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ORIGINAL ARTICLE

Sequencing of the insulin receptor (*INSR*) gene reveals association between gene variants in exon and intron 13 and schizoaffective disorder

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Abstract

OBJECTIVES: From the hypothesis that an impaired insulin and insulin-like growth factor cellular signalling in brain and body might result in the development of schizophrenia or related psychoses, I in this study chose to thoroughly investigate the insulin receptor gene in patients with schizophrenia or schizoaffective disorder and controls.

METHODS: For identification of single nucleotide polymorphisms (SNPs) of interest in the insulin receptor gene, targeted whole gene sequencing of DNA using the SOLiD technology was first carried out in two subgroups of the study population: 1) 49 schizophrenia or schizoaffective disorder patients with heredity for schizophrenia or related psychoses, and 2) 25 controls. Ten possible SNPs of interest were identified and these were then typed by standard methods in the whole study population consisting of 105 patients with schizophrenia or schizoaffective disorder and 60 controls.

RESULTS: In comparisons between schizophrenia patients, schizoaffective disorder patients and controls, overall significant differences were found in genotype distribution and allele frequency for SNP rs2229431 in exon 13, and tendencies towards overall significant differences were found for SNP rs12610022 in intron 13, but not for the other 8 possible SNPs investigated. It was the patients with schizoaffective disorder who differed in genotype distributions and allele frequencies compared to both the patients with schizophrenia and the controls, whereas between the patients with schizophrenia and the controls, no significant differences were found.

CONCLUSION: The results of this study show that two gene variants in exon and intron 13 of the insulin receptor gene confer risk specifically for schizoaffective disorder.

INTRODUCTION

The literature on schizophrenia and the related schizoaffective disorder provides strong evidence for a role of genetic factors in their aetiologies (Bertelsen & Gottesman, 1995; Craddock *et al.* 2005). A variety of genes, each with small or moderate effect, have been suggested to be involved in schizophrenia (Gottesman & Shields, 1967), and to date about 145 such genetic loci have been reported (Allen *et al.* 2008; Chen *et al.* 2015; Forero *et al.* 2016; Giegling *et al.* 2017; Kang *et al.* 2016, 2018; Li *et al.* 2017; Pardiňas *et al.* 2018; Ptacek *et al.* 2011; Ripke *et al.* 2014; Rujescu, 2012; Schwab & Wildenauer, 2013; Sekar *et al.* 2016; Vacic *et al.* 2011). For schizoaffective disorder, where in general there is a genetic overlap with schizophrenia and/ or affective disorder in transmission (Abrams, 1984; Bertelsen & Gottesman, 1995; Cardno & Owen, 2014), a number of genes with small or moderate effect that are shared in common with schizophrenia and/ or affective disorder have been identified. Association to single nucleotide polymorphisms (SNPs) in the Disrupted in Schizophrenia 1 (*DISC1*) gene on chromosome 1q42 has been found for both schizoaffective disorder, schizophrenia and bipolar disorder (Hodgkinson *et al.* 2004), while to SNPs in the $GABA_A$ receptor genes association has been found solely for schizoaffective disorder bipolar type, but not for schizoaffective disorder non-bipolar type, bipolar disorder or schizophrenia (Breuer *et al.* 2011; Craddock *et al.* 2010; Green *et al.* 2010); and to the SNP rs7341475 in the reelin (*RELN*) gene on chromosome 7q22 association has been found for schizoaffective disorder and schizophrenia in women, but not in men, and not for bipolar disorder in either men or women (Pisanté *et al.* 2009; Shifman *et al.* 2008). Patients with schizoaffective disorder or affective disorders have also been reported to be more likely to carry two copies of the most common brain-derived neurotrophic factor (*BDNF*) haplotype, when compared with patients with schizophrenia or healthy controls (Lencz *et al.* 2009). Furthermore, three patients with schizoaffective disorder have been found to have different point mutations in the calreticulin (*CALR*) gene on chromosome 19p13.3-p13.2 (Aghajani *et al.* 2006; Nunes *et al.* 2008; Olad Nabi *et al.* 2009) and 7 other patients of a five-generation family, diagnosed with schizophrenia or schizoaffective disorder (bipolar type), have been found to carry a deletion at the chromosome 9p24.2 locus containing the *SLC1A1* glutamate transporter gene (Myles-Worsley *et al.* 2013). However, the main genetic factor(s) associated with schizophrenia and that (those) distinguishing schizoaffective disorder from schizophrenia still remain(s) to be found.

