Association between body mass index and insulin receptor substrate-4 (*IRS-4*) gene polymorphisms in patients with schizophrenia

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Abstract

OBJECTIVES: The insulin receptor substrate-4 (IRS-4) protein is highly expressed in the hypothalamus that plays a main role in the regulation of body weight. Therefore, as overweight and obesity are more frequent in patients with schizophrenia compared to the general population, this study was undertaken to investigate potential associations between body mass index (BMI) or height and polymorphisms in the *IRS-4* gene in schizophrenia patients and healthy controls.
METHODS: The *IRS-4* gene of 93 patients and 59 controls was screened for DNA sequence variations, and then 10 detected single nucleotide polymorphisms (SNPs) were investigated in relation to BMI and height of patients and controls.
RESULTS: Mean BMI was higher in patients than in controls, whereas there was no difference regarding height. Significant associations were found between patients' BMI and genotypes of six SNPs (rs1801164, rs80131334, rs41307415, rs73253702, rs1801162, rs2073114) or a haplotype including these six SNPs. In contrast, no associations were found between BMI or height of controls, or height of patients, and the genotypes or haplotypes.
CONCLUSION: This study clearly demonstrates associations between BMI and *IRS-4* variants in schizophrenia patients, but not in healthy controls, pointing to a possible involvement of *IRS-4* in the control of body weight in schizophrenia.

INTRODUCTION

The literature on the schizophrenia illness provides strong evidence for a role of genetic factor(s) in its aetiology (Craddock *et al.* 2005). There are also clear indications that schizophrenia is a systemic disorder and not only a brain disease (Flyckt 2001; Wiesel 2007). Therefore, we sought for a common molecular basis for schizophrenia abnormalities in brain and body, and found an interesting hypothesis, described more in detail in a recent study (Melkersson *et al.* 2011) that impaired cellular signalling via the insulin receptor (IR) and possibly also via the insulin-like growth factor 1 receptor (IGF-1R) might underlie known abnormalities associated with schizo-

phrenia in both the central nervous system (CNS) (i.e. structural and functional changes) and in peripheral organ systems (i.e. growth dysregulation, impaired glucose tolerance, lowered resting energy expenditure and neuromuscular dysfunction). Several studies have also reported significantly higher prevalence of overweight and obesity in patients with schizophrenia compared to the general population (Allison *et al.* 1999; Melkersson *et al.* 2004). So, possibly, there exists also a dysregulation of body weight in patients with schizophrenia that is attributed to the same molecular basis as the other known abnormalities in schizophrenia, i.e. to the insulin/ IGF-1 signalling system in cells.

The IR and IGF-1R are both present in the CNS and peripheral organs in humans (McCowen & Smith 2005; Rui & White 2004; Sara *et al.* 1982). No significant associations between polymorphisms in the *IGF-1R* or *IGF-1* genes and schizophrenia have been found (Bonvicini *et al.* 2010; Gunnell *et al.* 2007). 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. Gunnell *et al.* (2007) reported no association between a SNP in the *IRS-1* gene and schizophrenia. On the other hand, in our own recent study (Melkersson *et al.* 2011), although no main associations between SNPs in the *IRS-4* gene and schizophrenia were found, a single patient with schizophrenia and a G/A mutation in the *IRS-4* gene was reported. We also found a higher frequency of the G/G genotype of the *IRS-4* SNP rs1801164 in patients with familial schizophrenia, a SNP that in addition is located at the same gene position as the reported G/A mutation in the schizophrenia patient (Melkersson *et al.* 2011).

Regarding the IRS 1–4 proteins, animal studies have demonstrated that in contrast to mice lacking IRS-1 or IRS-2, 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; Nilsson *et al.* 2006; Perrin *et al.* 2007; Ryan *et al.* 2003; Vita *et al.* 2000; Wozniak *et al.* 1993). The IRS-3 protein has also so far been identified only in rodents (Björnholm *et al.* 2002). Furthermore, the IRS-4 protein is highly expressed in the hypothalamus (Bischof & Wevrick 2005; Numan & Russell 1999), where it is supposed to interact not only with the IR and IGF-1R, but also with the leptin receptor (LR) that is involved in the regulation of food intake and energy expenditure (Wauman *et al.* 2008; White 2002). Therefore, as the prevalence of overweight and obesity is higher in patients with schizophrenia compared to the general population, the aim of this study was to investigate potential associations between body mass index (BMI) or height and polymorphisms in the *IRS-4* gene in schizophrenia patients and healthy controls subjects.

