CLINICAL STUDY

Genetic variation at the *IGF1* locus shows association with post-stroke outcome and to circulating IGF1

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Abstract

Objective: In humans, serum IGF1 (s-IGF1) is associated with outcome after ischemic stroke (IS). Therefore variation at the *IGF1* locus could also associate with both IS and s-IGF1. We investigated whether genetic variation at the *IGF1* locus is associated with i) s-IGF1, ii) IS occurrence, iii) IS severity, and iv) post-stroke outcome.

Design/methods: Patients ($n=844$; 66% males, mean age 56 years) and community controls ($n=668$) were included from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLISIS). Post-stroke outcome was evaluated with the modified Rankin Scale at 3 and 24 months after index stroke, and baseline stroke severity with the Scandinavian Stroke Scale. s-IGF1 was determined in patients and after random selection in 40 of the controls.

Results: Eleven single nucleotide polymorphisms (SNPs) were selected in the *IGF1* gene. In healthy controls the major allele of rs7136446 was associated with higher s-IGF1, whereas in patients no such association was found. No SNP was associated with IS, nor with stroke severity. After multivariate correction for presence of diabetes, smoking, and hypertension, the major allele of rs7136446 was associated with favorable functional outcome 24-months post-stroke (odds ratio 1.46; 95% CI 1.09–1.96).

Conclusion: Variation in rs7136446 of the *IGF1* gene associates with post-stroke outcome in relatively young IS patients. Also, rs7136446 associates with s-IGF1 in controls but not in IS, which indicates that IS perturbs a normal genetic impact on s-IGF1 levels.

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Introduction

Insulin-like growth factor 1 (IGF1) affects brain plasticity after brain injuries as well as cognition and mood in the healthy brain. In line with this, compounds that affect mood and plasticity after brain injuries also affect local IGF1. Specifically, serotonin reuptake inhibitors, which affects local brain IGF1 levels in experimental animals (1), also improve stroke outcome in humans (2). Furthermore, circulating serum IGF1 (s-IGF1) is associated with better recovery after ischemic stroke (IS) (3, 4). Circulating s-IGF1 is regulated by growth hormone from the pituitary, but also by other factors such as age, metabolic state, and possibly physical activity (5, 6). Specifically, s-IGF1 is lower in higher ages and in subjects with the metabolic syndrome, whereas physical activity is at least partially associated with higher s-IGF1. These factors act on s-IGF1 via degradation/stability and on *de novo*

synthesis of IGF1 protein. Part of this is determined by primary transcriptional mechanisms in the *IGF1* gene locus. Variations at the *IGF1* gene locus include several single nucleotide polymorphisms (SNPs), and a 192 base-pair cytosine–adenine repeat polymorphism (192 bp CA-repeat) in the promoter region. The absence of the 192 bp CA-repeat is associated with lower levels of s-IGF1 (7), an increased risk of IS and a lower long-term survival after IS (8). This variant also shows association with an increased risk of myocardial infarction (MI) and diabetes (7). Another study reported an association between rs2229765 in the *IGF1* receptor gene (*IGF1R*) and IS (9). However, to our knowledge, no other genetic variants in the *IGF1* or *IGF1R* genes have been investigated in relation to IS occurrence or post-stroke outcome. The objectives of this study were therefore to investigate whether genetic variation in *IGF1* is associated with s-IGF1, IS occurrence, stroke severity, and/or post-stroke outcome.

Subjects and methods

The design of Sahlgrenska Academy Study on Ischemic Stroke (SAHLIS) has been reported elsewhere (4, 10). Briefly, patients (<70 years) with first-ever or recurrent acute IS were recruited consecutively at four Stroke Units in western Sweden between 1998 and 2008 ($n=844$). Caucasian community controls ($n=668$) from the same geographic area were randomly selected from population registers to match the sex and average age of cases. For the first 600 cases, data on functional outcome (modified Rankin Scale (mRS)) after 3 and 24 months or stroke severity (Scandinavian Stroke Scale (SSS)) acutely were available. From these subjects, s-IGF1 levels in 407 patients and in 40 randomly selected controls were available from a previous study (4). The s-IGF1s of these subjects were analyzed in 2008 in one series of experiments. The frequency of previous hypertension, diabetes mellitus, and smoking was recorded. Participants or next of kin provided written informed consent. This study was approved by the Ethics Committee of the University of Gothenburg. Data and characteristics of each distinct subgroup of samples have been reported previously (4, 10, 11), and is also compiled in Table 1.