Since there 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 found an interesting 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/ or the insulin-like growth factor 1 receptor (IGF1R) might underlie known abnormalities associated with schizophrenia in both the central nervous system (CNS) and in peripheral organ systems.

The INSR is present in both the CNS and peripheral organs in humans (McCowen & Smith, 2005; Rui & White, 2004; Sara *et al.* 1982). Interestingly, a genome-wide linkage scan in schizoaffective disorder has shown suggestive evidence for linkage at chromosome 19p13.2 (Hamshere *et al.* 2005), i.e. relatively close to the *INSR* gene that is located on chromosome 19p13.3-13.2. However, there are no earlier reports published on the *INSR* gene and schizoaffective disorder, and no association has been found between an SNP in the *INSR* gene and schizophrenia (Kim *et al.* 2013). As regards 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 previously been investigated in relation to schizophrenia, although not to schizoaffective disorder (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 in schizophrenia patients both between an *IRS-2* SNP and auditory hallucinations, and between *IRS-4* SNPs and family history or body mass index, have been reported, 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 this hypothesis that impaired signalling via the INSR and/ or IGF1R on cells of brain and body might result in development of schizophrenia and related psychoses, I in this study chose to thoroughly investigate the whole *INSR* gene in patients with schizophrenia or schizoaffective disorder and controls.

MATERIAL AND METHODS

Consecutive out-patients from psychiatric polyclinics in the region of Stockholm, Sweden and with the diagnoses of schizophrenia or schizoaffective disorder according to DSM-5 criteria (American Psychiatric Association, 2013) were invited to participate in this study. In total 105 patients gave their written informed consent to participate. Characteristics of the patients are described in Table 1. All patients were unrelated Caucasian individuals. They were in full or partial remission regarding psychotic symptoms, and were all receiving long-term

Tab. 1. Characteristics of the study population

Diagnosis, DSM-5	Schizophrenia	Schizoaffective disorder	Control subjects
Number, n	94	11	60
Men: Women, n	47: 47	3: 8	17: 43
Age[a], year	44 (9)	47 (12)	45 (11)
Duration of disease[a], year	18 (9)	24 (12)	–
Heredity for psychosis, n [%]	37 [40][b,c]	6 [55][d]	0 [0]
DM and/ or heredity for DM, n [%]	48 [52][b,e]	9 [82][e]	0 [0][e]

Abbreviations: DM = diabetes mellitus, n = number
[a] The data are given as mean and standard deviation
[b] Two patients were adoptees and lacked knowledge of their relatives
[c] I.e. having one or more first-, second-, third- or fourth-degree relatives, excluding siblings, with schizophrenia or related psychosis (Melkersson, 2009)
[d] I.e. having one or more first-, second-, third- or fourth-degree relatives, excluding siblings, with schizoaffective disorder, schizophrenia and/ or affective disorder
[e] DM type 1, type 2 or other types

therapy with antipsychotics. Control subjects were 60 unrelated Caucasian individuals from the Stockholm County or the nearby Uppsala County who gave written informed consent to participate in the study (Table 1). They were all healthy individuals with no heredity for psychotic disorder or diabetes mellitus (DM) type 1, type 2 or other types. The study was approved by the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden.

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.

