The insulin receptor substrate-4 (*IRS-4*) gene and schizophrenia: no evidence for a main genetic factor, however one report of a single schizophrenia patient with a mutation

Kristina Melkersson [1], Bengt Persson [2], Thorbjörn Hongslo [3]

1 Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
2 Science for Life Laboratory, Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden
3 National Veterinary Institute, Uppsala, Sweden

Correspondence to: Kristina Melkersson, MD., PhD.
Department of Molecular Medicine and Surgery, Karolinska Institutet
c/o Sollentuna Psychiatric Polyclinic, Nytorpsvägen 10
SE-191 35 Sollentuna, Sweden.
TEL: +46-8-58730405; FAX: +46-8-351318; E-MAIL: Kristina.Melkersson@ki.se

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Abstract

OBJECTIVES: Since there are clear indications that schizophrenia is a systemic disorder, we sought for a common molecular basis for schizophrenia abnormalities in brain and body. Our hypothesis was that an impaired insulin/ insulin-like growth factor signalling in cells might underlie both structural and functional brain changes and peripheral abnormalities in schizophrenia. No associations between polymorphisms in the genes for insulin-like growth factor 1 or its receptor and schizophrenia have been reported. However, the insulin receptor substrates 1-4 linking both the insulin and insulin-like growth factor 1 receptors with intracellular pathways have not been extensively studied in schizophrenia. In this study, we therefore chose to study the insulin receptor substrate-4 (*IRS-4*) gene as a candidate gene in schizophrenia.
METHODS: The *IRS-4* gene of 93 patients and 59 control subjects was screened for DNA sequence variations, followed by case-control analyses of 10 detected single nucleotide polymorphisms.
RESULTS: No significant genotype, allele or haplotype associations were found with the schizophrenia illness. However, one female patient with paranoid schizophrenia had an *IRS-4* gene mutation at position 107863596, resulting in a change in amino acid coding from histidine to tyrosine at position 879.
CONCLUSIONS: Although this study supports the view that the *IRS-4* gene is not of major importance for the aetiology of the vast majority of schizophrenia cases, our finding of this single patient with schizophrenia and a mutation in the *IRS-4* gene may point to that the insulin/ insulin-like growth factor signalling system in cells is still of interest in the future search for schizophrenia genes.

ORIGINAL ARTICLE

INTRODUCTION

The literature on the schizophrenia illness provides strong evidence for a role of genetic factor(s) in its aetiology (Craddock *et al*. 2005). A variety of genes, each with small or moderate effect, have been suggested to be involved in the aetiology of the disorder (Gottesman & Shields 1967), and in recent years there have been reports on such genes as those encoding α1C subunit of the L-type voltage-dependent calcium channel (CACNA1C), catechol-O-methyltransferase (COMT), disrupted in schizophrenia 1 (DISC1), dysbindin (DTNBP1), G72, golli myelin-basic-protein (MBP), neuregulin 1 (NRG1), neurexin 1 (NRXN1), serotonin receptor 2A (HTR2A), zinc finger 804A (ZNF804A) and others (Egan *et al*. 2001; Chumakov *et al*. 2002; Straub *et al*. 2002; Stefansson *et al*. 2002; Craddock *et al*. 2005; O'Donovan *et al*. 2008; Baruch *et al*. 2009; Green *et al*. 2009; Melkersson *et al*. 2009; Rujescu *et al*. 2009; Stefansson *et al*. 2009; Nyegaard *et al*. 2010). However, the main genetic factor(s) associated with schizophrenia still remain(s) to be found (Crow 2007).

Since there are clear indications that schizophrenia is a systemic disorder and not only a brain disease (Flyckt 2001; Wiesel 2007), we sought for a common molecular basis for schizophrenia abnormalities in brain and body. Interestingly, we found that impaired cellular signalling via the insulin receptor (IR) and possibly also via the insulin-like growth factor 1 receptor (IGF-1R) hypothetically might underlie known abnormalities associated with schizophrenia in both the central nervous system (CNS) (i.e. structural and functional changes) (Wozniak *et al*. 1993; Vita *et al*. 2000) and in peripheral organ systems (i.e. growth dysregulation, impaired glucose tolerance, lowered resting energy expenditure and neuromuscular dysfunction) (Meltzer 1976; Ryan *et al*. 2003; Nilsson *et al*. 2006; Perrin *et al*. 2007).