Genotyping was performed using the Golden Gate assay (Illumina, Inc., San Diego, CA, USA) at the SNP&SEQ Technology platform (www.genotyping.se), with support from Uppsala University and Knut and

Alice Wallenberg Foundation. The genotyping was performed blinded to case/control status. Allele frequencies in the different genotypes are shown in Table 2. The statistical power of the analysis was calculated using the Genetic Power Calculator at <http://pnu.mgh.harvard.edu/~purcell/gpc/> (12).

Eleven SNPs were selected to capture the common variation in *IGF1* based on data from the HapMap project on the CEU population (release 23) (13). The linkage disequilibrium (LD) blocks were defined using Haploview 4.1 and the method solid spine of LD (©2003–2006 Broad Institute of MIT and Harvard, MA). Nine of the 11 SNPs in *IGF1* were found to be situated in two different LD blocks. The remaining two single SNPs were on either side of these two blocks, making four LD 'blocks' in total (Supplementary Figure 1, see section on supplementary data given at the end of this article). Hence, to correct for multiple testing according to Bonferroni, the crude *P* values were multiplied by four. The 'Tagger' program in HaploView was used to select this minimal set of tagSNPs, such that all alleles had a minor allele frequency (MAF) > 0.1 and were correlated at an $r^2 > 0.8$.

Statistical evaluation was performed using SPSS versions 16.0 and 17.0 (SPSS, Inc.). Associations between single SNPs and the various factors were investigated using an additive model. Data on functional outcome was dichotomized (favorable outcome, mRS 0–2 vs unfavorable outcome, mRS 3–6).

Table 1 Baseline characteristics and missing data of SAHLIS participants in the overall samples, as well as in the subset of subjects with s-IGF1 data. There are more patients with complete genotype data than those with data on outcome as shown. Data on male (sex), presence of diabetes, hypertension, smoking, stroke severity (according to the Scandinavian Stroke Scale (SSS)), stroke outcome after 3 and 24 months (according to the modified Ranking scale (mRS), where mRS = 0–2 is a favorable outcome) are presented. Data are shown as mean 95% CI and s.d., or number (*n*) and percentage (%). Missing data are presented and based on total numbers included in the study. However, data on percentages of 'yes' or 'favorable outcome' are based only on the total numbers of subjects with available data.

	All subjects			Subjects (s-IGF1)	
	Controls	Patients		Controls	Patients
Total (<i>n</i>)	668	844	600	40	407
Age (years; mean $\pm$ s.d. ($\pm$ 95% CI))	56 $\pm$ 10.4 (0.8)	56.3 $\pm$ 10.5 (0.7)	56.7 $\pm$ 10.3 (0.8)	55.3 $\pm$ 12.6 (3.9)	54.9 $\pm$ 10.1 (1.0)
Sex (males; <i>n</i> (%))	392 (58.7)	554 (65.6)	385 (64.2)	24 (60.0)	259 (63.6)
Diabetes (<i>n</i> (%))					
Yes	33 (4.9)	153 (18.1)	114 (19)	1.0 (2.5)	75 (18.4)
Missing	2 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)
Hypertension (<i>n</i> (%))					
Yes	230 (34.4)	487 (57.7)	354 (59)	10 (25)	221 (54.3)
Missing	1 (0.1)	11 (1.3)	8 (1.3)	0 (0)	0 (0)
Currently smoking (<i>n</i> (%))					
Yes	131 (19.6)	324 (38.4)	233 (38.8)	11 (27.5)	157 (38.6)
Missing	0 (0)	4 (0.5)	3 (0.5)	0 (0)	0 (0)
Stroke severity (SSS; mean $\pm$ s.d. ($\pm$ 95% CI))			44.6 $\pm$ 12.1 (1.0)		46.4 $\pm$ 14.1 (1.4)
Missing			14 (2.3)		0 (0)
Stroke outcome (3 months; <i>n</i> (%))					
mRS 0–2			438 (77.0)		288 (75.2)
Missing			31 (5.2)		24 (5.9)
Stroke outcome (24 months; <i>n</i> (%))					
mRS 0–2			458 (77.4)		309 (79.8)
Missing			8 (1.3)		20 (4.9)

Table 2 Genotype frequencies for *IGF1* single nucleotide polymorphisms (SNP) in patients and controls. Descriptive data of the study regarding numbers of subjects with the specific alleles as indicated. Also the number of subjects with missing data is shown.

