGCACAC	94/ 55

Abbreviations: C=control subjects, INSR=insulin receptor, n=number, P=patients with schizophrenia
[a]From the Ensembl database, according to GRCh37.p13; GRCh38.p13 (http://www.ensembl.org)
[b]According to Kosaki *et al.* (1998) and McKeon *et al.* (1990)

close to that of insulin (Denley *et al.* 2004; Frasca *et al.* 1999). Moreover, studies in mouse fibroblasts have demonstrated that activation of INSR-A by insulin leads primarily to metabolic effects, whereas activation of INSR-A by IGF2 leads primarily to mitogenic effects, utilizing different intracellular signalling pathways (Frasca *et al.* 1999).

The gene for the INSR-A and -B, which is located on chromosome 19p13.3 - p13.2, has been little studied to date in schizophrenia. No association has been found between an SNP (rs2059806) in exon 8 of the *INSR* gene and schizophrenia (Hanis & Bertin, 1990; Kim *et al.* 2013), whereas associations have been reported between two SNPs (rs57476618 & rs2352954) in intron 2 and 10 of the *INSR* gene and diabetes mellitus (DM) and/ or heredity for DM in patients with schizophrenia (Melkersson, 2018). Two SNPs in non-coding regions of the insulin gene have also recently been shown to tend to associate with schizophrenia (Melkersson & Persson, 2022). Regarding the insulin receptor substrates (IRSs) 1-4, linking the INSR with intracellular pathways (Choi & Sung, 2000; Lavan *et al.* 1997; White, 1998; Xu *et al.* 1999), SNPs in the *IRSs 1-4* genes (located on chromosomes 2q36, 13q34, 7q22.1 and Xq22.3, respectively) have been investigated too in relation to schizophrenia (Gunnell *et al.* 2007; Kim *et al.* 2013; Melkersson, 2013; Melkersson & Persson, 2011, 2012; Melkersson *et al.* 2011). While no associations have been found between SNPs in the *IRS-1* gene and schizophrenia (Gunnell *et al.* 2007; Kim *et al.* 2013), an SNP in the *IRS-3* gene has been shown to be negatively associated with schizophrenia (Melkersson & Persson, 2012). Further, positive associations have been reported in patients with schizophrenia both between an *IRS-2* SNP and auditory hallucinations, and between *IRS-4* SNPs and family history or body mass index (BMI), as well as one case of a patient with schizophrenia and an *IRS-4* gene mutation (Kim *et al.* 2013; Melkersson, 2013; Melkersson & Persson, 2011; Melkersson *et al.* 2011).

From our previously-described hypothesis that impaired cellular signalling via the INSR-A, and probably also via the IGF1R, in brain and body may result in development of schizophrenia, in this study we chose to thoroughly investigate the whole *INSR* gene including its promoter region in schizophrenia.

Tab. 2. Data regarding 9 possible single nucleotide polymorphisms of interest in the insulin receptor gene in part II of the study

SNP numbering	SNP identification[a,b]	SNP position[a]	Polymorphism[c]	Gene location and known function
1X.	rs59765738	7207679; 7207668	A/**C**	intron 2
2X.	novel	7205068; 7205057	T/**C**	intron 2
3X.	rs57476618	7202999; 7202988	G/**T**	intron 2
4X.	novel	7167985; 7167974	T/**C**	exon 7
5X.	rs2352954	7152418; 7152407	A/**G**[d]	intron 10
6X.	novel	7150143; 7150132	G/**C**	intron 11
7X.	rs2229431	7141775; 7141764	G/**A**	exon 13, synonymous coding (asparagine)
8X.	rs112317501	7135987; 7135976	A/**G**[e]	intron 13
9X.	rs12610022	7135292; 7135281	A/**G**	intron 13

Abbreviation: SNP=single nucleotide polymorphism
[a]rs numbers and positions according to GRCh37.p13; GRCh38.p13 from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[b]No's 2X, 4X and 6X refer to potential novel SNPs not described in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[c]Polymorphism bases on the forward strand with the alternative base (=allele 2) written in bold text
[d]This SNP was located only 14 bases away from SNP rs2352955 (=SNP no 5Y), which was followed by a -1A, -2A or 3A deletion that is registered as rs375635473 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[e]The G-variant of this SNP was preceded by a 10A-, 11A- or 12A insertion that is registered as rs747721248 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and reported in SweGen (Ameur *et al.* 2017)

MATERIAL AND METHODS

Ethical approval

The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden.

Patients and control subjects

Consecutive out-patients from psychiatric polyclinics in the region of Stockholm, Sweden and with the diagnosis of schizophrenia according to DSM-5 criteria (American Psychiatric Association, 2013) were invited to participate in this study. In total 94 patients, 47 males and 47 females, gave their written informed consent to participate. The patients were structurally interviewed about mental and physical health in themselves and their relatives, and are described elsewhere in detail (Melkersson, 2009, 2018). In brief, all patients were unrelated Caucasian individuals. They were in full or partial remission regarding psychotic symptoms, and all were receiving long-term therapy with antipsychotics. Their mean (s.d.) age was 44 (9) years, and their duration of schizophrenia illness ranged from 0.5 to 42 years (mean (s.d.)=18 (9) years). Of the 94 patients, 37 had heredity for schizophrenia or related psychosis (i.e. they had one or more first-, second-, third- or fourth degree relatives, excluding siblings, with such a disorder). Control subjects were 60 unrelated Caucasian individuals (17 males and 43 females) from the Stockholm County or the nearby Uppsala County who gave written informed consent to participate in the study. The control subjects were also structurally interviewed about their own mental and physical health and also about that of their relatives. They were all healthy individuals with no heredity for psychotic disorder or DM type 1, type 2 or other types. Their mean (s.d.) age was 45 (11) years.

Collection and DNA-preparation of blood samples

Venous blood was taken in EDTA-containing tubes from all patients and control subjects and stored at -20°C until preparation of DNA. Genomic DNA was extracted from peripheral blood leukocytes by using a Genomic DNA Purification kit (Gentra Systems Inc., Minneapolis, MN, USA). The extracted DNA was frozen at -20°C until genotyped.