The IR and IGF-1R are both present in the CNS and peripheral organs in humans (Sara *et al*. 1982; Rui & White 2004; McCowen & Smith 2005). No significant associations between polymorphisms within the *IGF-1* or *IGF-1R* genes and schizophrenia have been found (Gunnell *et al*. 2007; Bonvicini *et al*. 2010). However, the insulin receptor substrates (IRSs) 1-4 linking both the IR and IGF-1R with intracellular pathways have – as yet – not been extensively genetically studied in schizophrenia. This would be worthwhile doing, especially to study the *IRS-4* gene (Lavan *et al*. 1997; Fantin *et al*. 1998). The IRS-3 protein has so far been identified only in rodents (Björnholm *et al*. 2002), leaving in the first place IRS-1, -2 and -4 of potential interest. Furthermore, animal studies have demonstrated that in contrast to mice lacking IRS-1 or IRS-2, the mice lacking IRS-4 exhibit mild defects in growth, reproduction, and glucose homeostasis (Fantin *et al*. 2000; Kahn & Saltiel 2005), which would seem to suggest possible similarities with abnormalities in brain and body found in schizophrenia (Meltzer 1976; Wozniak *et al*. 1993; Vita *et al*. 2000; Ryan *et al*. 2003; Nilsson *et al*. 2006; Perrin *et al*. 2007). Therefore, we chose to study the *IRS-4* gene as a functional candidate gene in schizophrenia. We first screened the whole *IRS-4* gene sequence of patients and control subjects for DNA sequence variations and then carried out a case-control study.

PATIENTS & METHODS

The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden. Consecutive out-patients from psychiatric polyclinics in the region of Stockholm, Sweden and with the diagnosis of schizophrenia according to DSM-IV criteria (American Psychiatric Association 1994) were asked to participate in the study. In total 93 patients, 46 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 the patient group is described elsewhere in detail (Melkersson 2009). In brief, all patients were unrelated Caucasian individuals. They were in full or partial remission regarding psychotic symptoms, and were all 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]. Control subjects were 59 unrelated Caucasian individuals (17 males and 42 females) who lived in the Stockholm County or in the nearby Uppsala County and 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 family history of psychotic disorder and diabetes mellitus (DM) (type 1, type 2 or other types). Their mean (s.d.) age was 45 (11) years.

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 sequenced.

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 cleaning of the PCR products 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, Mich., USA).

Tab. 1. Data regarding the 22 single nucleotide polymorphisms (SNPs) studied in the insulin receptor substrate-4 gene and immediate downstream

SNP numbering	SNP id[a,b]	SNP position[a] (NCBI build 36.3)	Polymorphism[c]	Amino acid position[a]	Non-synonymous/ synonymous coding
1.	rs17847228	107862359 [downstream]	T/C	---	----------
2.	rs41304472	107862390 [downstream]	A/T	---	----------
3.	rs28473027	107862462 [exon 1]	G/T	1257	Synonymous coding (arginine)
4.	rs28546943	107862543 [exon 1]	C/A	1230	Non-synonymous coding (aspartic acid → tyrosine)
5.	SNP1	107862568 [exon 1]	C/T	1221	Synonymous coding (arginine)
6.	rs17847227	107862759 [exon 1]	C/T	1158	Non-synonymous coding (alanine → threonine)
7.	SNP2	107862933 [exon 1]	C/T	1100	Non-synonymous coding (valine → isoleucine)
8.	rs34053928	107863100 [exon 1]	C/G	1044	Non-synonymous coding (arginine → proline)
9.	rs28712105	107863379 [exon 1]	G/A	951	Non-synonymous coding (proline → leucine)
10.	rs1801165	107863583 [exon 1]	T/G	883	Non-synonymous coding (lysine → threonine)
11.	rs1801164	107863596 [exon 1]	G/C	879	Non-synonymous coding (histidine → aspartic acid)
12.	SNP3	107863596 [exon 1]	G/A	879	Non-synonymous coding (histidine → tyrosine)
13.	rs1801163	107864481 [exon 1]	C/A	584	Non-synonymous coding (glycine → cysteine)
14.	rs34287560	107864707 [exon 1]	A/T	508	Non-synonymous coding (asparagine → lysine)
15.	rs17847225	107864746 [exon 1]	T/A	495	Synonymous coding (serine)
16.	SNP4	107864916 [exon 1]	T/A	439	Non-synonymous coding (serine → cysteine)
17.	SNP5/ rs80131334	107864998 [exon 1]	T/A	411	Synonymous coding (arginine)
18.	rs41307415	107864999 [exon 1]	C/T	411	Non-synonymous coding (arginine → glutamine)
19.	SNP6/ rs73253702	107866093 [exon 1]	G/A	46	Synonymous coding (threonine)
20.	rs1801162	107866131 [exon 1]	G/A	34	Non-synonymous coding (leucine → phenylalanine)
21.	rs2073114	107866168 [exon 1]	C/T	21	Synonymous coding (alanine)
22.	rs2073115	107866171 [exon 1]	C/T	20	Synonymous coding (alanine)