SNP information				Controls		Patients	
SNP no.	SNP name	Location	Genotype	<i>n</i>	% ^a	<i>n</i>	% ^a
1	rs2162679	Intron 1	A/A	466	71.4	574	72.1
			A/G	168	25.7	201	25.2
			G/G	18	2.7	21	2.6
			NA	16	2.3	48	5.6
2	rs12821878	Intron 2	A/A	47	7.0	44	5.2
			A/G	240	36.0	303	36.0
			G/G	379	56.9	493	58.6
			NA	2	0.2	4	0.4
3	rs1019731	Intron 2	A/A	18	2.7	18	2.1
			A/C	188	28.2	226	26.9
			C/C	460	69.0	596	70.9
			NA	2	0.2	4	0.4
4	rs7956547	Intron 2	A/A	374	56.2	447	53.2
			A/G	244	36.6	334	39.7
			G/G	47	7.0	59	7.0
			NA	3	0.4	4	0.4
5	rs5742632	Intron 2	A/A	387	58.1	461	54.9
			A/G	232	34.8	329	39.2
			G/G	46	6.9	49	5.8
			NA	3	0.4	5	0.5
6	rs9989002	Intron 2	A/A	55	8.2	73	8.7
			A/G	244	36.6	334	39.8
			G/G	367	55.1	431	51.4
			NA	2	0.2	6	0.7
7	rs7136446	Intron 2	A/A	300	45.1	344	41.0
			A/G	283	42.5	386	46.0
			G/G	82	12.3	109	12.9
			NA	3	0.4	5	0.5
8	rs10860865	Intron 2	A/A	46	6.9	57	6.7
			A/C	236	35.4	329	39.1
			C/C	384	57.6	454	54.0
			NA	2	0.2	4	0.4
9	rs5742671	Intron 2	A/A	22	3.3	26	3.0
			A/G	173	25.9	246	29.2
			G/G	471	70.7	568	67.6
			NA	2	0.2	4	0.4
10	rs1520220	Intron 3	C/C	464	69.6	559	66.5
			C/G	177	26.5	249	29.6
			G/G	25	3.7	32	3.8
			NA	2	0.2	4	0.4
11	rs6214	Exon 4	A/A	101	15.1	117	13.9
			A/G	301	45.1	394	46.9
			G/G	264	39.6	329	39.1
			NA	2	0.2	4	0.4

^aRegarding genotype frequencies for each SNP, the percentages are calculated with respect to the total number of genotyped controls or patients (excluding subject with missing data, not available (NA)). For NA the percentages are calculated relative total number of controls or patients.

Logistic regression was used to assess the relative frequencies of the major allele for each SNP in i) patients and controls, ii) patients surviving the IS, and iii) favorable vs unfavorable outcome. Stroke severity and s-IGF1 were evaluated by linear regression. Odds ratios (OR) or linear coefficients (*B*) and 95% CIs were with respect to the major allele and age- and sex-corrected. The vascular risk factor confounders of smoking, hypertension, and diabetes were also corrected for in an additional analysis. Assuming a multiplicative genetic model, the ORs that can be detected with 80% power at

a significance level of 5% are in the range of 1.23–1.37, with MAF=0.42–0.11 of the high risk allele. All genotype distributions were compatible with the Hardy–Weinberg equilibrium ($P > 0.05$, not shown), and the genotyping success rates were 95–100% (Table 2). The statistical significance level was $P < 0.05$. For further details on study design, patient examination, genotyping methods, protein measurements, and statistical analyses please see the [Supplementary information](#), see section on [supplementary data](#) given at the end of this article.

Results

Baseline characteristics of SAHLSIS are summarized in Table 1. Age and sex distributions were similar in controls and patients, including the group in which s-IGF1 was analyzed. However, hypertension, diabetes, and smoking were more common in patients, as would be expected. Eleven SNPs were selected to capture the common variation in the *IGF1* gene based on the CEU population from the HapMap project (Supplementary Figure 1). The genotype frequencies in controls and IS patients are presented in Table 2.