Sanger sequencing of DNA

DNA-sequencing of *INSR* gene regions of special interest (Table 1) was conducted in the whole study population by using the Sanger method. Genomic DNA was amplified by polymerase chain reaction (PCR), carried out in a Gene Amp® PCR System 2700 (Applied Biosystems, Foster City, CA, USA), followed by PCR product cleaning with Shrimp Alkaline Phosphatase and Exonuclease I (Fermentas International Inc., Burlington, Canada). Thereafter, the PCR fragments were sequenced in both directions, using BigDye® Terminator v3.1. sequencing kit (Applied Biosystems, Foster City, CA, USA), and analyzed by means of capillary electrophoresis in an ABI Prism 3730 Sequencer (Applied Biosystems, Foster City, CA, USA). Post-sequencing editing and alignment of sequences were made with the program Sequencher ™4.5 (Gene Codes Corporation, Ann Arbor, MI, USA).

SOLiD sequencing of DNA

Targeted DNA-sequencing of the whole *INSR* gene and its promoter region (Araki *et al.* 1987; Mamula *et al.* 1988; McKeon *et al.* 1990; Seino *et al.* 1989) was carried out in two subgroups of the study population: 1) 48 schizophrenia patients having either one or more first-, second-, third- or fourth-degree relatives with schizophrenia or related psychosis (n=37), or one or more siblings with such a disorder (n=11), and 2) 25 control subjects, by using the SOLiD technology (Applied Biosystems, Foster City, CA, USA). A standard SOLiD DNA fragment library was prepared for each sample and emulsion PCR was carried out according to the instructions from Applied Biosystems. Sequencing of DNA was then conducted with Applied Biosystems' SOLiD 4 system, using a 50 base pair read length on glass slides according to the protocol of the manufacturer. Thereafter, the reads were mapped to the reference sequence of the *INSR* gene including its promoter region (i.e. 19:7296034 to 19:7112219, according to GRCh37.p13, or 19:7296023 to 19:7112208, according to GRCh38.p13; http://www.ensembl.org). An *INSR* gene position was considered as a possible SNP of interest, where the following four criteria pertained: 1) the differences between patients and control subjects in mean reads and frequency of reads for the alternative base were above 30 and 6 % units, respectively, 2) at least three of the 48 patients had reads for the alternative base, 3) mean reads for the alternative base in the patients having reads was equal to or above 40 % and 4) the difference in frequency of reads for the alternative base between patients having one or more relatives, excluding siblings, with schizophrenia or related psychosis (n=37) and patients having only siblings with such a disorder (n=11) was equal to or above 11 % units. In total 9 such possible SNPs were identified (Table 2), which then were typed in the whole study population.

Typing of SNPs

The SNPs no's 1X, 2X, 3X, 7X & 9X (Table 2) and 1 & 3 (Table 3) were typed by Taqman® SNP Genotyping assays (Applied Biosystems/ Life Technologies, Foster City, CA, USA), the SNPs no's 4X, 5X, 5Y (including the deletion variation rs375635473), 6X & 8X (Table 2) were typed by Sanger sequencing, and the SNPs no's 40, 41 & 42 (Table 3) were typed by means of a pyrosequencing-based method described by Holmberg and colleagues (2005). In addition, the exact length of the insertion preceding SNP no. 8X (Table 2) was determined, using DNA Fragment Analysis by Capillary Electrophoresis (Applied Biosystems/ Life Technologies, Foster City, CA, USA).

Determination of height and BMI

Height and weight were measured, and BMI was calculated as weight divided by the square of height (kg/m^2) (Labhart, 1986).

Statistical methods

Categorial data were summarized using frequency counts and percentages. Haplotypes (Hs) were calculated as previously described (Stephens & Scheet, 2005; Stephens *et al.* 2001). Differences in genotype, allele and H frequencies between all patients and control subjects, or between subgroups of patients and control subjects, were examined with Chi-square test or Fisher's exact test. A p-value of less than 0.05 was considered statistically significant. The statistical analyses were conducted using the statistical programs SAS System 9.4 (SAS Institute Inc., Cary, NC, USA) and Statistica 13.5 (TIBCO Software Inc., USA).

RESULTS

Part I of the study

Four regions in the *INSR* gene possibly involved in the discrimination of INSR-A from INSR-B (Kosaki *et al.* 1998; McKeon *et al.* 1990), and the remaining 8 exons in the *INSR* gene coding the α-subunit of the INSR together with parts of their surrounding introns, were Sanger sequenced in all patients with schizophrenia and control subjects (Table 1), and compared to the corresponding reference sequences of the gene. Total 37 polymorphic-distributed SNPs were identified (Table 3: no's 2 & 4-39), of which one was a novel SNP (Table 3: no. 32) not previously described in the dbSNP, and therefore registered by us.

Additional five known SNPs of potential interest, located throughout the *INSR* gene and its promoter but outside the Sanger sequenced regions, were typed in all patients with schizophrenia and control subjects (Table 3: no's 1, 3 & 40-42). Of these five SNPs, four were polymorphic-distributed (Table 3: no's 1, 3 & 40-41) and one monomorphic-distributed (Table 3: no. 42) in our study population.

Genotype distributions and allele frequencies for the total 41 identified polymorphic-distributed *INSR* SNPs in patients with schizophrenia and control subjects, together with results of single association analyses, are given in Table 4. No significant differences in genotype distributions and allele frequencies for the 41 SNPs were found between patients with schizophrenia and control subjects (Table 4), or between subgroups of schizophrenia patients with (n=32-37) or without (n=48-55) heredity for schizophrenia or related psychosis (data not shown). However, between subgroups of schizophrenia patients with DM type 1 and/ or heredity for DM type 1, DM type 2 and/ or heredity for DM type 2, no DM and heredity for DM and control subjects, significant differences in genotype distributions and/ or allele frequencies were found for two of the 41 SNPs: rs2962 and rs2252673, in that the schizophrenia patients with DM type 2 and/ or heredity for DM type 2 differed in genotype distributions and/ or allele frequencies, compared with the schizophrenia patients with no DM

Tab. 3. Data regarding the single nucleotide polymorphisms studied in the insulin receptor gene in part I of the study

