

## RESEARCH ARTICLE

## Open Access

# Variations in apolipoprotein D and sigma non-opioid intracellular receptor 1 genes with relation to risk, severity and outcome of ischemic stroke

Håkan Lövkvist<sup>1,2,7\*</sup>, Ann-Cathrin Jönsson<sup>3</sup>, Holger Luthman<sup>4</sup>, Katarina Jood<sup>5</sup>, Christina Jern<sup>5</sup>, Tadeusz Wieloch<sup>6</sup> and Arne Lindgren<sup>1,2</sup>

## Abstract

**Background:** In experimental studies, the apolipoprotein D (APOD) and the sigma receptor type 1 (SIGMAR1) have been related to processes of brain damage, repair and plasticity.

**Methods:** We examined blood samples from 3081 ischemic stroke (IS) patients and 1595 control subjects regarding 10 single nucleotide polymorphisms (SNPs) in the *APOD* (chromosomal location 3q29) and *SIGMAR1* (chromosomal location 9p13) genes to find possible associations with IS risk, IS severity (NIHSS-score) and recovery after IS (modified Rankin Scale, mRS, at 90 days). Simple/multiple logistic regression and Spearman's rho were utilized for the analyses.

**Results:** Among the SNPs analyzed, rs7659 within the *APOD* gene showed a possible association with stroke risk (OR = 1.12; 95% CI: 1.01-1.25;  $P = 0.029$ ) and stroke severity (NIHSS  $\geq 16$ ) (OR = 0.70; 95% CI: 0.54-0.92;  $P = 0.009$ ) when controlling for age, sex and vascular risk factors for stroke. No SNP showed an association with stroke recovery (mRS).

**Conclusions:** We conclude that the SNP rs7659 within the *APOD* gene might be related to risk and severity of ischemic stroke in patients.

**Keywords:** Stroke, Genetics, *APOD*, *SIGMAR1*, mRS, NIHSS

## Background

Stroke is a major cause of death and the main cause of adult disability. Approximately 20 to 30% of all ischemic stroke patients die in the acute stages of the stroke episode while more than one third of those who survive remain dependent of daily next-of-kin support or community care six months after stroke onset [1,2]. Still, five years after stroke onset, two thirds of the survivors have some neurologic impairment and disability [3]. Recanalization of occluded vessels after embolic stroke is the only therapeutic intervention available to treat acute ischemic stroke (IS), while no pharmacological treatment that stimulate brain repair or plasticity and that might enhance recovery of lost

function is at hand. However, rehabilitative training such as task-oriented practice [4] and long-term progressive resistance training [5], may enhance recovery of lost brain functions.

The multifactorial and complex features of stroke impose a considerable challenge for the understanding of the pathology and for the development of new therapies. Multiple environmental factors including co-morbidities increase the risk of stroke [6]. Likewise, stroke severity is dependent on the type of stroke, density of ischemia and duration of vessel occlusion, and is also influenced by several toxic mechanisms, most identified in experimental animal models of stroke [7]. Finally, brain repair involves mechanisms differentially activated in time and space, and include inflammation, brain remodelling and relearning of activated neural networks [8,9]. Genetic factors influence the impact of these innate cellular mechanisms and environmental factors, affecting risk for stroke, as well as the

\* Correspondence: [hakan.lovkvist@skane.se](mailto:hakan.lovkvist@skane.se)

<sup>1</sup>Department of Clinical Sciences Lund, Neurology, Lund University, Lund, Sweden

<sup>2</sup>Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden

Full list of author information is available at the end of the article

severity of brain damage and the subsequent functional outcome [10].

Previous clinical studies have shown that allelic variants within the *PDE4D* gene, chromosome 9p21 and the ABO locus may be associated with IS risk [11-13]. Also variants in *HDAC9*, as well as in chromosome 6p21.1 and 9p21, have shown association with large vessel IS [14-17], and variants in *PITX2* and *ZFXH3* may affect cardioembolic stroke risk according to other studies made [18,19]. In contrast to the situation regarding IS risk, reports on genetic factors contributing to outcome after stroke are scarce. However, a study has reported that the apolipoprotein E (APOE)  $\epsilon$ 2 polymorphism might contribute to variability in outcomes after hemorrhagic stroke [20]. Likewise, an association was found between polymorphisms of the *COX-2* and Glycoprotein IIIa genes on functional outcome 90-days after IS [21]. These studies clearly demonstrate the potential of genetic analysis in identifying mechanisms involved in functional recovery of stroke patients. More recently, genetic variations in the human dopamine system were associated with motor learning after stroke [22]. This indicates the potential of genetic analysis in identifying relevant mechanisms involved in stroke and therapeutic targets.