For identification of SNPs of interest in the *INSR* gene, targeted whole *INSR* gene sequencing of DNA using the SOLiD technology (Applied Biosystems, Foster City, CA, USA) was carried out in two subgroups of the study population: 1) 49 schizophrenia or schizoaffective disorder patients with heredity for schizophrenia or related psychoses, i.e. having one or more first-, second-, third- or fourth-degree relatives, including siblings, with such a disorder (Melkersson, 2009), and 2) 25 control subjects. 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 was

Tab. 2. Data regarding the 10 possible single nucleotide polymorphisms of interest in the insulin receptor gene

SNP numbering	SNP identification[a,b]	SNP position[a]	Polymorphism[c]	Gene location and known function
1.	rs59765738	7207679	A>**C**	intron 2
2.	novel	7205068	T>**C**	intron 2
3.	rs57476618	7202999	G>**T**	intron 2
4.	novel	7167985	T>**C**	exon 7
5.	rs2352954	7152418	A>**G**	intron 10
6.	novel	7150143	G>**C**	intron 11
7.	rs11882912	7149898	G>**T**	intron 11
8.	rs2229431	7141775	G>**A**	exon 13, synonymous coding (Asn)
9.	rs112317501	7135987	A>**G**[d]	intron 13
10.	rs12610022	7135292	A>**G**	intron 13

Abbreviations: Asn = asparagine, SNP = single nucleotide polymorphism
[a] rs numbers and positions from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
[b] No's 2, 4 and 6 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] 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)