PATIENTS & 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-IV criteria (American Psychiatric Association 1994) were asked to participate in this study. In total 93 patients, 46 males and 47 females, gave their written informed consent to participate and the patient group is described elsewhere in detail (Melkersson 2009). In brief, all patients were unrelated Caucasian individuals. 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). They were in full or partial remission regarding psychotic symptoms, and were all receiving long-term therapy with antipsychotics (1st generation antipsychotics, n=30; clozapine or olanzapine, n=37; other 2nd generation antipsychotics, n=7; more than one type of antipsychotics, n=19). 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. 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. There was no difference in age found between patients and control subjects.

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).

Collection and DNA preparation of blood samples

Venous blood was taken in EDTA-containing tubes 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.

DNA sequencing

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

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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).

Data analyses

Pairwise linkage disequilibrium (LD) and haplotypes for the polymorphic-distributed *IRS-4* SNPs were calculated using the PHASE program version 2.1. (Stephens & Scheet 2005; Stephens *et al.* 2001). Data are presented as mean and standard deviation (s.d.). To compare BMI or height between patients and control subjects when controlling for gender, two-way analysis of variance (ANOVA) was used. In comparison between different groups of genotypes or haplotypes regarding BMI and height, one-way ANOVA was employed, and when controlling for gender, a two-way ANOVA was performed. In case of a significant interaction between group and gender, simple effects were tested within gender. These analyses were done separately for patients and control subjects. For the patients, in addition to gender, we also controlled for heredity for schizophrenia, occurrence of DM and/ or heredity for DM, and type of antipsychotics. For these analyses we used main effects ANOVA. A *p*-value of less than 0.05 was considered statistically significant. The statistical analyses were performed using the statistical program Statistica 9.0 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

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 sequenced and compared to the reference sequence (15,16). The *IRS-4* gene is located on chromosome Xq22.3, from position 107.866.295 to 107.862.368, and consists of one exon (15,16). Besides 16 known SNPs described in the National Centre for Biotechnology Information (NCBI) and Ensembl SNP databases (15,16), six novel SNPs (Table 1; SNP1-6) were revealed, which are described in detail in our previous study (Melkersson *et al.* 2011). Of these total 22 SNPs, 10 were polymorphic-distributed (Table 1; 1–3, 5–11), one was what is referred to as a mutation (Table 1; 4), and 11 were monomorphic-distributed. Between pairs of the 10 polymorphic-distributed SNPs, complete or nearly complete LD was found between 7 pairs of SNPs (Figure 1). Of them, three SNPs appeared in addition to be closely linked to one another (Figure 1; SNP5/ rs80131334, rs41307415 and SNP6/ rs73253702).

Mean BMI was higher in patients than in control subjects, whereas no difference was found between patients and control subjects regarding height (Table 2). Associations between BMI or height of patients and control subjects and their genotype groups (i.e. allele 1/ allele 1 versus allele 1/ allele 2 + allele 2/ allele 2) for all 10 polymorphic-distributed *IRS-4* SNPs were investigated. The associations between BMI of patients and the genotype groups of the 10 SNPs are shown in Table 3. Significant associations were found between patients' BMI and the genotype groups of rs1801164, SNP5/rs80131334, rs41307415, SNP6/ rs73253702, rs1801162 or rs2073114 (not corrected as well as corrected for gender, heredity for schizophrenia, occurrence of DM and/ or heredity for DM, and type of antipsychotics) (Table 3). It was found that patients who carried the allele 1/ allele 1 of one of these six SNPs had higher BMI compared with patients who