The apolipoprotein D (APOD) has been suggested to be related to stroke not only by virtue of its ability to influence trafficking of lipids but also by modulating oxidative stress, synaptic plasticity and cell death [23,24]. Moreover, APOD appears to be associated with several neurological diseases and normal ageing [25], schizophrenia [26], Alzheimer's disease (AD) [27,28] and Parkinson's disease (PD) [29]. APOD levels increase with age [30], with higher levels in women than in men [25]. Also, in experimental models of stroke [31] and trauma [32], the levels of APOD are elevated. Polymorphisms of the *APOD* gene have been associated with increased risk of AD [28,33]. The general increase of APOD levels in a broad range of disease states suggest that the protein may be induced in response to stress. Indeed, APOD appears to be an anti-oxidant [34] dependent on the integrity of the Met93 of this lipoprotein [35]. In animal models of stroke, increased APOD levels are correlated with better functional recovery, implying a possible function of APOD in the repair processes after stroke [31].

Whilst the apolipoproteins are trafficking lipids among cells [23], the sigma receptor type 1 (SIGMAR1, sometimes also denoted SIG1R or OPR1) is involved in signalling and trafficking of lipids and proteins within cells [36]. Through these mechanisms the SIGMAR1 may modulate cell death and brain plasticity in experimental models of stroke [37]. The SIGMAR1 appears to play a central role in central nervous system (CNS) diseases since polymorphisms in the *SIGMAR1* gene are associated with depression [38], schizophrenia [39] and alcoholism [40] as well as AD [41].

With this background we aimed to investigate whether polymorphisms in the *APOD* and *SIGMAR1* genes influence stroke severity as well as functional outcome in patients suffering from IS. By including a group of control subjects we also assessed these polymorphisms' possible impact on IS risk.

## Methods

### Study subjects

The study was approved by the ethical committee at Lund University, Lund (application 543/2008). We included 2241 consecutive first-ever IS patients of all ages from Lund Stroke Register (LSR) and 840 first-ever or recurrent IS patients below 70 years of age from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), Gothenburg. Both LSR and SAHLSIS have been described previously [42,43]. Patients were included if they had clinical symptoms of IS, confirmed by CT or MR or autopsy of the brain, provided DNA for analysis, and if they or their next of kin had given informed consent to participate. Exclusively for the IS risk association assessments we also included control subjects from the same geographical areas with age and gender distribution similar to those in the IS cohort. The 1595 control subjects (929 from LSR, 666 from SAHLSIS) were randomly selected from Swedish population registers from the same areas and matched for age and gender to the patients. The SAHLSIS sample included younger participants (with range 18–69 years) than the LSR sample (with range 17 to 102 years). The proportion of men was thus larger in the SAHLSIS sample (Table 1).

### Definition of stroke severity and stroke recovery (outcome)

For LSR patients, initial stroke severity was assessed using the NIH stroke scale (NIHSS) in the acute phase after stroke onset [44]. For SAHLSIS patients, initial stroke severity was assessed using the Scandinavian Stroke Scale (SSS) [45]. These SSS scores were transformed to NIHSS scores through the algorithm  $NIHSS = 25.68 - 0.43 * SSS$  [46]. A NIHSS score of 8 or above but below 16 was considered to indicate a moderately severe stroke, and a score of 16 or above was considered to indicate a severe stroke [47].

For SAHLSIS, mRS at 3 months was assessed using the original scale 0–5 at a follow-up visit with a neurologist. For LSR, stroke outcome was assessed using Riksstroke data at 3 months after stroke. We used a translation algorithm to calculate mRS grades from a set of self-reported functional outcome questions available in Riksstroke data [48]. The Riksstroke data do not distinguish between mRS-grades 0, 1 and 2. However, as mRS-grade 2 is regarded as the upper limit for independence of help/support and the patient disability information relevant for this study is provided by mRS-grades 3, 4 and 5, we merged