[a]rs numbers and positions from the Ensembl and NCBI SNP databases (21,22)
[b]SNP1-6 refer to six novel SNPs not described in the Ensembl and NCBI SNP databases (21,22)
[c]Allele 1/ allele 2

Pairwise linkage disequilibrium (LD) and haplotypes were calculated using the PHASE program version 2.1. (Stephens *et al*. 2001; Stephens & Scheet 2005). Statistical differences in genotype, allele and haplotype frequencies between patients and control subjects were examined by cross tables and Fisher's exact test. The same statistical method was used to investigate the association between clinical parameters such as family history of schizophrenia and subtype of schizophrenia on one hand and the groups of genotypes or haplotypes on the other. In comparison between different groups of genotypes or haplotypes regarding age at onset of schizophrenia, one-way analysis of variance (ANOVA) was used. To control for gender, logistic regression analysis or two-way ANOVA were employed. A *p*-value of less than 0.05 was considered statistically significant. The statistical analyses were performed using the statistical programs SAS version 9.1. (SAS Institute Inc., Cary, NC, USA) and Statistica for Windows (Statsoft Inc., Tulsa, Okla., USA).

RESULTS

Firstly, the DNA sequence of the *IRS-4* gene along with 8 base pairs upstream and 9 base pairs downstream (i.e. from position 107.866.303 to 107.862.359) in patients (n=93) and control subjects (n=59) was compared to the reference sequence (21,22). The *IRS-4* gene is located on chromosome Xq22.3, from position 107.866.295 to 107.862.368, and consists of one exon (21,22). Besides 16 known SNPs described in the National Centre for Biotechnology Information (NCBI) and Ensembl SNP databases (21,22), six novel SNPs (1–6) were revealed (Table 1). Of these six SNPs, three are non-synonymous (Table 1; SNP2, SNP3 and SNP4). One female patient had what is referred to as a mutation: the G/A genotype instead of G/G, G/C or C/C at the same position as the previously known SNP with rs number 1801164, resulting in a change in amino acid coding from histidine to tyrosine at position 879 (Table 1; SNP3). During the study, two of the six novel SNPs (Table 1; SNP5 and

Tab. 2A. Genotype and allele frequencies regarding the polymorphic-distributed single nucleotide polymorphisms (SNPs) in the insulin receptor substrate-4 (*IRS-4*) gene in all schizophrenia patients (P) compared with control subjects (C).

SNP[a]	Polymorphism[b]	Genotype frequencies (%) P (n=93) vs C (n=59)							Allele frequencies[d] (%) Male P (n=46) vs C (n=17)			Female P (n=47) vs C (n=42)		
		1-(1)[c]	1-2	2-(2)[c]	1-(1)[c]	1-(2)	2-(2)[c]	*p*-value	1	1	*p*-value	1	1	*p*-value
5.	C/T	100.00	0.00	0.00	98.31	1.69	0.00	0.39	100.00	100.00	1.00	100.00	98.81	0.47
7.	C/T	98.92	1.08	0.00	100.00	0.00	0.00	1.00	100.00	100.00	1.00	98.94	100.00	1.00
11.	G/C[e]	80.43	14.13	5.43	72.88	18.64	8.47	0.56	89.13	82.35	0.67	84.04	82.14	0.84
16.	T/A	98.92	0.00	1.08	100.00	0.00	0.00	1.00	97.83	100.00	1.00	100.00	100.00	1.00
17.	T/A	93.55	3.23	3.23	93.22	3.39	3.39	1.00	93.48	94.12	1.00	96.81	95.24	0.71
18.	C/T	93.55	3.23	3.23	93.22	3.39	3.39	1.00	93.48	94.12	1.00	96.81	95.24	0.71
19.	G/A	93.55	3.23	3.23	93.22	3.39	3.39	1.00	93.48	94.12	1.00	96.81	95.24	0.71
20.	G/A	92.47	4.30	3.23	93.22	3.39	3.39	1.00	93.48	94.12	1.00	95.74	95.24	1.00
21.	C/T	80.65	13.98	5.38	72.88	18.64	8.47	0.56	89.13	82.35	0.67	86.17	82.14	0.54
22.	C/T	88.17	9.68	2.15	79.66	15.25	5.08	0.35	95.65	88.24	0.29	90.43	86.90	0.49