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

SNP numbering	SNP id[a,b]	SNP position[a] (NCBI build 36.3)	Polymorphism[c]	Amino acid position[a]	Non-synonymous/ synonymous coding
1.	SNP1/ rs137853894	107862568 [exon 1]	C/T	1221	Synonymous coding (arginine)
2.	SNP2/ rs137853895	107862933 [exon 1]	C/T	1100	Non-synonymous coding (valine → isoleucine)
3.	rs1801164	107863596 [exon 1]	G/C	879	Non-synonymous coding (histidine → aspartic acid)
4.	SNP3/ rs1801164	107863596 [exon 1]	G/A	879	Non-synonymous coding (histidine → tyrosine)
5.	SNP4/ rs137853896	107864916 [exon 1]	T/A	439	Non-synonymous coding (serine → cysteine)
6.	SNP5/ rs80131334	107864998 [exon 1]	T/A	411	Synonymous coding (arginine)
7.	rs41307415	107864999 [exon 1]	C/T	411	Non-synonymous coding (arginine → glutamine)
8.	SNP6/ rs73253702	107866093 [exon 1]	G/A	46	Synonymous coding (threonine)
9.	rs1801162	107866131 [exon 1]	G/A	34	Non-synonymous coding (leucine → phenylalanine)
10.	rs2073114	107866168 [exon 1]	C/T	21	Synonymous coding (alanine)
11.	rs2073115	107866171 [exon 1]	C/T	20	Synonymous coding (alanine)

[a] rs numbers and positions from the Ensembl and NCBI SNP databases (15,16)
[b] SNP1-6 refer to six novel SNPs, described in detail in our previous study (Melkersson *et al.* 2011)
[c] Allele 1/ allele 2

Tab. 2. Body mass index (BMI) and height in patients with schizophrenia and healthy control subjects.

	All patients (n=93)	Male patients (n=46)	Female patients (n=47)	All control subjects (n=59)	Male control subjects (n=17)	Female control subjects (n=42)
BMI (kg/m²) Mean (s.d.)	29(6)[#]	29(6)	29(7)	23(3)	24(3)	23(3)
Height (m) Mean (s.d.)	1.74(0.10)	1.81(0.08)[##]	1.67(0.06)	1.71(0.10)	1.82(0.08)[##]	1.67(0.07)

[#]Significantly different compared to the control subjects independent of gender, $p<0.0001$
[##]Significantly different compared to the female individuals independent of patient- or control group, $p<0.0001$

Tab. 3. Genotype groups of the 10 polymorphic-distributed single nucleotide polymorphisms (SNPs) in the insulin receptor substrate-4 (*IRS-4*) gene in relation to body mass index (BMI) in 93 patients with schizophrenia and 59 healthy control subjects.

SNP	SNP1/ rs137853894		SNP2/ rs137853895		rs1801164		SNP4/ rs137853896		SNP5/ rs80131334		rs41307415		SNP6/ rs73253702		rs1801162		rs2073114		rs2073115	
Genotype group[a]	C/C	C/T+T/T	C/C	C/T+T/T	G/G	G/C+C/C	T/T	T/A+A/A	T/T	T/A+A/A	C/C	C/T+T/T	G/G	G/A+A/A	G/G	G/A+A/A	C/C	C/T+T/T	C/C	C/T+T/T
Number of patients (n)[b]	92/92	0/92	91/92	1/92	73/91[c]	18/91[c]	91/92	1/92	86/92	6/92	86/92	6/92	86/92	6/92	85/92	7/92	74/92	18/92	81/92	11/92
BMI (kg/m²)[d] Mean (s.d.)	29(7)	---	29(7)	30(0)	30(7)[#]	25(5)	29(6)	40(0)	29(6)[##]	22(4)	29(6)[###]	22(4)	29(6)[####]	22(4)	29(6)[#####]	23(4)	30(7)[######]	25(5)	29(7)	27(5)
Number of control subjects (n)	58/59	1/59	59/59	0/59	43/59	16/59	59/59	0/59	55/59	4/59	55/59	4/59	55/59	4/59	55/59	4/59	43/59	16/59	47/59	12/59
BMI (kg/m²) Mean (s.d.)	23(3)	20(0)	23(3)	---	24(3)	23(2)	23(3)	---	23(3)	24(2)	23(3)	24(2)	23(3)	24(2)	23(3)	24(2)	24(3)	23(2)	24(3)	22(2)