**Table 1 Characteristics of control subjects and ischemic stroke (IS) cases**

	LSR		SAHLSIS		Combined	
	Controls (N = 929)	IS cases (N = 2241)	Controls (N = 666)	IS cases (N = 840)	Controls (N = 1595)	IS cases (N = 3081)
<b>Age</b> , Median (min, max)	76 (17, 96)	76 (18, 102)	58 (18, 70)	58 (18, 69)	66 (17, 96)	69 (18, 102)
<b>Male sex</b> , Number (%)	529 (57)	1169 (52)	391 (59)	550 (66)	920 (58)	1719 (56)
<b>Diabetes mellitus</b> , n	925	2139	664	840	1589	2979
Number (%)	69 (8)	550 (26)	33 (5)	153 (18)	102 (6)	703 (24)
<b>Hypertension</b> , n	925	2183	665	829	1590	3012
Number (%)	438 (47)	1479 (68)	230 (35)	485 (58)	668 (42)	1964 (65)
<b>Current smoking</b> , n	927	2193	666	836	1593	3029
Number (%)	92 (10)	423 (19)	131 (20)	323 (39)	223 (14)	746 (25)
<b>NIHSS at stroke onset</b> , n	–	1983	–	581	–	2564
0–7, Number (%)	–	1482 (75)	–	448 (77)	–	1930 (75)
8–15, Number (%)	–	338 (17)	–	92 (16)	–	430 (17)
16–, Number (%)	–	163 (8)	–	41 (7)	–	204 (7)
<b>mRS at 3 months</b> , n	–	1157	–	565	–	1722
0–2, Number (%)	–	625 (54)	–	435 (77)	–	1060 (62)
3, Number (%)	–	196 (17)	–	80 (14)	–	276 (16)
4, Number (%)	–	111 (10)	–	41 (7)	–	152 (9)
5, Number (%)	–	92 (8)	–	2 (<1)	–	94 (5)
Deceased, Number (%)	–	133 (12)	–	7 (1)	–	140 (8)

LSR = Lund Stroke Register, SAHLSIS = the Sahlgrenska Academy Study on Ischaemic Stroke, mRS = modified Rankin Scale, NIHSS = NIH stroke scale, N = gross sample size, n = net sample size after removal of missing values. All percentages are based on net sample sizes.

mRS-values 0, 1 and 2 into a value of 1 [49]. In addition to the original mRS grades 0–5, we added mRS grade 6 for individuals who had died at follow up for both samples.

### Phenotypes

Definitions of intermediate phenotypes diabetes mellitus, hypertension and current smoking, and IS pathogenetic subtypes (i.e. large vessel disease, LVD; small vessel disease, SVD; and cardioembolic stroke, CE; have been described previously [11,50,51].

### Selection of genetic variants and genotyping

Seven SNPs in *APOD* and five SNPs in *SIGMAR1* (or in the immediate vicinity of these regions) were selected using two different criteria: (1) SNPs serving as markers were selected based on their low pairwise linkage disequilibrium and a population frequency of 5% or more for the two gene regions (N = 7); (2) SNPs representing non-synonymous genetic variants with low population frequency but still above 0.1% in European populations were chosen based on their probable impact on protein function (N = 6). One of these latter non-synonymous variants, rs1800866 in *SIGMAR1*, is frequent enough to also be used as a marker. The genotypings were performed at our local lab in Malmö, Sweden using Sequenom technology, except for

rs76929107 at the *APOD* locus and rs1800866 at the *SIGMAR1* locus that were genotyped at LGC Genomics (former KBioscience), UK (<http://www.lgcgenomics.com>), using IPLEX on a MassARRAY platform (Sequenom, San Diego, CA, USA).

We scored the minor allele count of each SNP, i.e. 2, 1 or 0, and used these in additive models. Monomorphic SNPs were excluded from further analyses.

### Statistical methods

All included SNPs were tested for possible departure from Hardy-Weinberg equilibrium by chi-square test with one degree of freedom. These tests were performed on the control subjects included solely for the IS risk association analyses.

The possible association of each selected SNP with IS risk (i.e. IS patients versus control subjects) was analyzed by use of simple logistic regression, and multiple logistic regression controlling for age, gender, diabetes mellitus, hypertension and current smoking [11]. For the stroke severity response variable we used Spearman rank correlation as well as simple and logistic multiple regression with dichotomized stroke severity response (with risk category defined by NIHSS  $\geq$  8 and NIHSS  $\geq$  16, respectively) [47]. We also assessed functional outcome in a likewise manner (with risk category defined as mRS  $\geq$  3).

By using non-parametric statistics for the assessments of the possible impact of polymorphisms on the NIHSS and mRS scores, we were able to obtain effect measures and *P*-values that were not distorted by incorrect assumptions about these non-continuous variables.