SNP numbering	SNP identification[a]	SNP position[a]	Polymorphism[b]	Gene location and known function
1.	rs2860189	7294402; 7294391	C/**T**	5′ near gene, promoter
2.	rs774495674	7293697; 7293686	C/**T**	intron 1
3.	rs3745544	7267939; 7267928	G/**A**	intron 1
4.	rs891087	7184518; 7184507	G/**A**[d]	exon 3, synonymous coding (aspartic acid)
5.	rs72549237	7174633; 7174622	C/**T**	exon 4, non-synonymous coding (valine → isoleucine)
6.	rs2229435	7174619; 7174608	A/**G**	exon 4, synonymous coding (serine)
7.	rs72549236	7174581; 7174570	C/**A**	intron 4
8.	rs13306458	7174573; 7174562	C/**T**	intron 4
9.	rs1469712102	7174529; 7174518	C/**T**	intron 4
10.	rs41412545	7172526; 7172515	C/**A**	intron 4
11.	rs8113777	7172209; 7172198	C/**T**	intron 5
12.	rs2860178	7170517; 7170506	A/**G**	intron 6
13.	rs7252268	7170505; 7170494	C/**A**	intron 6
14.	rs41505247	7168240; 7168229	C/**G**	intron 6
15.	rs2245143	7168213; 7168202	C/**G**/**T**	intron 6
16.	rs2860177	7167951; 7167940	T/**G**	intron 7
17.	rs41315976	7167867; 7167856	G/**A**	intron 7
18.	rs1366234	7167817; 7167806	C/**A**	intron 7
19.	rs2229429	7166388; 7166377	G/**A**[d]	exon 8, synonymous coding (aspartic acid)
20.	rs2059806	7166376; 7166365	C/**T**	exon 8, synonymous coding (alanine)
21.	rs3815902	7166138; 7166127	G/**A**	intron 8
22.	rs2059807	7166109; 7166098	A/**G**	intron 8
23.	rs2352953	7163326; 7163315	T/**C**	intron 8

and heredity for DM (Tables 3 & 5: no's 28 & 34). In addition, a significant association was found between SNP rs41505247 (Table 3: no. 14) and BMI of patients with schizophrenia [C/C-genotype (n=89) versus C/G- + G/G-genotypes (n=4), mean (s.d.): 29 ± 6 versus 36 ± 5, p=0.04 (corrected for gender)].

Part II of the study

Targeted whole *INSR* gene sequencing of DNA with the SOLiD technology was carried out in the two divided subgroups of the study population, and 9 more possible SNPs of interest were identified and typed in the whole study population (Table 2). Six of these 9 possible SNPs were polymorphic-distributed (Table 2: no's 1X, 3X, 5X, 7X, 8X & 9X), whereas three were monomorphic-distributed and could not be verified as SNPs (Table 2: no's 2X, 4X & 6X). Moreover, SNP no. 8X (Table 2: rs112317501) was preceded by an insertion variation that is registered as rs747721248 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and reported in SweGen (Ameur *et al.* 2017), and SNP no. 5X (Table 2: rs2352954) was located only 14 bases away from SNP rs2352955 (=SNP no. 5Y), which was followed by a -1A, -2A or 3A deletion variation that is registered as rs375635473 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP).

Genotype distributions and allele frequencies for all seven polymorphic-distributed SNPs (no. 5Y included) in the *INSR* gene in patients with schizophrenia and control subjects, together with results of single association analyses, are given in Table 6. No significant differences in genotype distributions and allele frequencies for the seven SNPs were found between patients with schizophrenia and control subjects (Table 6). However, between subgroups of schizophrenia patients with (n=37) or without (n=55) heredity for schizophrenia or related psychosis, tendencies towards significant differences in genotype distribution and allele frequency were found for SNP no. 7X (rs2229431) (p=0.08 and p=0.09, respectively), but not for the other six SNPs (data not shown). It was the G/G-genotype and G-allele of rs2229431 that tended to be more common in the schizophrenia patients with heredity than in those without. The SNP rs2229431 associated also significantly with height of the patients with schizophrenia [G/G-genotype (n=84) versus G/A- + A/A-genotypes

SNP numbering	SNP identification[a]	SNP position[a]	Polymorphism[b]	Gene location and known function
24.	rs2245648	7163230; 7163219	T/**C**	intron 8
25.	rs2245649	7163214; 7163203	T/**C**	intron 8
26.	rs2963	7163154; 7163143	G/**A**	exon 9, synonymous coding (leucine)
27.	rs2245655	7163140; 7163129	T/**G**	exon 9, synonymous coding (proline)
28.	rs2962	7163065; 7163054	G/**A**	exon 9, synonymous coding (phenylalanine)
29.	rs6413501	7152775; 7152764	C/**T**	exon 10, synonymous coding (threonine)
30.	rs6413502	7152717; 7152706	G/**T**	intron 10
31.	rs3745548	7152703; 7152692	C/**T**	intron 10
32.	SNP-C[c]	7152640; 7152629	C/**T**	intron 10
33.	rs1339060405	7150647; 7150636	T/**C**	intron 10
34.	rs2252673	7150418; 7150407	C/**G**	intron 11
35.	rs67009449	7143153; 7143142	A/-	intron 11
36.	rs749049187	7142951; 7142940	C/**A**	exon 12, synonymous coding (serine)
37.	rs2229434	7142921; 7142910	C/**T**	exon 12, synonymous coding (threonine)
38.	rs2229430	7142843; 7142832	C/**G**	exon 12, synonymous coding (alanine)
39.	rs13306451	7142824; 7142813	T/**C**	intron 12
40.	rs1799815	7125519; 7125508	G/**A**	exon 17, synonymous coding (tyrosine)
41.	rs1799816	7125518; 7125507	C/**T**	exon 17, non-synonymous coding (valine → methionine)
42.	rs13306449	7117134; 7117123	T/**C**	exon 22, non-synonymous coding (tyrosine → cysteine)

Abbreviation: SNP=single nucleotide polymorphism
[a]rs numbers and positions according to GRCh37.p13; GRCh.38.p13 from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[b]Polymorphism bases on the forward strand with the alternative allele (=allele 2 or 3) written in bold text
[c]SNP-C refers to a novel SNP not previously described in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and therefore is registered by us
[d]This SNP may also present with the alternative bases C or T, but none of these were detected in our study population

(n=9), mean (s.d.): 1.74 ± 0.10 versus 1.70 ± 0.13, p=0.03 (corrected for gender)], but not of the control subjects [p=0.69 (corrected for gender)]. Furthermore, between subgroups of schizophrenia patients with DM type 1 and/ or heredity for DM type 1, DM type 2 and/ or heredity for DM type 2, no DM and heredity for DM and control subjects, significant differences in genotype distributions and/ or allele frequencies were found for four of the seven SNPs: rs59765738, rs57476618, rs2352954 and rs2352955 (Tables 2 & 5: no's 1X, 3X, 5X & 5Y). For rs59765738 and rs57476618, it was the schizophrenia patients with DM type 1 and/ or heredity for DM type 1 who differed in genotype distributions and/ or allele frequencies, compared with the schizophrenia patients with DM type 2 and/ or heredity for DM type 2, the schizophrenia patients with no DM and heredity for DM and/ or the control subjects (Table 5: no's 1X & 3X), and for rs2352954 and rs2352955, it was the schizophrenia patients with DM type 2 and/ or heredity for DM type 2 who differed in genotype distributions and/ or allele frequencies, compared with the schizophrenia patients with no DM and heredity for DM (Table 5: no's 5X & 5Y).