The s-IGF1 levels in SAHLSIS have been previously reported (4) and were used to investigate whether *IGF1* SNPs are associated with s-IGF1 levels. In the healthy controls, the major allele of rs7136446 was associated with elevated s-IGF1 (Table 3). However, in IS cases, there were no significant associations between s-IGF1 and any of the *IGF1* SNPs either acutely or at 3 months post-stroke (Table 3). Additional correction for smoking, hypertension, and diabetes did not alter the associations (not shown).

To identify possible associations between the *IGF1* SNPs and IS, we performed multivariable logistic regression adjusting for age and sex. Occurrence of IS was not associated with any *IGF1* SNP (Table 4). Additional correction for smoking, hypertension, and diabetes did not alter the association (not shown).

Elevated s-IGF1 has been shown to associate with enhanced post-stroke recovery. In analogy to that, different *IGF1* expression due to genotype variants could be associated with neuroprotective action and reduce acute stroke severity. This was evaluated by linear regression analysis of the SSS with respect to *IGF1* SNPs (Table 5). Interestingly, there was a trend for the association of the major allele of rs9989002 (i.e. SNP 6) with less severe IS, i.e. higher SSS scores. Additional correction for smoking, hypertension, and diabetes gave similar associations (not shown).

Association between *IGF1* variants and post-stroke outcome was analyzed for each SNP with regard to favorable functional outcome (i.e. mRS=0–2) after 3 and 24 months. The major allele of SNP rs7136446 was associated with favorable functional outcome 24 months after IS, after correction for age, sex, smoking, hypertension, and diabetes (i.e. SNP 7, Table 6). However, after Bonferroni adjustment of *P* values, no SNP showed association with functional outcome after 3 months (Table 6).

Another measure of functional outcome after stroke is survival. When survival 3- and 24-months post-stroke was analyzed, no association was detected for any of the SNPs (after Bonferroni's adjustment, *P*>0.3 for all SNPs, not shown).

Discussion

This is the first study to investigate the genetic variation at the *IGF1* locus in relation to IS and s-IGF1 using a tagSNP approach. The main finding was that the SNP rs7136446 showed association to functional outcome 24 months after index stroke. In healthy controls, the major allele of the same SNP was associated with higher levels of s-IGF1. In contrast, in IS patients, rs7136446 was not associated with s-IGF1. For the major allele of rs9989002, there was a trend toward an association with less initial stroke severity. None of the SNPs analyzed were associated with occurrence of IS compared with controls.

Here, we present the novel data that functional independence (mRS 0–2) 24 months after index stroke is associated with the major allele of rs7136446. A similar trend was observed 3 months after index stroke, however this did not withstand correction for multiple testing (crude, *P*=0.029 and adjusted, *P*=0.116). If this SNP is associated with post-stroke recovery, one would expect the association to increase with time after stroke. Indeed, this is what we observed.

Table 3 Association between *IGF1* SNPs and serum IGF1 levels in patients and controls in SAHLSIS. Linear coefficients (*B*) and 95% CIs are calculated with respect to the major allele corrected for age and sex. The *B* value is expressed so that a positive value represents elevated s-IGF1 in the major allele.