SNP rs7659 was significantly associated with stroke severity in a first-step test. We therefore performed subsequent analyses involving subgroups including study group, gender, and age (</≥70 years) [50,52]. SPSS software

(PASW/SPSS, version 18, IBM Corporation, Armonk, NY, USA) was used as a computational tool for these assessments.

## Results

### Ischemic stroke risk

Table 2 displays (1) the frequencies of all ten non-monomorphic SNPs for LSR and SAHLSIS joined together, and (2) the results of association analyses of these SNP

**Table 2 Analysis of association between ischemic stroke risk and ten *APOD* and *SIGMAR1* SNPs**

SNP*	Allele pair	Control subj. number of genotypes	IS patients number of genotypes	Crude OR (95% CI)	Multiple LR** OR (95% CI)
<b><i>SIGMAR1</i>:</b>					
rs11559048	CC	1530	2695	0.51 (0.21-1.26)	0.58 (0.91-1.20)
	CT	8	9	<i>P</i> = 0.145	<i>P</i> = 0.491
	TT	1	-		
rs1800866	TT	1128	2141	1.03 (0.92-1.16)	1.02 (0.90-1.16)
	TG	401	795	<i>P</i> = 0.615	<i>P</i> = 0.768
	GG	46	88		
rs12001648	CC	1393	2420	0.95 (0.79-1.15)	0.85 (0.70-1.04)
	CT	165	286	<i>P</i> = 0.595	<i>P</i> = 0.120
	TT	9	8		
rs7036351	GG	1130	1944	1.03 (0.91-1.16)	1.02 (0.89-1.16)
	GA	399	693	<i>P</i> = 0.675	<i>P</i> = 0.789
	AA	40	77		
rs3808873	GG	833	1475	1.00 (0.89-1.11)	0.98 (0.88-1.10)
	GA	491	887	<i>P</i> = 0.950	<i>P</i> = 0.779
	AA	91	153		
<b><i>APOD</i>:***</b>					
rs76929107	CC	1540	2943	1.06 (0.74-1.51)	1.10 (0.75-1.59)
	CT	44	91	<i>P</i> = 0.761	<i>P</i> = 0.634
	TT	1	1		
rs5952	TT	1548	2710	1.90 (0.52-6.92)	2.23 (0.57-8.75)
	TC	3	10	<i>P</i> = 0.329	<i>P</i> = 0.251
	CC	-	-		
rs34697430	GG	435	769	1.00 (0.92-1.09)	1.01 (0.92-1.11)
	GA	784	1317	<i>P</i> = 0.966	<i>P</i> = 0.895
	AA	349	623		
rs7659	AA	803	1306	1.11 (1.01-1.22)	1.12 (1.01-1.25)
	AG	617	1177	<i>P</i> = 0.038	<i>P</i> = 0.029
	GG	130	240		
rs823510	TT	880	1576	0.99 (0.89-1.09)	0.99 (0.89-1.11)
	TG	590	981	<i>P</i> = 0.805	<i>P</i> = 0.884
	GG	82	164		

\*)All genotypes were conforming to the Hardy-Weinberg equilibrium criterion (with *P* = 0.093 or more when using a chi-square test on the control subjects), except for rs11559048 that showed a significant Hardy-Weinberg disequilibrium (*P* < 0.001).

\*\*)ORs obtained by multiple logistic regression analysis controlling for covariates age, gender, diabetes mellitus, hypertension and current smoking.

\*\*\*)Two additional *APOD* encoding SNPs, rs5954 and rs5955, were genotyped but not included in this study due to monomorphic traits.

frequencies against IS risk. All SNPs except rs11559048 conformed to the Hardy-Weinberg equilibrium criterion (Table 2). One SNP, rs7659 within the *APOD* gene region, was associated with IS risk (OR = 1.11; 95% CI: 1.01-1.22;  $P = 0.038$  when tested by univariate analysis, and OR = 1.12; 95% CI: 1.01-1.25;  $P = 0.029$  when using multiple logistic regression analysis controlling for covariates age, gender, diabetes mellitus, hypertension and current smoking). However, none of these  $P$ -values were significant when considering Bonferroni correction for multiple testing.