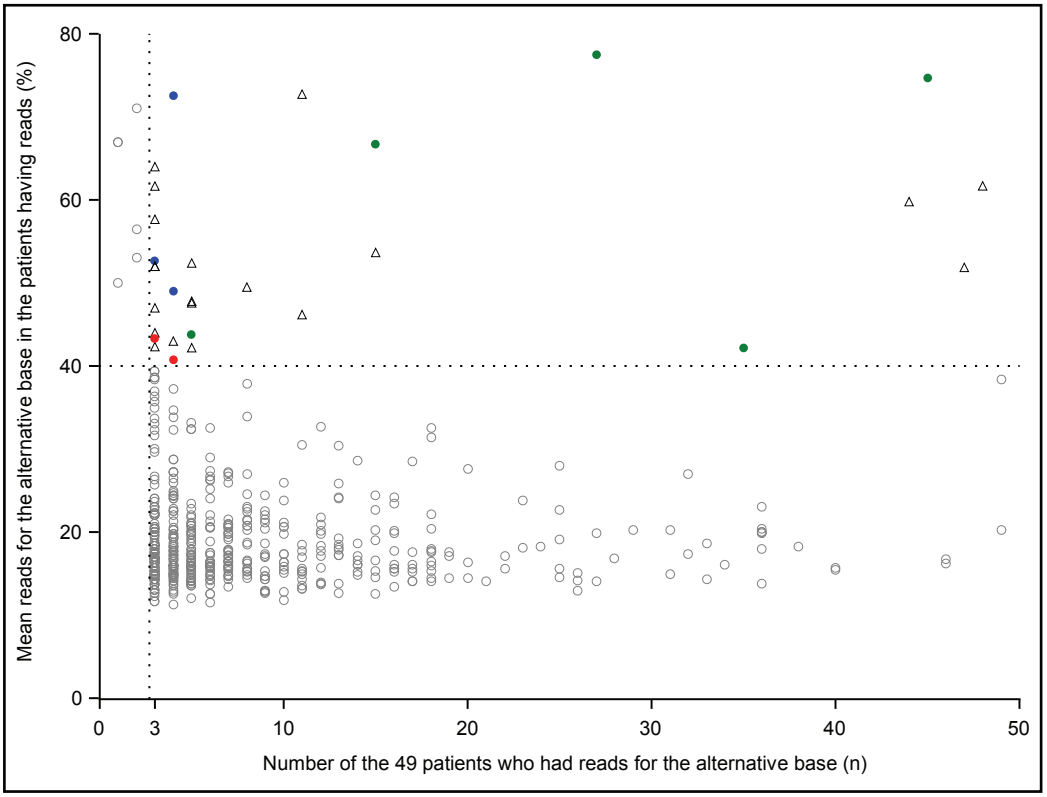

Fig. 1. Positions in the insulin receptor gene presenting with reads for the alternative base and described in accordance with the four criteria given in the text. Filled circles (●,●,●) indicate positions fulfilling all four criteria, unfilled triangles (Δ) indicate positions fulfilling criteria 1, 2 and 3, and unfilled circles (○) indicate positions fulfilling criteria 1 and 2, or 1 and 3, whereas positions fulfilling only criterium 1 not are shown.

then performed 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 from position 19:7112219 to 19:7296034 (http://www.ensembl.org/ release 64, September 2011). 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 49 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 psychoses (n = 37, Table 1) and patients having only siblings with such a disorder (n = 12) was equal to or above 11 % units. In total 10 such possible SNPs were identified (Figure 1, Table 2), which then in a second step of the study were typed in the whole study population by the following methods: the SNPs no's 1-3, 8 and 10 were typed by TaqMan® SNP genotyping Assays according to the instructions of the manufacturer (Applied Biosystems/ Life Technologies, Foster City, CA, USA), the SNPs no's 4-6 and 9 were typed by Sanger sequencing, and the SNP no 7 was typed by both these methods. In addition, the exact length of the insertion preceding SNP no 9 was determined, using DNA Fragment Analysis by Capillary Electrophoresis (Applied Biosystems/ Life Technologies, Foster City, CA, USA).

Categorial data were summarized using frequency counts and percentages. Associations between genotype distributions or allele frequencies and disease (schizophrenia, schizoaffective disorder and controls) were analysed by the chi-square or Fisher's exact tests. The same statistical methods were used to investigate associations both between the variable heredity for schizophrenia or related psychoses in combination with schizophrenia on one hand and the groups of genotype or allele on the other, and between the variable DM and/ or heredity for DM in combination with schizophrenia on one hand and the groups of genotype or allele on the other. A *p*-value of less than 0.05 was considered statistically significant. The statistical analyses were performed using the statistical programs Statistica 13.0 (Dell Inc., Tulsa, OK, USA) and SAS System 9.4 (SAS Institute Inc., Cary, NC, USA).

Tab. 3. Genotype distributions and allele frequencies regarding the seven single nucleotide polymorphisms studied in the insulin receptor gene in schizophrenia patients, schizoaffective disorder patients and control subjects

SNP[a]	Polymorphism[b]	Numbers of SP, SAP and C	Genotype frequencies (%)										Allele frequencies (%)[d]			
			SP			SAP			C			*p*-value[c]	SP	SAP	C	*p*-value[c]
			1-1	1-2	2-2	1-1	1-2	2-2	1-1	1-2	2-2					
1.	A>**C**	94/ 10/ 55	28.72	42.55	28.72	40.00	30.00	30.00	25.45	52.73	21.82	0.603	50.00	55.00	51.82	0.890
3.	G>**T**	94/ 10/ 55	11.70	40.43	47.87	10.00	40.00	50.00	9.09	32.73	58.18	0.817	68.09	70.00	74.55	0.498
5.	A>**G**	94/ 11/ 58	3.19	29.79	67.02	9.09	27.27	63.64	1.72	34.48	63.79	0.632	81.91	77.27	81.03	0.867
7.	G>**T**	94/ 10/ 56	97.87	0.00	2.13	100.00	0.00	0.00	100.00	0.00	0.00	0.586	97.87	100.00	100.00	0.241
8.	G>**A**	94/ 10/ 56	90.43	9.57	0.00	70.00	20.00	10.00	94.64	5.36	0.00	**0.034**[f]	95.21	80.00	97.32	**0.005**[h]
9.	A>**G**[e]	94/ 11/ 60	87.23	12.77	0.00	81.82	18.18	0.00	90.00	8.33	1.67	0.416	93.62	90.91	94.17	0.847
10.	A>**G**	94/ 11/ 60	89.36	10.64	0.00	72.73	18.18	9.09	91.67	6.67	1.67	0.073[g]	94.68	81.82	95.00	**0.048**[i]