[a]Same SNP numbering as in Table 1
[b]Allele 1/ allele 2
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male controls carry only one allele (allele 1 or allele 2)
[d]Only highest allele frequency is shown
[e]One female patient had a mutation: the genotype G/A and is not included in the calculations regarding this SNP

Tab. 2B. Genotype frequencies regarding the polymorphic-distributed single nucleotide polymorphisms (SNPs) in the insulin receptor substrate-4 (*IRS-4*) gene in schizophrenia patients without diabetes mellitus (DM) and family history of DM (P) compared with control subjects (C).

SNP[a]	Polymorphism[b]	Genotype frequencies (%) P (n=43) vs C (n=59)						
		1-(1)[c]	1-2	2-(2)[c]	1-(1)[c]	1-(2)	2-(2)[c]	*p*-value
5.	C/T	100.00	0.00	0.00	98.31	1.69	0.00	1.00
7.	C/T	100.00	0.00	0.00	100.00	0.00	0.00	1.00
11.	G/C[d]	80.95	9.52	9.52	72.88	18.64	8.47	0.44
16.	T/A	100.00	0.00	0.00	100.00	0.00	0.00	1.00
17.	T/A	93.02	2.33	4.65	93.22	3.39	3.39	1.00
18.	C/T	93.02	2.33	4.65	93.22	3.39	3.39	1.00
19.	G/A	93.02	2.33	4.65	93.22	3.39	3.39	1.00
20.	G/A	93.02	2.33	4.65	93.22	3.39	3.39	1.00
21.	C/T	81.40	9.30	9.30	72.88	18.64	8.47	0.44
22.	C/T	88.37	6.98	4.65	79.66	15.25	5.08	0.41

[a]Same SNP numbering as in Table 1
[b]Allele 1/ allele 2
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male controls carry only one allele (allele 1 or allele 2)
[d]One female patient had a mutation: the genotype G/A and is not included in the calculations regarding this SNP

SNP6) were reported in the databases, thus supporting our findings. The other four novel SNPs (Table 1; SNP1, SNP2, SNP3 and SNP4) are registered by us in the dbSNP.

Secondly, groups of patients and control subjects were evaluated regarding the 22 (i.e. the 6 novel and 16 known) SNPs. Eleven of the 22 SNPs were polymorphic-distributed (Table 1; 5, 7, 11, 12, 16–22), and 11 were monomorphic-distributed (Table 1; 1–4, 6, 8–10, 13–15). Complete LD was found between rs1801164 and rs2073114, SNP5 and rs41307415, SNP5 and SNP6, and rs41307415 and SNP6, but not between the other

pairwise-combinations of the polymorphic-distributed SNPs (Figure 1). Genotype and allele frequencies in patients and control subjects, together with results of single association analyses, are presented in Tables 2A and 2B. No significant genotype or allele associations were found with the schizophrenia illness, either when comparing all schizophrenia patients with control subjects (Table 2A), or when comparing a subgroup of 43 schizophrenia patients without DM and heredity for DM (i.e. type 1, type 2 and other types of DM) with control subjects (Table 2B). Neither did the haplotype analyses reveal any significant associations with the schizophrenia illness (Tables 3, 4A and 4B).

Thirdly, associations between the clinical parameters family history of schizophrenia (presence/ absence), subtype of schizophrenia (paranoid/ nonparanoid) or age at onset of schizophrenia of the 93 patients and their *IRS-4* genotypes or haplotypes were investigated. Significant differences in genotype distributions were found for rs1801164 and rs2073114 between patients with and without a family history of schizophrenia (Table 5; p=0.02). It was found that the G/G genotype of rs1801164 or the linked C/C genotype of rs2073114 were more common in patients with a family history of schizophrenia. However, no other significant associations between the clinical parameters and genotypes or haplotypes were found (data not shown).