[a]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)
[b]Complete data was missing in one patient
[c]One female patient had what is referred to as a mutation at this gene position (Melkersson *et al*. 2011) and is not included in this calculation
[d]The *p*-values are given not corrected as well as corrected for gender, heredity for schizophrenia, occurrence of diabetes mellitus (DM) and/ or heredity for DM, and type of antipsychotics
[#]Significantly different compared to the patients with the G/C + C/C genotypes, $p=0.009$ (not corrected) and $p=0.01$ (corrected)
[##]Significantly different compared to the patients with the T/A + A/A genotypes, $p=0.005$ (not corrected) and $p=0.007$ (corrected)
[###]Significantly different compared to the patients with the C/T + T/T genotypes, $p=0.005$ (not corrected) and $p=0.007$ (corrected)
[####]Significantly different compared to the patients with the G/A + A/A genotypes, $p=0.005$ (not corrected) and $p=0.007$ (corrected)
[#####] Significantly different compared to the patients with the G/A + A/A genotypes, $p=0.008$ (not corrected) and $p=0.01$ (corrected)
[######]Significantly different compared to the patients with the C/T + T/T genotypes, $p=0.009$ (not corrected) and $p=0.009$ (corrected)

SNP1/ rs137853894	SNP1/ rs137853894								
SNP2/ rs137853895	0.000	SNP2/ rs137853895							
rs1801164	0.001	0.019	rs1801164						
SNP4/ rs137853896	0.000	0.000	0.001	SNP4/ rs137853896					
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/ rs1801164 that is located at the same gene position as rs1801164 and is referred to as a mutation (Melkersson *et al.* 2011) is not included in these LD data.

carried the allele 1/ allele 2 or allele 2/ allele 2 of the SNPs (Table 3). In contrast, no associations were found between BMI of control subjects and genotype groups of the 10 SNPs (not corrected as well as corrected for gender) (Table 3). Between height and the genotype groups of the 10 SNPs, no significant associations were found, either for patients or control subjects (data not shown).

Body mass index and height of patients and control subjects were also investigated in relation to estimated haplotypes based on the 10 polymorphic-distributed SNPs of the *IRS-4* gene (Table 4). The haplotype 3, but not the haplotypes 1 or 2, associated with BMI of patients (Table 5). It was found that patients not carrying haplotype 3 had higher BMI compared to patients carrying this haplotype (Table 5). In contrast, none of the haplotypes 1, 2 or 3 associated with BMI of control subjects. Between height and the haplotypes 1, 2 or 3, no significant associations were found, either for patients or control subjects (data not shown).

Tab. 4. Estimated haplotypes based on the 10 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,d]	Frequency (%)
H1. CCGTTCGGCC	84.4
H2. CCCTTCGGTT	9.2
H3. CCCTATAATC	4.6

[a]One female patient had what is referred to as a mutation at gene position 107863596 (Melkersson *et al*. 2011) and is not included in this calculation
[b]Alleles are numbered 1-3, 5-11 as in Table 1
[c]Since the *IRS-4* gene is located on the X-chromosome, male patients and male control subjects carry only one or none haplotype set (H or –)
[d]All haplotypes with estimated frequencies >4.5% in the overall study population

DISCUSSION

In this study, based on DNA sequencing of the whole *IRS-4* gene followed by analyses of 10 detected SNPs in relation to BMI and height, patients with schizophrenia carrying allele 1/ allele 1 genotypes of SNPs in the *IRS-4* gene had higher BMI compared with patients carrying allele 1/ allele 2 or allele 2/ allele 2 genotypes. Total six different SNPs and one haplotype including these six SNPs were associated with BMI in patients, but not with BMI in control subjects, or with height in patients or 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 *IRS-4* variants with small effect, our present findings point to an involvement of *IRS-4* in the control of body weight in schizophrenia.

The patients with schizophrenia in this study had higher mean BMI compared with healthy control subjects, which is fully in line with previous studies, reporting a significant higher prevalence of overweight (BMI ≥ 25 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) in patients with schizophrenia compared to the general population (Allison *et al*. 1999; Melkersson *et al*. 2004). In addition, it was the patients with schizophrenia, but not the healthy controls subjects, carrying the allele 1/ allele 1 genotypes of the six *IRS-4* SNPs, who had higher BMI, suggesting an increased risk for weight gain exclusively in schizophrenia if carrying these *IRS-4* genotypes. This is a novel finding as the *IRS-4* gene not before has been reported to belong to the group of known obesity genes, either in schizophrenia (Allen *et al*. 2008; Jassim *et al*. 2011; Perez-Iglesias *et al*. 2010) or generally (Hebebrand *et al*. 2010; Hofker & Wijmenga 2009).