Abbreviations: C = control subjects, CI = confidence interval, OR = odds ratio, SAP = schizoaffective disorder patients, SNP = single nucleotide polymorphisms, SP = schizophrenia patients, vs = versus

[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] Statistically significant *p*-values are written in bold text and borderline significant *p*-values are underlined

[d] Only highest allele frequency is shown

[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)

[f] SAP vs SP: p = **0.034**, SAP vs C: p = **0.040**, SP vs C: p = 0.536

[g] SAP vs SP: p = 0.054, SAP vs C: p = 0.111, SP vs C: p = 0.374

[h] SAP vs SP: p = **0.026**, for A-allele OR (95% CI) = 4.97 (1.38-17.96), SAP vs C: p = **0.010**, for A-allele OR (95% CI) = 9.08 (1.86-44.38), SP vs C: p = 0.545, for A-allele OR (95% CI) = 1.83 (0.48-6.89)

[i] SAP vs SP: p = **0.045**, for G-allele OR (95% CI) = 3.96 (1.13-13.90), SAP vs C: p = **0.049**, for G-allele OR (95% CI) = 4.22 (1.08-16.44), SP vs C: p = 0.902, for G-allele OR (95% CI) = 1.07 (0.38-3.02)

RESULTS

Seven of the 10 possible SNPs of interest were polymorphic-distributed in the whole study population (Table 2: no's 1, 3, 5 and 7-10; Figure 1: red- or green filled circles), whereas three were monomorphic-distributed and could not be verified as SNPs (Table 2: no's 2, 4 and 6; Figure 1: blue filled circles). In addition, one of the seven verified SNPs (Table 2: no 9) was preceded by a deletion/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).

Tab. 4. Genotype distributions and allele frequencies regarding the seven single nucleotide polymorphisms studied in the insulin receptor gene in schizophrenia patients with or without heredity for schizophrenia or related psychosis

SNP[a]	Polymorphism[b]	Numbers of SPHer and SPnoHer	Genotype frequencies (%)							Allele frequencies (%)[d]		
			SPHer			SPnoHer			*p*-value[c]	SPHer	SPnoHer	*p*-value[c]
			1-1	1-2	2-2	1-1	1-2	2-2				
1.	A>**C**	37/ 55	32.43	40.54	27.03	27.27	45.45	27.27	0.850	52.70	50.00	0.765
3.	G>**T**	37/ 55	13.51	32.43	54.05	10.91	47.27	41.82	0.375	70.27	65.45	0.525
5.	A>**G**	37/ 55	5.41	27.03	67.57	1.82	30.91	67.27	0.665	81.08	82.73	0.845
7.	G>**T**	37/ 55	97.30	0.00	2.70	98.18	0.00	1.82	1.000	97.30	98.18	1.000
8.	G>**A**	37/ 55	97.30	2.70	0.00	85.45	14.55	0.00	0.079	98.65	92.73	0.087
9.	A>**G**[e]	37/ 55	94.59	5.41	0.00	81.82	18.18	0.00	0.114	97.30	90.91	0.127
10.	A>**G**	37/ 55	94.59	5.41	0.00	85.45	14.55	0.00	0.306	97.30	92.73	0.320

Abbreviations: SNP = single nucleotide polymorphism, SPHer = schizophrenia patients with heredity for schizophrenia or related psychosis, SPnoHer = schizophrenia patients without heredity for schizophrenia or related psychosis

[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] Borderline significant *p*-values are underlined

[d] Only highest allele frequency is shown

[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)