Regarding the insertion variation rs747721248, preceding SNP no. 8X (Table 2), no significant differences in genotype distribution and allele frequency were found between patients with schizophrenia (n=94) and control subjects (n=60), or between subgroups of schizophrenia patients with DM type 1 and/ or heredity for DM type 1 (n=4), DM type 2 and/ or heredity for DM type 2 (n=42), no DM and heredity for DM (n=44) and control subjects (n=60) (data not shown). However, between subgroups of schizophrenia patients with (n=37) or without (n=55) heredity for schizophrenia or related psychosis, tendencies towards significant differences in genotype distribution and allele frequency were found (p=0.05 and p=0.06, respectively), in that this insertion tended to be less common in the schizophrenia patients with heredity than in those without (Figure 1).

Regarding the deletion variation rs375635473 near SNPs no's 5X & 5Y (Table 2), no significant differences in genotype distribution and allele frequency were found between patients with schizophrenia (n=94) and control subjects (n=58), or between subgroups of schizophrenia patients with (n=37) or without

Tab. 4. Genotype distributions and allele frequencies for the 41 single nucleotide polymorphisms in the insulin receptor gene in patients with schizophrenia compared with control subjects in part I of the study

SNP[a]	Polymorphism[b]	Numbers of P and C (n/ n)	Genotype distributions (%) P 1-1	P 1-2	P 2-2	C 1-1	C 1-2	C 2-2	*p-value*	Allele frequencies (%)[c] P	C	*p-value*
1.	C/**T**	94/ 59	69.15	30.85	0.00	74.58	25.42	0.00	0.58	84.57	87.29	0.62
2.	C/**T**	86/ 60	100.00	0.00	0.00	96.67	3.33	0.00	0.17	100.00	98.33	0.17
3.	G/**A**	94/ 60	98.94	0.00	1.06	100.00	0.00	0.00	1.00	98.94	100.00	0.52
4.	G/**A**	93/ 54	84.95	15.05	0.00	85.19	14.81	0.00	1.00	92.47	92.59	1.00
5.	C/**T**	94/ 55	98.94	1.06	0.00	100.00	0.00	0.00	1.00	99.47	100.00	1.00
6.	A/**G**	94/ 55	98.94	1.06	0.00	94.55	5.45	0.00	0.14	99.47	97.27	0.14
7.	C/**A**	94/ 55	98.94	1.06	0.00	98.18	1.82	0.00	1.00	99.47	99.09	1.00
8.	C/**T**	94/ 55	100.00	0.00	0.00	98.18	1.82	0.00	0.37	100.00	99.09	0.37
9.	C/**T**	94/ 55	98.94	1.06	0.00	100.00	0.00	0.00	1.00	99.47	100.00	1.00
10.	C/**A**	94/ 56	94.68	5.32	0.00	98.21	1.79	0.00	0.41	97.34	99.11	0.42
11.	C/**T**	94/ 56	72.34	26.60	1.06	76.79	21.43	1.79	0.73	85.64	87.50	0.73
12.	A/**G**	94/ 55	32.98	47.87	19.15	30.91	50.91	18.18	0.94	56.91	56.36	1.00
13.	C/**A**	94/ 55	67.02	31.91	1.06	70.91	29.09	0.00	0.82	82.98	85.45	0.63
14.	C/**G**	94/ 55	95.74	4.26	0.00	98.18	1.82	0.00	0.65	97.87	99.09	0.65
15.	C/**G**/**T**	94/ 55	**1-1** 47.87;	**1-2** 30.85;	**2-2** 6.38;	**1-3** 8.51;	**2-3** 6.38;	**1-1** 40.00;	**1-2** 41.82;	**2-2** 9.09;	**1-3** 7.27;	**2-3** 1.82 0.48	63.30	60.91	0.71
16.	T/**G**	94/ 55	55.32	38.30	6.38	47.27	43.64	9.09	0.60	74.47	69.09	0.35
17.	G/**A**	94/ 55	98.94	1.06	0.00	94.55	5.45	0.00	0.14	99.47	97.27	0.14
18.	C/**A**	94/ 55	55.32	38.30	6.38	50.91	40.00	9.09	0.78	74.47	70.91	0.50
19.	G/**A**	94/ 56	64.89	34.04	1.06	73.21	25.00	1.79	0.41	81.91	85.71	0.43
20.	C/**T**	94/ 55	55.32	38.30	6.38	49.09	43.64	7.27	0.76	74.47	70.91	0.50
21.	G/**A**	94/ 55	56.38	37.23	6.38	50.91	40.00	9.09	0.74	75.00	70.91	0.50

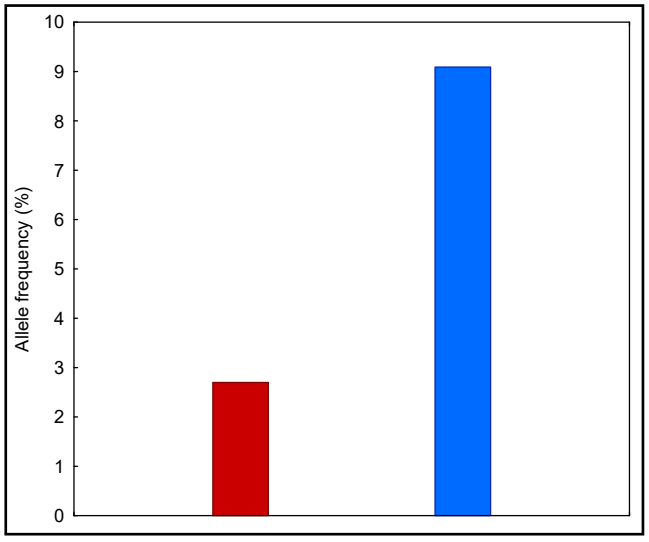

Fig. 1. Allele frequency of the 10A-, 11A- or 12A insertion variation rs747721248 in schizophrenia patients with (n=37; red bar) or without (n=55; blue bar) heredity for schizophrenia or related psychosis.

(n=55) heredity for schizophrenia or related psychosis, or between subgroups of schizophrenia patients with DM type 1 and/ or heredity for DM type 1 (n=4), DM type 2 and/ or heredity for DM type 2 (n=42), no DM and heredity for DM (n=44) and control subjects (n=58) (data not shown).