### Stroke severity and functional outcome

The results of the assessments of the ten non-monomorphic SNPs of *APOD* and *SIGMAR1* against stroke severity are presented in Table 3. Analyses using Spearman's Rho suggested that variations in one SNP, the *APOD*-encoding rs7659, is associated with NIHSS (Rho = -0.048;  $P = 0.023$ ), while multiple logistic regression considering a NIHSS cut-off point of 16 provided an OR = 0.70; 95% CI: 0.54-0.92;  $P = 0.009$ . Also, an association (OR = 0.65; 95% CI: 0.46-0.91;  $P = 0.012$ ) between the *SIGMAR1* encoding rs12001648 and medium-severe stroke onset risk (NIHSS  $\geq 8$ ) was found. When a subgroup of patients aged 70 years or above was tested against the severe IS onset indicator (defined as NIHSS  $\geq 16$ ), an association between stroke severity and variants of SNP rs7659 within the *APOD* region was noticed (OR = 0.63; 95% CI: 0.45-0.88;  $P = 0.006$ ). These results are shown in Table 4. Still, none of these tests implied any significant association when

considering Bonferroni-correction. However, when considering the pathogenetic stroke main subtype CE as a subgroup for assessment we found SNP rs7659 to be significantly associated with stroke severity defined by the NIHSS > 16 cut point (OR = 0.59; 95% CI: 0.40-0.85;  $P = 0.005$ ; results shown in Table 4).

None of the ten non-monomorphic SNPs significantly affected functional outcome after stroke (Table 5).

### Discussion

With this large study sample comprising a total of 3081 IS patients we were able to perform analyses aimed to find possible impact of selected polymorphisms encoding for *APOD* and *SIGMAR1* on (1) stroke severity and (2) stroke outcome, respectively. By adding 1595 control subjects not suffering any stroke onset from the same geographical areas and with the same age and gender distribution as the IS patients, we have also been able to examine possible effect of these polymorphisms on IS risk.

The conclusions from the non-parametric Spearman correlation analyses (NIHSS and mRS-scores, respectively, against SNP variations) were based upon  $P$ -values obtained by using a "conservative" approach providing high adequacy at the cost of some statistical power loss. The transformation of these numerical variables into dichotomized indicators (coded 1 or 0) also caused information loss. On the other hand this enabled us to focus on possible threshold effects when examining e.g. the genetic effect on IS severity (by using NIHSS cutpoints 0-7 vs. 8 or above, or 0-15 vs. 16 or above).

**Table 3 Analysis of association between stroke severity (NIHSS) and ten *APOD* and *SIGMAR1* SNPs**

SNP	NIHSS score		NIHSS; dichotomous indicator of medium-severe (vs. mild) ischemic stroke onset (NIHSS $\geq 8$ )				NIHSS; dichotomous indicator of severe (vs. mild-medium) ischemic stroke onset (NIHSS $\geq 16$ )				
	Estimated Spearman's Rho	$P$ -value	Simple logistic regression OR (95% CI)	$P$ -value	Multiple logistic regression OR (95% CI)	$P$ -value	Simple logistic regression OR (95% CI)	$P$ -value	Multiple logistic regression OR (95% CI)	$P$ -value	
<b><i>SIGMAR1</i>:</b>											
rs11559048	0.023	0.284	1.86 (0.44-7.79)	0.399	1.74 (0.40-7.69)	0.462	—*)	—	—*)	—	
rs1800866	-0.012	0.554	1.02 (0.87-1.22)	0.772	0.98 (0.81-1.17)	0.804	0.97 (0.75-1.25)	0.822	0.87 (0.64-1.17)	0.347	
rs12001648	-0.044	0.040	0.77 (0.56-1.05)	0.094	0.65 (0.46-0.91)	0.012	1.01 (0.66-1.56)	0.967	0.75 (0.48-1.27)	0.283	
rs7036351	0.000	0.984	1.04 (0.87-1.25)	0.670	0.99 (0.81-1.21)	0.922	0.94 (0.71-1.25)	0.664	0.84 (0.61-1.16)	0.290	
rs3808873	0.011	0.627	1.04 (0.88-1.23)	0.619	1.07 (0.90-1.27)	0.457	0.79 (0.61-1.03)	0.084	0.77 (0.57-1.03)	0.079	
<b><i>APOD</i>:</b>											
rs76929107	0.004	0.826	0.85 (0.50-1.46)	0.555	0.74 (0.40-1.36)	0.327	0.97 (0.45-2.12)	0.948	1.17 (0.50-2.73)	0.722	
rs5952	0.022	0.309	1.54 (0.38-6.18)	0.542	1.40 (0.34-5.79)	0.641	—*)	—	—*)	—	
rs34697430	0.011	0.599	1.07 (0.93-1.23)	0.334	1.08 (0.94-1.25)	0.280	1.16 (0.95-1.42)	0.154	1.20 (0.96-1.50)	0.116	
rs7659	-0.048	0.023	0.89 (0.77-1.04)	0.141	0.85 (0.72-1.00)	0.044	0.84 (0.66-1.06)	0.135	0.70 (0.54-0.92)	0.009	
rs823510	0.013	0.538	1.03 (0.88-1.21)	0.680	1.00 (0.85-1.19)	0.965	1.06 (0.83-1.34)	0.658	0.96 (0.73-1.25)	0.736	

Ischemic stroke patients from Lund Stroke Register and the Sahlgrenska Academy Study on Ischaemic Stroke. NIHSS = NIH stroke scale. \*) Cannot be estimated due to monomorphism among patients with NIHSS score of 16 or above.