Tab. 5. Genotype distributions and allele frequencies regarding the seven single nucleotide polymorphisms studied in the insulin receptor gene in schizophrenia patients with or without diabetes mellitus and/ or heredity for diabetes mellitus[a]

SNP[b]	Polymorphism[c]	Numbers of SPDM and SPnoDM	Genotype frequencies (%)							Allele frequencies (%)[e]		
			SPDM			SPnoDM			*p*-value[d]	SPDM	SPnoDM	*p*-value[d]
			1-1	1-2	2-2	1-1	1-2	2-2				
1.	A>**C**	48/ 44	35.42	43.75	20.83	22.73	43.18	34.09	0.253	57.29 (A) 42.71 (C)	44.32 (A) 55.68 (C)	0.104
3.	G>**T**	48/ 44	18.75	43.75	37.50	4.55	38.64	56.82	0.054	59.38	76.14	**0.018**[g]
5.	A>**G**	48/ 44	0.00	20.83	79.17	6.82	38.64	54.55	**0.015**	89.58	73.86	**0.007**[h]
7.	G>**T**	48/ 44	100.00	0.00	0.00	95.45	0.00	4.55	0.226	100.00	95.45	0.051
8.	G>**A**	48/ 44	91.67	8.33	0.00	88.64	11.36	0.00	0.732	95.83	94.32	0.739
9.	A>**G**[f]	48/ 44	89.58	10.42	0.00	84.09	15.91	0.00	0.435	94.79	92.05	0.555
10.	A>**G**	48/ 44	91.67	8.33	0.00	86.36	13.64	0.00	0.511	95.83	93.18	0.523

Abbreviations: CI = confidence interval, OR = odds ratio, SNP = single nucleotide polymorphism, SPDM = schizophrenia patients with diabetes mellitus and/ or heredity for diabetes mellitus, SPnoDM = schizophrenia patients without diabetes mellitus and/ or heredity for diabetes mellitus

[a] Type 1, type 2 or other types

[b] Same SNP numbering as in Table 2

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

[d] Statistically significant *p*-values are written in bold text and borderline significant *p*-values are underlined

[e] Only highest allele frequency is shown, except for SNP no 1

[f] 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)

[g] For T-allele OR (95% CI) = 0.46 (0.24-0.87)

[h] For G-allele OR (95% CI) = 3.04 (1.35-6.84)

Genotype distributions and allele frequencies of the seven verified SNPs in the *INSR* gene in patients with schizophrenia, patients with schizoaffective disorder and control subjects, together with results of single association analyses, are given in Table 3. In comparisons between the three groups, overall significant differences were found in genotype distribution and allele frequency for SNP rs2229431 in exon 13, and a tendency towards overall significant difference or overall significant difference was found in genotype distribution and allele frequency for SNP rs12610022 in intron 13 (Table 3: no's 8 and 10; Figure 1: red filled circles). Pairwise analyses showed that it was the patients with schizoaffective disorder who differed in genotype distributions and allele frequencies compared to both the patients with schizophrenia and the control subjects, whereas between the patients with schizophrenia and the control subjects, no significant differences were found (Table 3). As regards the deletion/insertion variation rs747721248 preceding SNP no 9 (Table 2), no overall significant differences in genotype distribution and allele frequency were found between schizophrenia patients, schizoaffective disorder patients and control subjects.

When comparing subgroups of schizophrenia patients with or without heredity for schizophrenia or related psychoses (Table 1), tendencies towards significant differences in genotype distribution and allele frequency were found for SNP rs2229431, but not for the other six SNPs (Table 4: no 8). 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 (Table 4: no 8). In a further subgroup analysis comparing schizophrenia patients with or without DM and/ or heredity for DM (Table 1), a tendency towards significant difference or significant differences were found in genotype distribution and allele frequency for SNP rs57476618 in intron 2 and SNP rs2352954 in intron 10, but not for the other five SNPs (Table 5: no´s 3 and 5). Regarding the deletion/insertion variation rs747721248, tendencies towards significant differences in genotype distribution and allele frequency were found between subgroups of schizophrenia patients with (n = 37) or without (n = 55) heredity for schizophrenia or related psychoses (p = 0.054 and p = 0.060, respectively) in that this insertion tended to be less common in the schizophrenia patients with heredity than in those without. However, between subgroups of schizophrenia patients with (n = 48) or without (n = 44) DM and/ or heredity for DM, no differences were found.