Tab. 3. Estimated haplotypes based on the polymorphic-distributed single nucleotide polymorphisms in the insulin receptor substrate-4 (*IRS-4*) gene, together with their frequencies in the overall study population (n=151)[a]

Haplotypes[b,c]	Frequency (%)
H1. CCGTTCGGCC	84.4
H2. CCCTTCGGTT	9.2
HX. All rare[d]	<5.0

[a]One female patient had a mutation: the G/A genotype at position 107863596 and the haplotypes H1/ CCATTCGGCC and is not included in this calculation
[b]Alleles are numbered 5, 7, 11, 16–22 as in Table 1
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male controls carry only one or none haplotype set (H or –)
[d]All haplotypes with estimated frequencies <5.0% in the overall study population (n=151)

Tab. 4A. Frequencies of estimated haplotypes based on the polymorphic-distributed single nucleotide polymorphisms in the insulin receptor substrate-4 (*IRS-4*) gene in all schizophrenia patients (P) compared with control subjects (C).

Haplotypes[a,b]	Haplotype frequencies (%) P (n=92) vs C (n=59)						
	–/(–)[c]	–/H	H/(H)[c]	–/(–)[c]	–/H	H/(H)[c]	*p*-value
H1. CCGTTCGGCC	7.53	13.98	78.49	8.47	20.34	71.19	0.52
H2. CCCTTCGGTT	88.17	9.68	2.15	79.66	15.25	5.08	0.35
HX. All rare[d]	89.25	6.45	4.30	91.53	5.08	3.39	1.00

[a]One female patient had a mutation: the G/A genotype at position 107863596 and the haplotypes H1/ CCATTCGGCC and is not included in this calculation
[b]Alleles are numbered 5, 7, 11, 16–22 as in Table 1
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male controls carry only one or none haplotype set (H or –)
[d]All haplotypes with estimated frequencies <5% in both patients and control subjects

Tab. 4B. Frequencies of estimated haplotypes based on the polymorphic-distributed single nucleotide polymorphisms in the insulin receptor substrate-4 (*IRS-4*) gene in schizophrenia patients without diabetes mellitus (DM) and family history of DM (P) compared with control subjects (C)

Haplotypes[a,b]	Haplotype frequencies (%) P (n=43) vs C (n=59)						
	–/(–)[c]	–/H	H/(H)[c]	–/(–)[c]	–/H	H/(H)[c]	*p*-value
H1. CCGTTCGGCC	9.30	11.63	79.07	8.47	20.34	71.19	0.52
H2. CCCTTCGGTT	88.37	6.98	4.65	79.66	15.25	5.08	0.41
HX. All rare[d]	90.70	4.65	4.65	91.53	5.08	3.39	1.00

[a]One female patient had a mutation: the G/A genotype at position 107863596 and the haplotypes H1/ CCATTCGGCC and is not included in this calculation
[b]Alleles are numbered 5, 7, 11, 16–21 as in Table 1
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male controls carry only one or none haplotype set (H or –)
[d]All haplotypes with estimated frequencies <5% in both patients and control subjects

Tab. 5. The genotype distributions of the single nucleotide polymorphisms (SNPs) rs1801164 and rs2073114 in the insulin receptor substrate-4 gene in association with family history of schizophrenia in 93 patients studied

SNP	rs1801164[b]			rs2073114		
Polymorphism[a] Family history of schizophrenia Percentage of patients[c] (%)	G/G	G/C	C/C	C/C	C/T	T/T
Presence (n=36)	94.29	5.71	0.00[#]	94.44	5.56	0.00[#]
Absence (n=55)	70.91	20.00	9.09	70.91	20.00	9.09

[a]Allele 1/ allele 2
[b]One female patient had the genotype G/A and is not included in the calculations regarding this SNP
[c]Two patients were adoptees and lacked knowledge about their relatives
[#]Significantly different compared to the patients without a family history of schizophrenia, p=0.02 (not controlled as well as controlled for gender)

SNP1	**SNP1**									
SNP2	0.000	**SNP2**								
rs1801164	0.001	0.019	**rs1801164**							
SNP4	0.000	0.000	0.001	**SNP4**						
SNP5/ rs80131334	0.000	0.000	0.293	0.000	**SNP5/ rs80131334**					
rs41307415	0.000	0.000	0.293	0.000	1.000	**rs41307415**				
SNP6/ rs73253702	0.000	0.000	0.293	0.000	1.000	1.000	**SNP6/ rs73253702**			
rs1801162	0.000	0.000	0.314	0.000	0.934	0.934	0.934	**rs1801162**		
rs2073114	0.001	0.019	1.000	0.001	0.294	0.294	0.294	0.314	**rs2073114**	
rs2073115	0.000	0.030	0.617	0.001	0.005	0.005	0.005	0.005	0.617	**rs2073115**