SNP no.	Acute patients (n=384)	<i>P</i> values		3 months patients (n=369)	<i>P</i> values		Healthy controls (n=40)	<i>P</i> values	
	<i>B</i> (±95% CI)	Crude	Adjusted	<i>B</i> (±95% CI)	Crude	Adjusted	<i>B</i> (±95% CI)	Crude	Adjusted
1	7.5 (−4.4 to 19.4)	0.217	>0.5	2.1 (−8.6 to 12.8)	>0.5	>0.5	−3.2 (−27.2 to 20.8)	>0.5	>0.5
2	−0.3 (−10.4 to 9.9)	>0.5	>0.5	0.1 (−9.1 to 9.2)	>0.5	>0.5	−19.9 (−37.1 to −2.7)	0.025	0.098
3	−5.7 (−17.7 to 6.4)	0.356	>0.5	−1.6 (−12.3 to 9.0)	>0.5	>0.5	−8.6 (−31.4 to 14.3)	0.453	>0.5
4	5.0 (−4.8 to 14.8)	0.316	>0.5	1.9 (−7.2 to 10.9)	>0.5	>0.5	5.1 (−14.1 to 24.2)	>0.5	>0.5
5	−0.2 (−10.6 to 10.1)	>0.5	>0.5	−0.8 (−10.4 to 8.8)	>0.5	>0.5	4.7 (−14.8 to 24.2)	>0.5	>0.5
6	−1.4 (−10.7 to 8.0)	>0.5	>0.5	−4.7 (−13.3 to 3.9)	0.287	>0.5	−0.5 (−18.0 to 17.0)	>0.5	>0.5
7	−4.4 (−13.3 to 4.4)	0.325	>0.5	−5.4 (−13.5 to 2.8)	0.197	>0.5	19.3 (5.2 to 33.4)	0.009	0.034
8	−4.3 (−14.0 to 5.5)	0.392	>0.5	−6.7 (−15.6 to 2.2)	0.137	>0.5	9.1 (−10.9 to 29.2)	0.361	>0.5
9	−3.5 (−15.2 to 8.3)	>0.5	>0.5	−5.5 (−16.4 to 5.3)	0.314	>0.5	9.7 (−11.3 to 30.8)	0.354	>0.5
10	−6 (−17.1 to 5.1)	0.29	>0.5	−7.0 (−17.2 to 3.2)	0.179	>0.5	5.0 (−22.9 to 32.9)	>0.5	>0.5
11	2.3 (−6.9 to 11.5)	>0.5	>0.5	3.7 (−4.5 to 12.0)	0.37	>0.5	14.2 (−1.5 to 30.0)	0.076	>0.5

Crude *P* values are adjusted for multiple comparisons according to Bonferroni as shown.

Table 4 Minor allele frequencies (MAF) for *IGF1* SNPs in controls and patients in SAHLSIS, as well as odds ratios (ORs) for the association between the *IGF1* SNPs and ischemic stroke. MAF are shown in the analyses with logistic regression. ORs and 95% CIs are calculated with respect to the major allele corrected for age and sex. For IS control/patient comparisons, an OR > 1 represents an increased frequency of the major allele in the controls (i.e. associates with protection against IS).

SNP no.	MAF (%) control	MAF (%) patient	OR ($\pm$ 95% CI)	P values	
				Crude	Adjusted
1	15.6	15.3	1.02 (0.83–1.25)	> 0.5	> 0.5
2	25.1	23.3	1.11 (0.94–1.31)	0.233	> 0.5
3	16.8	15.6	1.09 (0.9–1.33)	0.387	> 0.5
4	25.4	26.9	0.92 (0.78–1.09)	0.326	> 0.5
5	24.4	25.4	0.94 (0.80–1.11)	0.466	> 0.5
6	26.6	28.6	0.90 (0.77–1.05)	0.181	> 0.5
7	33.6	36.0	0.90 (0.77–1.05)	0.168	> 0.5
8	24.6	26.4	0.91 (0.77–1.07)	0.237	> 0.5
9	16.3	17.7	0.89 (0.74–1.08)	0.251	> 0.5
10	17.0	18.6	0.90 (0.75–1.08)	0.253	> 0.5
11	37.8	37.4	1.01 (0.87–1.18)	> 0.5	> 0.5

Both crude and P values adjusted for multiple comparisons according to Bonferroni are shown.

The magnitude of association is moderate (OR $\sim$ 1.5), but still this SNP is probably of potential significance in relation with IS because the same SNP is associated with endogenous s-IGF1. Statistical correction for the confounding covariates cannot adjust for the presence of a specific SNP which has been presented life-long. However, covariate correction can be used to test whether an association is mediated by the confounders. If vascular diseases were to mediate the effect of rs713446 on outcome, the association would be weaker after correction and *vice versa*. Therefore, the increased strength of association between rs713446 and outcome after correction for vascular risk factors indicates an association that is primarily independent of vascular confounders.