Of the six SNPs in the *IRS-4* gene which were associated with higher BMI in patients with schizophrenia, close LD was found between the two SNPs

Tab. 5. Haplotypes, based on the 10 polymorphic-distributed single nucleotide polymorphisms in the insulin receptor substrate-4 gene, in relation to body mass index (BMI) in 92 patients with schizophrenia[a] and 59 healthy control subjects.

Haplotype[b]	H1		H2		H3	
Haplotype group[c]	not H1 carrier	H1 carrier	not H2 carrier	H2 carrier	not H3 carrier	H3 carrier
Number of patients (n)[d]	7/91	84/91	80/91	11/91	85/91	6/91
BMI (kg/m^2) Mean (s.d.)	26(7)	29(6)	29(7)	27(5)	29(6)[#]	22(4)
Number of control subjects (n)	5/59	54/59	47/59	12/59	55/59	4/59
BMI (kg/m^2) Mean (s.d.)	22(2)	23(3)	24(3)	22(2)	23(3)	24(2)

[a]One female patient had what is referred to as a mutation at gene position 107863596 (Melkersson *et al*. 2011) and is not included in this calculation
[b]Haplotypes as described in Table 4
[c]Since the *IRS-4* gene is located on the x-chromosome, male patients and male control subjects carry only one or none haplotype set (H or –)
[d]Complete data was missing in one patient
[#]Significantly different compared to the patients carrying H3, *p*=0.005 (not corrected) and *p*=0.008 (corrected for gender, heredity for schizophrenia, occurrence of diabetes mellitus (DM) and/ or heredity for DM, and type of antipsychotics)

rs1801164 and rs2073114 and between the four SNPs SNP5/rs80131334, rs41307415, SNP6/rs73253702 and rs1801162. Three of the six SNPs were in addition non-synonymous coding (rs1801164, rs41307415, rs1801162), i.e. each of them causes a change of amino acid coding in the IRS-4 protein, leading to possible alterations in both structure and function of this protein.

Although structural similarities, the IRSs -1, -2 and -4 differ in their sites and levels of tissue expression, indicating divergent intracellular signalling roles for these proteins (Fantin *et al.* 2000; Uchida *et al.* 2000). Whereas IRS-1 and IRS-2 show widespread expression at relatively high levels in brain and body, IRS-4 exhibits more limited expression at relatively lower levels, mainly in brain, fibroblasts, kidney, lymph nodes, ovary, prostate, spinal cord, thymus, thyroid glands, trachea and possibly also in pancreatic beta cells (Mertens *et al.* 2011; Numan & Russell 1999; Uchida *et al.* 2000; White 2002). One interesting exception, however, is that the IRS-4 protein is highly expressed in the hypothalamus (Bischof & Wevrick 2005; Numan & Russell 1999), which also contains IRs, IGF-1Rs and LRs that might require this protein for signal transduction (Unger & Betz 1998; Wauman *et al.* 2008). Both insulin and leptin regulate energy expenditure and food intake by acting on hypothalamic neurons, and cross-talk between the insulin and leptin signal transduction pathways is well documented. Both hormones can activate the IRS – phosphatidylinositol 3-kinase pathway in the hypothalamic arcuate nucleus (Niswender *et al.* 2003; 2001), where also IRS-4 is expressed (Numan & Russell 1999), and neuron-specific IR knockout mice display obesity with leptin resistance (Brüning *et al.* 2000). However, the function of the IRS-4 protein in the hypothalamus and brain is as yet unknown (Chiba *et al.* 2009). Neither is it known whether the higher BMI in our patients with schizophrenia carrying the risk genotypes (i.e. allele 1/ allele 1) of the six *IRS-4* SNPs only is caused of an altered IRS-4 protein, or rather mainly can be explained by another associated polymorphism in another gene and its protein product. The latter explanation seems to be the most probable, since previously, no significant associations between the schizophrenia illness in itself and SNPs in the *IRS-4* gene have been found (Melkersson *et al.* 2011).

In conclusion, the present findings clearly demonstrate associations between BMI and *IRS-4* in schizophrenia patients, but not in healthy control subjects, pointing to a possible involvement of *IRS-4* in the control of body weight in schizophrenia. These findings may also tell us that the insulin/ IGF signalling system in cells still is an interesting focus in the future search for schizophrenia genes.

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