Fig. 1. Pairwise linkage disequilibrium (LD) data indicated as r^2 values for the polymorphic-distributed single nucleotide polymorphisms (SNPs) in the insulin receptor substrate-4 gene. SNP3, i.e. the mutation at the same position as the SNP with rs number 1801164, is not included in these LD data

DISCUSSION

In this study, based on DNA sequencing of the whole *IRS-4* gene followed by case-control analyses of 10 detected SNPs, no significant associations between *IRS-4* gene variants and the schizophrenia illness were found. Negative results were observed in both comparisons of genotype, allele and haplotype frequencies between patients and control subjects. Although the number of 93 patients and 59 control subjects included in this study does not allow definite conclusions to be drawn regarding gene variants with small effect, our results clearly point to no major involvement of the *IRS-4* gene in the aetiology of schizophrenia.

It might be argued that our patient and control subject groups differed as regards occurrence of DM and a family history of DM, and that therefore our results might have been confounded. However, this can be ruled out because our results did not change when we subgrouped our patients and compared the 43 patients without DM and a family history of DM with the 59 control subjects who all were healthy individuals with no family history of psychotic disorder and DM. Furthermore, data from an association study of 324 patients with type 2 DM and 267 control subjects with normal glucose tolerance indicate that polymorphisms in the *IRS-4* gene not are associated with type 2 DM (Almind *et al*. 1998), which also is consistent with the finding that mice lacking IRS-4 only show a slightly impaired response in the oral glucose tolerance test and no diabetic phenotype (Fantin *et al*. 2000). Neither have any associations between type 1 DM or other types of DM and *IRS-4* polymorphisms been reported.

The female patient who had a mutation in the *IRS-4* gene had a diagnosis of paranoid schizophrenia according to DSM-IV criteria (American Psychiatric Association 1994). Since this mutation is expected to change the amino acid coding from histidine to tyrosine (21), and the IRS-4 protein may be involved in neuronal growth and function in several areas of the brain (Numan & Russell 1999; Ye *et al*. 2002; Goren *et al*. 2004; Kahn & Saltiel 2005; Chiba *et al*. 2009), it might be possible that it is this *IRS-4* gene mutation that underlies this patient's schizophrenia. If so, this is the first described patient with schizophrenia and a mutation in the *IRS-4* gene on the X-chromosome. In comparison, 16 other

genes from the X-chromosome have been associated with cases of non-syndromic mental retardation (Ross *et al*. 2005). However, we are not aware of any non-syndromic X-chromosome-linked schizophrenia cases before having been reported (Ross *et al*. 2005). On the other hand, schizophrenia or schizophrenia-spectrum disorders earlier have been described in X-chromosome-linked syndromes such as adrenoleukodystrophy, Coffin-Lowry syndrome, fragile X syndrome and Lujan-Fryns syndrome (Reiss *et al*. 1988; De Hert *et al*. 1996; Szpak *et al*. 1996; Hanauer *et al*. 2002).

Since patients with familial schizophrenia are a more homogeneous patient group from an aetiological view than are patients with sporadic, non-familial schizophrenia (Melkersson 2009), the familial form of schizophrenia may also be expected to be associated with a higher genetic loading than the non-familial form (Faraone *et al*. 2000). Therefore, our finding of a higher frequency of the G/G genotype of the non-synonymous coding polymorphism rs1801164, which in addition is located at the same gene position as the female patient's G/A mutation found in this study, further might point to a connection between *IRS-4* (or another associated variation in another gene) and susceptibility to schizophrenia.

We conclude that although the present investigation supports the view that the *IRS-4* gene is not of major importance for the aetiology of the vast majority of schizophrenia cases, it is possible that our finding of this single patient with schizophrenia and an *IRS-4* gene mutation tells us that the insulin/ IGF signalling system in cells is still an interesting focus in the future search for schizophrenia genes.