Previously, it was shown that another measure of outcome, namely survival, is affected by genetic variation in *IGF1*. Specifically, increased death rates after IS were reported in non-carriers of the 192 bp CA-repeat (8). We did not find any statistically significant associations between any SNP and survival. However, in contrast to the aforementioned study, SAHLSIS is a study of relatively young stroke sufferers (mean age 56 years). Therefore, the number of deceased patients after 3 and 24 months is relatively low (1.2 and 4.1% respectively). Thus, the lack of association may simply reflect an overall lack of power regarding survival in this analysis.

Higher s-IGF1 concentrations were associated with the major allele of rs7136446 in healthy controls in this study. This is opposite to what was reported in a study that included only healthy, premenopausal female participants, wherein the major alleles of rs7136446 and rs9989002, both analyzed in our study, were associated with slightly (5–7%) lower s-IGF1 levels (14). Verheus *et al.* did not, however, correct for multiple testing in the analysis of 16 SNPs, and also the found differences in s-IGF1 were quite small in magnitude.

Interestingly, the major allele of rs7136446 has recently been associated with maximal muscle force production in 148 Finnish males (15). One could speculate that patients with higher maximal muscle force may recover muscle strength faster, and thus gain better functional independence. This would be compatible with our results, but of course primary neuronal mechanisms could also account for the association to outcome.

In control subjects, circulating s-IGF1 was 37% higher (not shown) in the most common genotype than in the least common genotype. However, this association was not present at all in the patients. Although the power to detect differences in the healthy control group was lower ($n=40$) than that in patients ($n=407$), the associations appear genuine as we corrected for multiple comparisons (Table 3). As

Table 5 Association between *IGF1* SNPs and baseline stroke severity as measured by the Scandinavian Stroke Scale (SSS). Linear coefficients (B) and 95% CIs are calculated with respect to the major allele corrected for age and sex. For stroke severity the B value is expressed so that a positive value represents less severe IS in the major allele.

SNP no.	Stroke severity SSS B ($\pm$ 95% CI)	P values	
		Crude	Adjusted
1	1.7 (–0.2 to 3.7)	0.084	0.336
2	1.2 (–0.4 to 2.9)	0.137	> 0.5
3	1.4 (–0.5 to 3.3)	0.151	> 0.5
4	0.8 (–0.8 to 2.4)	0.347	> 0.5
5	0.4 (–1.2 to 2.1)	> 0.5	> 0.5
6	1.8 (0.3 to 3.3)	0.022	0.086
7	1.2 (–0.2 to 2.7)	0.098	0.391
8	–1.4 (–3.0 to 0.2)	0.077	0.309
9	–0.8 (–2.7 to 1.1)	0.4	> 0.5
10	1.0 (–0.9 to 2.8)	0.303	> 0.5
11	–0.3 (–1.7 to 1.2)	> 0.5	> 0.5

Crude P values are adjusted for multiple comparisons according to Bonferroni as shown.

Table 6 Association between *IGF1* SNPs and favorable post-stroke outcome at 3 and 24 months after index stroke in SAHLSIS. Minor allele frequencies (MAF) are shown for mRS 0–2 and 3–6. Odds ratios (OR) and 95% CIs are calculated with respect to the major allele corrected for age and sex. An OR > 1 represents the OR of having a favorable outcome (i.e. mRS 0–2) with respect to the major allele.