Concerning subgroups of schizoaffective disorder patients with or without heredity for psychoses, or with or without DM and/ or heredity for DM (Table 1), differences in genotype distributions and allele frequencies for the seven SNPs and the deletion/insertion variation rs747721248 could not be determined with certainty since there were too few patients in the subgroups compared.

DISCUSSION

In this study, investigating associations between *INSR* SNPs of potential interest and schizophrenia or schizoaffective disorder, I found that patients with schizoaffective disorder more often carried the A-allele of SNP rs2229431 in exon 13 and the G-allele of SNP rs12610022 in intron 13, compared to patients with schizophrenia and controls. To the best of my knowledge, this is the first report showing that *INSR* gene variants confer risk specifically for schizoaffective disorder. The odds ratios for the A- and G-alleles were also relatively high, 9.08 and 4.22, respectively, indicating rather large effect contributions to the disease risk (Owen, 2012).

In addition, I found that the reference (i.e. the G/G-genotype and G-allele) of the SNP rs2229431 tended to be more common in the patients with heredity for schizophrenia or related psychoses than in those without heredity, indicating that this SNP also may distinguish the core group of schizophrenia patients with heredity from that without. Furthermore, the reference (i.e. no 10A-, 11A- or 12A-insertion) of the deletion/insertion variation rs747721248 tended to be more common in the schizophrenia patients with heredity than in those without heredity. Previously, only one *INSR* SNP, rs2059806 located in exon 8 (Hanis & Bertin, 1990), has been investigated in relation to schizophrenia, but not found to be associated (Kim *et al.* 2013).

The SNP rs2229431, which is located in exon 13 of the *INSR* gene, is synonymous coding for asparagine. Thus, it does not in itself cause a direct change in amino acid coding. However, it may be an SNP that either alone or together with the intronic-located SNP rs12610022, has a regulatory function on amino acid coding of the INSR (Hindorff *et al.* 2009). Neither can it be completely ruled out that this SNP, alone or together with rs12610022, is linked to another nearby SNP in linkage disequilibrium that is the causative variant (Hindorff *et al.* 2009).

This genetic distinction of the *INSR* gene between schizoaffective disorder and schizophrenia found in this study may be connected to other known discrepancies between the two disorders, such as to differences in clinical symptoms, electroencephalography (EEG) correlates, serum autoantibodies to serotonin, interleukin-1ß mRNA expression levels in post-mortem brains and comorbidity of type 1 DM (Fillman *et al.* 2013; Garakh *et al.* 2015; Juvonen *et al.* 2007; Schott *et al.* 2003).

In this present study, positive associations were also found between the *INSR* SNPs rs57476618 in intron 2 or rs2352954 in intron 10 and DM and/ or heredity for DM in patients with schizophrenia, of whom the majority had type 2 DM and/ or heredity for type 2 DM. In comparison, there are no other studies published on *INSR* SNPs in relation to DM in schizophrenia patients; but in relation to DM in otherwise healthy individuals, the *INSR* SNPs rs2059806 in exon 8 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 type 2 DM and insulin resistance (Bodhini *et al.* 2012; Malodobra *et al.* 2011; Quederni *et al.* 2009; Wang *et al.* 2012).

In conclusion, the main results in this study show that two SNPs in the *INSR* gene – rs2229431 in exon 13 and rs12610022 in intron 13 – confer risk specifically for schizoaffective disorder.

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