Parts I and II of the study

Estimated Hs, based on the SNPs in the *INSR* gene that were significantly associated with a) DM type 1 and/ or heredity for DM type 1 (HX), or b) DM type 2 and/ or heredity for DM type 2 (HY), and their frequencies in the whole study population are described in Table 7. No significant differences in frequencies of H-distributions and H-alleles were found for HX or HY between patients with schizophrenia (n=86-94) and control subjects (n=55) (data not shown). However, between subgroups of schizophrenia patients with or without heredity for schizophrenia or related psychosis, tendencies towards significant differences in frequencies of H-distributions and H-alleles were found for HX_4, and between subgroups of schizophrenia patients with

SNP[a]	Polymorphism[b]	Numbers of P and C (n/ n)	Genotype distributions (%) P 1-1	P vs C 1-2	P 2-2	C 1-1	C 1-2	C 2-2	*p-value*	Allele frequencies (%)[c] P	vs	C	*p-value*
22.	A/**G**	94/ 56	20.21	39.36	40.43	17.86	46.43	35.71	0.70	60.11		58.93	0.90
23.	T/**C**	94/ 55	87.23	12.77	0.00	90.91	9.09	0.00	0.60	93.62		95.45	0.61
24.	T/**C**	94/ 55	56.38	38.30	5.32	69.09	23.64	7.27	0.18	75.53		80.91	0.32
25.	T/**C**	94/ 55	87.23	12.77	0.00	90.91	9.09	0.00	0.60	93.62		95.45	0.61
26.	G/**A**	94/ 55	87.23	12.77	0.00	90.91	9.09	0.00	0.60	93.62		95.45	0.61
27.	T/**G**	94/ 55	87.23	12.77	0.00	90.91	9.09	0.00	0.60	93.62		95.45	0.61
28.	G/**A**	94/ 55	82.98	17.02	0.00	83.64	14.55	1.82	0.46	91.49		90.91	0.84
29.	C/**T**	86/ 60	98.84	1.16	0.00	98.33	1.67	0.00	1.00	99.42		99.17	1.00
30.	G/**T**	86/ 60	96.51	3.49	0.00	93.33	6.67	0.00	0.45	98.26		96.67	0.45
31.	C/**T**	86/ 60	98.84	1.16	0.00	96.67	1.67	1.67	0.51	99.42		97.50	0.31
32.	C/**T**	86/ 60	100.00	0.00	0.00	98.33	1.67	0.00	0.41	100.00		99.17	0.41
33.	T/**C**	86/ 60	100.00	0.00	0.00	98.33	1.67	0.00	0.41	100.00		99.17	0.41
34.	C/**G**	86/ 60	3.49	33.72	62.79	1.67	33.33	65.00	0.95	79.65		81.67	0.76
35.	A/-	86/ 60	98.84	1.16	0.00	96.67	3.33	0.00	0.57	99.42		98.33	0.57
36.	C/**A**	86/ 60	98.84	1.16	0.00	100.00	0.00	0.00	1.00	99.42		100.00	1.00
37.	C/**T**	86/ 60	97.67	2.33	0.00	96.67	3.33	0.00	1.00	98.84		98.33	1.00
38.	C/**G**	86/ 60	97.67	2.33	0.00	96.67	3.33	0.00	1.00	98.84		98.33	1.00
39.	T/**C**	86/ 60	97.67	2.33	0.00	96.67	3.33	0.00	1.00	98.84		98.33	1.00
40.	G/**A**	82/ 60	86.59	13.41	0.00	90.00	10.00	0.00	0.61	93.29		95.00	0.62
41.	C/**T**	82/ 60	98.78	1.22	0.00	100.00	0.00	0.00	1.00	99.39		100.00	1.00

Abbreviations: C=control subjects, n=number, P=patients with schizophrenia, SNP=single nucleotide polymorphism
[a]Same SNP numbering as in Table 3
[b]Polymorphism bases on the forward strand with the alternative allele (=allele 2 or 3) written in bold text
[c]Only highest allele frequency is shown

DM type 1 and/ or heredity for DM type 1, DM type 2 and/ or heredity for DM type 2, no DM and heredity for DM and control subjects, significant differences in frequencies of H-distributions and H-alleles were found for HX_3 regarding DM type 1, and for HY_1-2,10-16 regarding DM type 2 (Table 8).

DISCUSSION

In this study, based on DNA-sequencing of the whole *INSR* gene and its promoter, followed by association analyses of total 50 identified gene variants (i.e. 48 SNPs and two insertion/ deletion variations) of possible interest, no differences in genotype distributions and allele frequencies were found between all patients with schizophrenia and control subjects. Nevertheless, subgroup analyses showed that the G/G-genotype and G-allele of the SNP rs2229431 in exon 13 and the no (10A-, 11A- or 12A) insertion of the insertion variation rs747721248 in intron 13 (i.e. in both cases, the reference variants) tended to be more common in schizophrenia patients with heredity for schizophrenia or related psychosis than in those without heredity, indicating that these two *INSR* gene variants may distinguish the group of schizophrenia patients with heredity from that without (Bigdeli *et al.* 2015). In addition, subgroup analyses showed association between the SNP rs2229431 and height in patients with schizophrenia. The involvement of the *INSR* gene in schizophrenia susceptibility has been little studied until now, but in accord with this study, one earlier study has reported no association between the *INSR* SNP rs2059806 in exon 8 (SNP no. 20 in Tables 3 & 4 in this study) and schizophrenia (Kim *et al.* 2013). Of interest in this context is also that it has been reported earlier that patients with a diagnosis of schizoaffective disorder more often carry the A-allele of the *INSR* SNP rs2229431, than patients diagnosed with schizophrenia (Melkersson, 2018), and that serotonin receptor 2A gene polymorphisms associate both with heredity and height in patients with schizophrenia (Melkersson & Hulting, 2009).