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REFERENCES

1 Almind K, Frederiksen SK, Ahlgren MG, Urhammer S, Hansen T, Clausen JO, et al (1998). Common amino acid substitutions in insulin receptor substrate-4 are not associated with type II diabetes mellitus or insulin resistance. Diabetologia. **41:** 969–974.
2 American Psychiatric Association (1994). Diagnostic and statistical manual of mental disorders, 4th ed. Washington DC: American Psychiatric Press.
3 Baruch K, Silberberg G, Aviv A, Shamir E, Bening-Abu-Shach U, Baruch Y, et al (2009). Association between golli-MBP and schizophrenia in the Jewish Ashkenazi population: are regulatory regions involved? Int J Neuropsychopharmacol. **12:** 885–894.
4 Björnholm M, He AR, Attersand A, Lake S, Liu SCH, Lienhard GE, et al (2002). Absence of functional insulin receptor substrate-3 (IRS-3) gene in humans. Diabetologia. **45:** 1697–1702.
5 Bonvicini C, Gennarelli M, Scassellati C, Bignotti S, Gardella R, Barlati S, et al (2010). Polymorphic CA repeat in IGF-1 gene: lack of association with schizophrenia. Psychiatr Genet. **20:** 44–45.
6 Chiba T, Inoue D, Mizuno A, Komatsu T, Fujita S, Kubota H, et al (2009). Identification and characterization of an insulin receptor substrate 4-interacting protein in rat brain: implications for longevity. Neurobiol Aging. **30:** 474–482.
7 Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, et al (2002). Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA. **99:** 13675–13680.
8 Craddock N, O'Donovan MC, Owen MJ (2005). The genetics of schizophrenia and bipolar disorder: dissecting psychosis [review]. J Med Genet. **42:** 193–204.
9 Crow TJ (2007). How and why genetic linkage has not solved the problem of psychosis: review and hypothesis. Am J Psychiatry. **164:** 13–21.
10 De Hert M, Steemans D, Theys P, Fryns J-P, Peuskens J (1996). Lujan-Fryns syndrome in the differential diagnosis of schizophrenia. Am J Med Genet. **67:** 212–214.
11 Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al (2001). Effect of COMT $Val^{108/158}$ Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA. **98:** 6917–6922.
12 Fantin VR, Sparling JD, Slot JW, Keller SR, Lienhard GE, Lavan BE (1998). Characterization of insulin receptor substrate 4 in human embryonic kidney 293 cells. J Biol Chem. **24:** 10726–10732.
13 Fantin VR, Wang Q, Lienhard GE, Keller SR (2000). Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. Am J Physiol Endocrinol Metab. **278:** E127–E133.
14 Faraone SV, Seidman LJ, Kremen WS, Toomey R, Pepple JR, Tsuang MT (2000). Neuropsychologic functioning among the nonpsychotic relatives of schizophrenic patients: the effect of genetic loading. Biol Psychiatry. **48:** 120–126.
15 Flyckt LK (2001). Schizophrenia as a systemic disorder – studies of peripheral and central biological functions [dissertation]. Stockholm, Sweden: Karolinska Institutet.
16 Goren HJ, Kulkarni RN, Kahn R (2004). Glucose homeostasis and tissue transcript content of insulin signaling intermediates in four inbred strains of mice: C57BL/6, C57BLKS/6, DBA/2, and 129X1. Endocrinology. **145:** 3307–3323.
17 Gottesman II, Shields J (1967). A polygenic theory of schizophrenia. Proc Natl Acad Sci USA. **58:** 199–205.
18 Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, et al (2009). The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry. **15:** 1016–1022.
19 Gunnell D, Lewis S, Wilkinson J, Georgieva L, Smith Davey G, Day INM, et al (2007). IGF1, growth pathway polymorphisms and schizophrenia: a pooling study. Am J Med Genet. **144B:** 117–120.
20 Hanauer A, Young ID (2002). Coffin-Lowry syndrome: clinical and molecular features [review]. J Med Genet. **39:** 705–713.
21 http://www.ensembl.org/ (release 46, August 2007).
22 http://www.ncbi.nlm.nih.gov/ (version 31.08.2007).
23 Kahn CR, Saltiel AR (2005). The molecular mechanism of insulin action and the regulation of glucose and lipid metabolism. In: Kahn CR, Weir GC, King GL, et al, editors. Joslin's Diabetes Mellitus, 14th ed. New York: Lippincott Williams & Wilkins. p.145–168.
24 Lavan BE, Fantin VR, Chang ET, Lane WS, Keller SR, Lienhard GE (1997). A novel 160-kDa phosphotyrosine protein in insulin-treated embryonic kidney cells is a new member of the insulin receptor substrate family. J Biol Chem. **272:** 21403–21407.
25 McCowen KC, Smith RJ (2005). Insulin-like growth factors. In: Kahn CR, Weir GC, King GL, et al, editors. Joslin's Diabetes Mellitus, 14th ed. New York: Lippincott Williams & Wilkins. p.169–178.
26 Melkersson K (2009). Familial and sporadic schizophrenia: a comparison of somatic diseases and abuse in patients and their relatives. Acta Neuropsychiatr. **21:** 4–10.
27 Melkersson K, Hulting A-L (2009). Serotonin receptor 2A gene polymorphisms and schizophrenia: association with family history, diagnostic subtype and height in patients. Neuroendocrinol Lett. **30:** 343–351.
28 Meltzer HY (1976). Neuromuscular dysfunction in schizophrenia [review]. Schizophr Bull. **2:** 106–136.