SNP no.	mRS 3 months					mRS 24 months				
	MAF (%)			P values		MAF (%)			P values	
	mRS 0–2	mRS 3–6	OR ($\pm$ 95% CI)	Crude	Adjusted	mRS 0–2	mRS 3–6	OR ($\pm$ 95% CI)	Crude	Adjusted
1	14.7	16.8	1.19 (0.81–1.75)	0.368	> 0.5	15.1	15.6	1.06 (0.72–1.56)	> 0.5	> 0.5
2	22.6	27.9	1.33 (0.97–1.83)	0.078	0.312	22.9	25.4	1.14 (0.83–1.58)	0.417	> 0.5
3	15.1	17.6	1.22 (0.84–1.77)	0.295	> 0.5	14.6	18.7	1.35 (0.94–1.94)	0.107	0.427
4	26.3	27.9	1.08 (1.48–0.79)	> 0.5	> 0.5	26.4	27.2	1.05 (0.77–1.44)	> 0.5	> 0.5
5	24.3	24.8	1.02 (0.73–1.42)	> 0.5	> 0.5	25.2	23.3	0.91 (0.65–1.27)	> 0.5	> 0.5
6	26.4	32.1	1.31 (1.77–0.97)	0.076	0.304	27.1	29.7	1.15 (0.85–1.55)	> 0.5	> 0.5
7	32.8	40.1	1.38 (1.03–1.85)	0.029	0.116	33.0	41.0	1.42 (1.07–1.9)	0.017	0.067^a
8	24.5	28.2	1.22 (0.89–1.67)	0.217	> 0.5	25.2	26.1	1.05 (0.77–1.44)	> 0.5	> 0.5
9	16.4	18.7	1.18 (0.81–1.71)	0.387	> 0.5	17.0	17.2	1.01 (0.7–1.47)	> 0.5	> 0.5
10	17.0	19.5	1.18 (0.83–1.68)	0.351	> 0.5	17.4	17.9	1.03 (0.72–1.47)	> 0.5	> 0.5
11	38.3	34.7	0.85 (0.63–1.14)	0.284	> 0.5	38	37.7	0.99 (0.74–1.32)	> 0.5	> 0.5

Crude P values are adjusted for multiple comparisons according to Bonferroni as shown.

^aAfter additional correction for confounders (smoking, hypertension, and diabetes) and Bonferroni's adjustment, this analysis reached statistical significance ($P=0.049$, OR 1.46, 95% CI 1.09–1.96). $P<0.05$ values after Bonferroni adjustment are in bold.

s-IGF1 is regulated by environmental factors (5), this may indicate that IS *per se* strongly affects the normal impact of this SNP on s-IGF1. Indeed, after experimental IS, local IGF1 in the brain is upregulated (16). As the blood–brain barrier is often injured after IS a local increase of IGF1 may disseminate into the circulation. In this way IS *per se* may be such a strong regulator of IGF1 synthesis, that a normal endogenous association between rs7136446 and s-IGF1 disappears.

While specific SNPs were associated with either stroke severity, stroke outcome, or s-IGF1, we did not observe any statistically significant associations between IS patients and controls in terms of frequency of the *IGF1* SNPs variations. This analysis is considered to be an indirect assessment of the relative risk of being afflicted by IS comparing genotypes of IS patients and healthy controls. This is in contrast to an earlier study which reported an association between noncarriers of a 192 bp CA-repeat in the *IGF1* promoter and risk of IS (8). Similarly, the absence of the CA-repeat has been associated with MI, as well as with lower levels of s-IGF1 (7). The tagSNP rs7965399 is in LD with the 192 bp CA-repeat (17); however it is uncommon in subjects of Caucasian origin (MAF 0.02, HapMap). Therefore, it did not meet the tagSNP selection criteria and was not included in this study. Two other SNPs, rs7956547 and rs2162679, have been reported to associate with MI in a Japanese study of 320 patients (18), but these two SNPs did not display significant associations with IS in this study. Notably, the associations with MI were not corrected for multiple testing of six SNPs, and are therefore uncertain.

Methodological and statistical strengths of this study include consecutive recruitment of well-characterized and relatively young IS cases and population-based

controls. This, in combination with the very high hospitalization rate (84–95%) for stroke (especially < 70 years) in Sweden (19), makes selection bias unlikely. In addition, few patients were lost to the follow-up. Weaknesses include the relatively small sample size and lack of replication in another geographic area. Although the associations we report are relatively weak, it should be noted that strict correction for multiple testing was made, which is in contrast to earlier studies of genetic variation.

In conclusion, our study indicates that the major allele of rs7136446 is associated with favorable functional outcome 24-months post-stroke and with s-IGF1 in healthy controls but not in patients. The results point toward a role for IGF1 in post-stroke outcome but not for stroke risk, but this needs validation in independent samples.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0486>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

N D Åberg and S Olsson planned, designed, performed, analyzed, and wrote the study. D Åberg, M Nilsson, and C Blomstrand planned and wrote the study. K Jood analyzed and wrote the study. T M Stanne and J Svensson wrote the study. J Isgaard and C Jern planned, designed, and wrote the study. All authors contributed to and have approved the final manuscript. N D Åberg and S Olsson contributed equally and are joint first authors, and J Isgaard and C Jern are in joint senior position.

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