Regarding DM type 1 and schizophrenia, subgroup analyses showed differences in genotype distributions and/ or allele frequencies between schizophrenia

Tab. 5. Genotype distributions and allele frequencies for single nucleotide polymorphisms in the insulin receptor gene in schizophrenia patients with diabetes mellitus (type 1 or 2) and/ or heredity for diabetes mellitus (type 1 or 2), schizophrenia patients with no diabetes mellitus and heredity for diabetes mellitus and control subjects in parts I and II of the study

SNP[a]	Polymorphism[b]	Number of	Overall comparison	Genotype distributions (%)							Allele frequencies (%)[c]		
		P-DM2, P-DM1, P-noDM and C (n/ n/ n/ n)	*p*-value[d] (genotype/ allele)	P-DM2			P-DM1 P-noDM C			*p*-value[d]	P-DM2	P-DM1 P-noDM C	*p*-value[d]
				1-1	1-2	2-2	1-1	1-2	2-2				
28.	G/**A**	42/ 4/ 44/ 55	0.07/ 0.07	95.24	4.76	0.00	100.00	0.00	0.00	1.00	97.62	100.00	1.00
							75.00	25.00	0.00	**0.01**		87.50	**0.01**
							83.64	14.55	1.82	0.18		90.91	0.05
5X.	A/**G**	42/ 4/ 44/ 58	0.12/ **0.04**	0.00	19.05	80.95	0.00	25.00	75.00	1.00	90.48	87.50	0.58
							6.82	38.64	54.55	**0.01**		73.86	**<0.01**
							1.72	34.48	63.79	0.12		81.03	0.07
5Y.	A/**G**	42/ 4/ 44/ 58	0.16/ 0.05	0.00	19.05	80.95	0.00	25.00	75.00	1.00	90.48	87.50	0.58
							6.82	36.36	56.82	**0.02**		75.00	**0.01**
							1.72	34.48	63.79	0.12		81.03	0.07
34.	C/**G**	37/ 4/ 42/ 60	0.37/ 0.16	0.00	27.03	72.97	0.00	25.00	75.00	1.00	86.49	87.50	1.00
							7.14	40.48	52.38	0.08		72.62	**0.03**
							1.67	33.33	65.00	0.70		81.67	0.38
		P-DM1, P-DM2, P-noDM and C (n/ n/ n/ n)		**P-DM1**			**P-DM2 P-noDM C**			***p*-value[d]**	**P-DM1**	**P-DM2 P-noDM C**	***p*-value[d]**
				1-1	**1-2**	**2-2**	**1-1**	**1-2**	**2-2**				
1X.	A/**C**	4/ 42/ 44/ 55	0.30/ 0.07	75.00	25.00	0.00	33.33	47.62	19.05	0.37	87.50	57.14	0.14
							22.73	43.18	34.09	0.09		44.32 (A)	**0.03**
							25.45	52.73	21.82	0.11		51.82	0.07
3X.	G/**T**	4/ 42/ 44/ 55	**0.01/ <0.01**	75.00	25.00	0.00	14.29	47.62	38.10	**0.02**	87.50	38.10 (G)	**0.01**
							4.55	38.64	56.82	**<0.01**		23.86 (G)	**<0.01**
							9.09	32.73	58.18	**<0.01**		25.45 (G)	**<0.01**

Abbreviations: C=control subjects, n=number, P-DM1=schizophrenia patients with diabetes mellitus type 1 and/ or heredity for diabetes mellitus type 1, P-DM2=schizophrenia patients with diabetes mellitus type 2 and/ or heredity for diabetes mellitus type 2, P-noDM=schizophrenia patients with no diabetes mellitus and heredity for diabetes mellitus, SNP=single nucleotide polymorphism

[a]Same SNP numbering as in Tables 2 and 3

[b]Polymorphism bases on the forward strand with the alternative allele (=allele 2) written in bold text

[c]Only highest allele frequency is shown, if nothing else is described

[d]Significant *p*-values ($p<0.05$) are written in bold text, and tendencies towards significant *p*-values ($0.05 \geq p \leq 0.09$) are underlined

patients with DM type 1 and/ or heredity for DM type 1 and schizophrenia patients with DM type 2 and/ or heredity for DM type 2, schizophrenia patients with no DM and heredity for DM, or control subjects for the SNPs rs59765738 and rs57476618 in intron 2, as well as for the H HX_3, consisting of the A- and G-alleles of these two SNPs. It was the A- and G-alleles (i.e. in both cases, the reference bases) of these two SNPs, separately or combined, that were more common in schizophrenia patients with DM type 1 and/ or heredity for DM type 1 than in schizophrenia patients with DM type 2 and/ or heredity for DM type 2, schizophrenia patients with no DM and heredity for DM, or control subjects. Interestingly, another variant of this H, HX_4, consisting of the C- and G-alleles of these two SNPs, tended to be more common in schizophrenia patients with heredity for schizophrenia or related psychosis than in those without heredity, indicating a possible link (i.e. the G-allele of rs57476618 in intron 2) between DM type 1 and schizophrenia. A possible link between these two diseases has also earlier been pointed out in register studies (Eaton *et al.* 2006; Finney, 1989; Juvonen *et al.* 2007; Melkersson, 2020; Melkersson & Wernroth, 2019) and in clinical studies on antibody reactivity and autoimmunity in schizophrenia (Melkersson & Bensing, 2021; Melkersson & Persson, 2022). In this context, it is also to be noted that the subgroup of schizophrenia patients with DM type 1 and/ or heredity for DM type 1 in this study consisted solely of schizophrenia patients with heredity for DM type 1 and not with DM type 1 itself (Melkersson & Bensing, 2021; Melkersson & Persson, 2022). To compare, there are no earlier studies published, reporting associations between *INSR* SNPs or Hs and specifically DM type 1 and/ or heredity for DM type 1 in patients with schizophrenia (Barrett *et al.* 2009; Melkersson, 2018; Pociot & Lernmark, 2016).

Regarding DM type 2 and schizophrenia, subgroup analyses showed differences in genotype distributions and/ or allele frequencies between schizophrenia patients with DM type 2 and/ or heredity for DM type

Tab. 6. Genotype distributions and allele frequencies for the seven single nucleotide polymorphisms in the insulin receptor gene in patients with schizophrenia, compared with control subjects in part II of the study

SNP[a]	Polymorphism[b]	Numbers of P and C (n/ n)	Genotype distributions (%) P 1-1	P vs C 1-2	P vs C 2-2	C 1-1	C 1-2	C 2-2	*p*-value	Allele frequencies (%)[c] P	P vs C	C	*p*-value
1X.	A/**C**	94/ 55	28.72	42.55	28.72	25.45	52.73	21.82	0.46	50.00		51.82	0.76
3X.	G/**T**	94/ 55	11.70	40.43	47.87	9.09	32.73	58.18	0.48	68.09		74.55	0.24
5X.	A/**G**	94/ 58	3.19	29.79	67.02	1.72	34.48	63.79	0.71	81.91		81.03	0.85
5Y.	A/**G**[d]	94/ 58	3.19	28.72	68.09	1.72	34.48	63.79	0.71	82.45		81.03	0.76
7X.	G/**A**	94/ 56	90.43	9.57	0.00	94.64	5.36	0.00	0.54	95.21		97.32	0.55
8X.	A/**G**[e]	94/ 60	87.23	12.77	0.00	90.00	8.33	1.67	0.41	93.62		94.17	0.85
9X.	A/**G**	94/ 60	89.36	10.64	0.00	91.67	6.67	1.67	0.37	94.68		95.00	0.90