29 Nilsson BM, Forslund AH, Olsson RM, Hambraeus L, Wiesel F-A (2006). Differences in resting energy expenditure and body composition between patients with schizophrenia and healthy controls. Acta Psychiatr Scand. **114:** 27–35.
30 Numan S, Russell DS (1999). Discrete expression of insulin receptor substrate-4 mRNA in adult rat brain. Brain Res Mol Brain Res. **72:** 97–102.
31 Nyegaard M, Demontis D, Foldager L, Hedemand A, Flint TJ, Sorensen KM, et al (2010). CACNA1C (rs1006737) is associated with schizophrenia. Mol Psychiatry. **15:** 119–121.
32 O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, et al (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet. **40:** 1053–1055.
33 Perrin MA, Chen H, Sandberg DE, Malaspina D, Brown AS (2007). Growth trajectory during early life and risk of adult schizophrenia. Br J Psychiatry. **191:** 512–520.
34 Reiss AL, Hagerman RJ, Vinogradov S, Abrams M, King RJ (1988). Psychiatric disability in female carriers of the fragile X chromosome. Arch Gen Psychiatry. **45:** 25–30.
35 Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, et al (2005). The DNA sequence of the human X chromosome. Nature. **434:** 325–337.
36 Rui L, White MF (2004). The role of insulin receptor substrate proteins in insulin signaling and metabolic regulation. In: LeRoith D, Taylor SI, Olefsky JM, editors. Diabetes mellitus. A fundamental and clinical text, 3rd ed. New York: Lippincott Williams & Wilkins. p.207–223.
37 Rujescu D, Ingason A, Cichon S, Pietiläinen OPH, Barnes MR, Toulopoulou T, et al (2009). Disruption of the neurexin 1 gene is associated with schizophrenia. Hum Mol Genet. **18:** 988–996.
38 Ryan MCM, Collins P, Thakore JH (2003). Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. Am J Psychiatry. **160:** 284–289.
39 Sara VR, Hall K, von Holtz H, Humbel R, Sjögren B, Wetterberg L (1982). Evidence for the presence of specific receptors for insulin-like growth factors 1 (IGF-1) and 2 (IGF-2) and insulin throughout the adult human brain. Neurosci Lett. **34:** 39–44.
40 Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, et al (2009). Common variants conferring risk of schizophrenia. Nature. **460:** 744–747.
41 Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al (2002). Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet. **71:** 877–892.
42 Stephens M, Scheet P (2005). Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet. **76:** 449–462.
43 Stephens M, Smith NJ, Donnelly P (2001). A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. **68:** 978–989.
44 Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, et al (2002). Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet. **71:** 337–348.
45 Szpak GM, Lewandowska E, Schmidt-Sidor B, Popow J, Kozlowski P, Lechowicz W, et al (1996). Adult schizophrenic-like variant of adrenoleukodystrophy. Folia Neuropathol. **34:** 184–192.
46 Wiesel FA (2007). Schizophrenia is a systemic disease. Genetic mechanisms and environmental factors interact in the disease development [in Swedish]. Lakartidningen. **104:** 2146–2147.
47 Vita A, Dieci M, Silenzi C, Tenconi F, Giobbio GM, Invernizzi G (2000). Cerebral ventricular enlargement as a generalized feature of schizophrenia: a distribution analysis on 502 subjects. Schizophr Res. **44:** 25–34.
48 Wozniak M, Rydzewski B, Baker SP, Raizada MK (1993). The cellular and physiological actions of insulin in the central nervous system. Neurochem Int. **22:** 1–10.
49 Ye P, Li L, Lund PK, D'Ercole AJ (2002). Deficient expression of insulin receptor substrate-1 (IRS-1) fails to block insulin-like growth factor-1 (IGF-1) stimulation of brain growth and myelination. Brain Res Dev Brain Res. **136:** 111–121.