Abbreviations: C=control subjects, n=number, P=patients with schizophrenia, SNP=single nucleotide polymorphism
[a]Same SNP numbering as in Table 2
[b]Polymorphism bases on the forward strand with the alternative base (=allele 2) written in bold text
[c]Only highest allele frequency is shown
[d]This SNP was followed by a -1A, -2A or 3A deletion that is registered as rs375635473 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[e]The G-variant of this SNP was preceded by a 10A-, 11A- or 12A insertion that is registered as rs747721248 in the dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and reported in SweGen (Ameur *et al.* 2017)

2 and schizophrenia patients with no DM and heredity for DM for the SNPs rs2962 in exon 9, rs2352954 and rs2352955 in intron 10, and rs2252673 in intron 11, as well as for the H HY_1-2, 10-16 which was constructed from these four SNPs. In addition, subgroup analyses showed association between the SNP rs41505247 in intron 6 and BMI in patients with schizophrenia. In comparison, there are no earlier studies published on SNPs or Hs in the *INSR* gene in relation to specifically DM type 2 or BMI in patients with schizophrenia (Melkersson, 2018), but interestingly, the *INSR* SNP rs2252673 in intron 11 has been reported to be associated with the polycystic ovary syndrome in which insulin resistance is included (Du *et al.* 2014; Feng *et al.* 2015). Association between *IRS-4* gene polymorphisms and BMI in patients with schizophrenia has been shown earlier as well (Melkersson & Persson, 2011). In otherwise healthy individuals, the *INSR* SNPs rs2059806 in exon 8, rs1799816 in exon 17 and rs3745551 in exon 22 have been reported to be associated with increased risk, and the *INSR* SNP rs1799817 in exon 17 to be associated with decreased risk, for DM type 2 and insulin resistance (Bodhini *et al.* 2012; Hart *et al.* 1996; Malodobra *et al.* 2011; Quederni *et al.* 2009; Sokhi *et al.* 2016; Wang *et al.* 2012), but in the patients with schizophrenia in this study, no associations were found regarding these four SNPs (i.e. no's 20 & 41 in Tables 3 & 4, and two not detected). Among risk genes for DM type 2 in otherwise healthy individuals other than the *INSR* gene, the *IGF2BP2* gene (coding the binding protein 2 to IGF2, which is one of the ligands to INSR-A; Denley *et al.* 2004; Frasca *et al.* 1999) has been suggested to play a role also in susceptibility to schizophrenia, whereas the *BCL11A*-, *UBE2E2*-, *SLC30A8*-, *HMG20A*- and *HNF4A* genes have been reported not to do that (Kajio *et al.* 2014; Zhang *et al.* 2012, 2013).

Tab. 7. Estimated haplotypes, based on the single nucleotide polymorphisms in the insulin receptor gene that were significantly associated with a) diabetes mellitus type 1 and/ or heredity for diabetes mellitus type 1 (HX), or b) diabetes mellitus type 2 and/ or heredity for diabetes mellitus type 2 (HY), and their frequencies in the whole study population[a]

Haplotypes	Frequency (%)
HX_1 [CT][b]	49.33
HX_2 [AT][b]	32.89
HX_3 [AG][b]	29.53
HX_4 [CG][b]	11.74
HY_1 [GGGG][c]	77.30
HY_2 [GAAC][c]	17.38
HY_3 [GGGC][c]	16.31
HY_4 [GAGG][c]	15.60
HY_5 [GGAC][c]	15.25
HY_6 [GAGC][c]	15.25
HY_7 [GAAG][c]	15.25
HY_8 [GGAG][c]	15.25
HY_9 [AGGG][c]	7.80
HY_10 [AAAC][c]	5.32
HY_11 [AGAC][c]	4.61
HY_12 [AAGC][c]	4.61
HY_13 [AGGC][c]	4.61
HY_14 [AAAG][c]	4.61
HY_15 [AGAG][c]	4.61
HY_16 [AAGG][c]	4.61

Abbreviation: H=haplotype
[a]n=141-152
[b]Alleles are numbered 1X and 3X as in Table 2
[c]Alleles are numbered 28, 5X, 5Y and 34 as in Tables 2 and 3

Tab. 8. Frequencies of estimated haplotypes based on the single nucleotide polymorphisms in the insulin receptor gene that were significantly associated with diabetes mellitus type 1 and/ or heredity for diabetes mellitus type 1 (HX), or diabetes mellitus type 2 and/ or heredity for diabetes mellitus type 2 (HY)

Haplotypes[a]	Number of	Overall comparison	H-distribution (%)							H-allele (%)		
	P-Her, P-noHer and C (n/ n/ n)	*p*-value[b] (H-distribution/ H-allele)	P-Her -/-	-/H	H/H	P-noHer C -/-	-/H	H/H	*p*-value[b]	P-Her H	P-noHer C H	*p*-value[b]
HX_4 [CG]	37/ 55/ 55	0.06/ 0.09	83.78	16.22	0.00	65.45	34.55	0.00	0.05	8.11	17.27	0.08
						81.82	18.18	0.00	0.81		9.09	0.82
	P-DM1, P-DM2, P-noDM and C (n/ n/ n/ n)		**P-DM1 -/-**	**-/H**	**H/H**	**P-DM2 P-noDM C -/-**	**-/H**	**H/H**	***p*-value[b]**	**P-DM1 H**	**P-DM2 P-noDM C H**	***p*-value[b]**
HX_3 [AG]	4/ 42/ 44/ 55	**0.01/<0.01**	0.00	25.00	75.00	38.10	47.62	14.29	**0.02**	87.50	38.10	**0.01**
						56.82	38.64	4.55	**<0.01**		23.86	**<0.01**
						58.18	32.73	9.09	**<0.01**		25.45	**<0.01**
	P-DM2, P-DM1, P-noDM and C (n/ n/ n/ n)		**P-DM2 -/-**	**-/H**	**H/H**	**P-DM1 P-noDM C -/-**	**-/H**	**H/H**	***p*-value[b]**	**P-DM2 H**	**P-DM1 P-noDM C H**	***p*-value[b]**
HY_1 [GGGG]	37/ 4/ 42/ 55	0.29/0.10	0.00	29.73	70.27	0.00	25.00	75.00	1.00	85.14	87.50	1.00
						7.14	47.62	45.62	**0.03**		69.05	**0.02**
						3.64	36.36	60.00	0.47		78.18	0.24
HY_2 [GAAC]	37/ 4/ 42/ 55	0.33/0.18	78.38	21.62	0.00	75.00	25.00	0.00	1.00	10.81	12.50	1.00
						54.76	42.86	2.38	**0.04**		23.81	**0.03**
						67.27	30.91	1.82	0.46		17.27	0.22
HY_10 [AAAC]	37/ 4/ 42/ 55	**0.01/0.02**	100.00	0.00	0.00	100.00	0.00	0.00	---	0.00	0.00	---
						78.57	21.43	0.00	**<0.01**		10.71	**<0.01**
						90.91	9.09	0.00	0.08		4.55	0.08
HY_11-16 [AGAC] [AAGC] [AGGC] [AAAG] [AGAG] [AAGG]	37/ 4/ 42/ 55	0.05/**0.02**	100.00	0.00	0.00	100.00	0.00	0.00	---	0.00	0.00	---
						83.33	16.67	0.00	**0.01**		8.33	**0.01**
						90.91	9.09	0.00	0.08		4.55	0.08

Abbreviations: C=control subjects, H=haplotype, n=number, P-DM1=schizophrenia patients with diabetes mellitus type 1 and/ or heredity for diabetes mellitus type 1, P-DM2=schizophrenia patients with diabetes mellitus type 2 and/ or heredity for diabetes mellitus type 2, P-noDM=schizophrenia patients with no diabetes mellitus and heredity for diabetes mellitus, P-Her=schizophrenia patients with heredity for schizophrenia or related psychosis, P-noHer=schizophrenia patients with no heredity for schizophrenia or related psychosis

[a]Same H numbering as in Table 7

[b]Significant *p*-values ($p<0.05$) are written in bold text, and tendencies towards significant *p*-values ($0.05 \geq p \leq 0.09$) are underlined

Insulin receptor-A is present in both the CNS and peripheral organs, while INSR-B is present mainly in peripheral organs (Moller *et al.* 1989; Mosthaf *et al.* 1990; Sara *et al.* 1982; Schulingkamp *et al.* 2000; Sesti *et al.* 1994), leaving INSR-A as being the INSR isoform of main interest in schizophrenia. Insulin as well is present in the CNS (Baskin *et al.* 1987), where it is suggested to have neuromodulatory functions and also to be of relevance to various aspects of the pathophysiology of schizophrenia, including the regulation of dopamine levels (Agarwal *et al.* 2020). In addition, insulin in vitro has been demonstrated to both inhibit [^{3}H] norepinephrine uptake and stimulate [^{3}H] serotonin uptake in neuronal cell cultures (Boyd *et al.* 1985; Wozniak *et al.* 1993). What role the 9 schizophrenia-associated *INSR* gene variants found in this study play is not fully known, but they are either synonymous coding for phenylalanine (rs2962 in exon 9) and asparagine (rs2229431 in exon 13) respectively, or located in introns which may have regulatory functions on the coding (Eddy, 2001) (i.e. rs59765738 & rs57476618 in intron 2 may regulate the coding of exon 2, rs41505247 in intron 6 may regulate the coding of exon 6, rs2352954 & rs2352955 in intron 10 may regulate the coding of exon 10, rs2252673 in intron 11 may regulate the coding of exon 11, and rs747721248 in intron 13 may regulate the coding of exon 13). It is also interesting that none of the 9 genetic variants were located in the gene regions known to possibly be directly involved in the alternative splicing of the INSR (Kosaki *et al.* 1998; McKeon *et al.* 1990).

A major strength of this study includes the fact that the whole *INSR* gene and its promoter were DNA-sequenced, which gives satisfactory information about the coding (i.e. the exons) as well as non-coding/ regulatory (i.e. the introns and promoter; Eddy, 2001;

McKeon *et al.* 1990; McKeon & Pham, 1991) regions of this gene in patients and control subjects. A further strength of the study includes its comprising of both a diagnostically homogeneous patient group consisting solely of patients diagnosed with schizophrenia, and a group of healthy control subjects with no heredity for either psychotic disorder, or DM type 1, type 2 or other types, allowing specific investigations of the involvement of the *INSR* gene in schizophrenia susceptibility. The limitation of the study, on the other hand, includes the comprising of a study population not large enough to certainly achieve sufficient statistical power in some of the subgroup analyses.

In conclusion, we show in this study for the first time associations in patients with schizophrenia between gene variants in the *INSR* gene and heredity for schizophrenia or related psychosis (rs2229431 in exon 13 and rs747721248 in intron 13), height (rs2229431 in exon 13), BMI (rs41505247 in intron 6), DM type 1 and/ or heredity for DM type 1 (rs59765738 & rs57476618 in intron 2), or DM type 2 and/ or heredity for DM type 2 (rs2962 in exon 9, rs2352954 & rs2352955 in intron 10, and rs2252673 in intron 11), as well as a linked-association between, on one hand, HX_3 (consisting of the A- and G-alleles of rs59765738 and rs57476618 in intron 2) and DM type 1 and/ or heredity for DM type 1, and on the other hand, HX_4 (consisting of the C- and G-alleles of the same two SNPs) and heredity for schizophrenia or related psychosis. These findings are in line with our previously-described hypothesis that impaired cellular signalling via the INSR-A, and probably also via the IGF1R, may underlie known abnormalities in the CNS and peripheral organs in schizophrenia (Melkersson & Persson, 2011, 2012; Melkersson *et al.* 2011), and also with one of our most recently-published studies, pointing to that an autoimmune-mediated process in the CNS, and to some extent in peripheral organs, underlies the development of a core group of schizophrenia cases and that the INSR-A and IGF1R and their ligands insulin, C-peptide and IGF1 may constitute antigen targets (Melkersson & Bensing, 2021). Further, these findings are supported by several studies by others, reporting INSR deficits in post-mortem brains, insulin-signalling abnormalities, altered IGF2 signalling, and association between an *IRS-2* SNP and auditory hallucinations in patients with schizophrenia (Kapogiannis *et al.* 2019; Kim *et al.* 2013; van Beveren *et al.* 2014; Wu *et al.* 2013; Yang *et al.* 2020; Zhao *et al.* 2006). Taken together, our findings in this study clearly point to that the *INSR* gene is involved in schizophrenia susceptibility.

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