**Table 4 Detailed assessment of possible association between stroke severity (NIHSS) and SNP rs7659 within APOD**

Subgroup	NIHSS score		NIHSS; dichotomous indicator of medium-severe (vs. mild) ischemic stroke onset (NIHSS ≥ 8)				NIHSS; dichotomous indicator of severe (vs. mild-medium) ischemic stroke onset (NIHSS ≥ 16)			
	Estimated Spearman's Rho	P-value	Simple logistic regression		Multiple logistic regression		Simple logistic regression		Multiple logistic regression	
			OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Total (N = 2221)	-0.048	0.023	0.89 (0.77-1.04)	0.141	0.85 (0.72-1.00)	0.044	0.84 (0.66-1.06)	0.135	0.70 (0.54-0.92)	0.009
			1676 controls		1618 controls		2023 controls		1947 controls	
			545 cases		495 cases		198 cases		166 cases	
Males (N = 1232)	-0.055	0.053	0.89 (0.72-1.11)	0.293	0.85 (0.68-1.06)	0.143	0.82 (0.58-1.17)	0.273	0.75 (0.52-1.10)	0.144
			971 controls		939 controls		1146 controls		1105 controls	
			261 cases		243 cases		86 cases		77 cases	
Females (N = 989)	-0.040	0.209	0.90 (0.73-1.11)	0.311	0.85 (0.68-1.07)	0.168	0.85 (0.62-1.16)	0.311	0.66 (0.46-0.96)	0.028
			705 controls		679 controls		877 controls		842 controls	
			284 cases		252 cases		112 cases		89 cases	
LSR (N = 1683)	-0.054	0.026	0.87 (0.73-1.03)	0.113	0.81 (0.67-0.98)	0.026	0.79 (0.61-1.03)	0.081	0.65 (0.48-0.88)	0.005
			1263 controls		1204 controls		1523 controls		1450 controls	
			420 cases		377 cases		160 cases		133 cases	
SAHLSIS (N = 538)	-0.032	0.454	0.97 (0.71-1.33)	0.858	0.94 (0.68-1.30)	0.702	1.03 (0.62-1.73)	0.902	0.86 (0.48-1.53)	0.605
			413 controls		412 controls		500 controls		497 controls	
			125 cases		118 cases		38 cases		33 cases	
Age < 70 (N = 1084)	-0.066	0.029	0.89 (0.70-1.13)	0.333	0.85 (0.67-1.09)	0.201	0.98 (0.66-1.47)	0.940	0.81 (0.51-1.27)	0.348
			868 controls		853 controls		1021 controls		1001 controls	
			216 cases		202 cases		63 cases		54 cases	
Age ≥ 70 (N = 1137)	-0.042	0.156	0.88 (0.72-1.08)	0.215	0.82 (0.66-1.02)	0.080	0.76 (0.57-1.01)	0.060	0.63 (0.45-0.88)	0.006
			808 controls		750 controls		1002 controls		946 controls	
			329 cases		266 cases		135 cases		112 cases	
LVD (N = 185)	-0.008	0.918	1.14 (0.70-1.87)	0.597	0.97 (0.57-1.66)	0.924	1.20 (0.54-2.67)	0.651	0.53 (0.17-1.65)	0.272
			133 controls		128 controls		170 controls		163 controls	
			52 cases		45 cases		15 cases		10 cases	
SVD (N = 500)	-0.019	0.667	0.77 (0.46-1.30)	0.329	0.81 (0.47-1.38)	0.432	N/A		N/A	
			461 controls		452 controls					
			39 cases		38 cases					
CE (N = 595)	-0.076	0.065	0.89 (0.70-1.15)	0.367	0.78 (0.59-1.03)	0.081	0.72 (0.52-1.00)	0.051	0.59 (0.40-0.85)	0.005
			364 controls		338 controls		484 controls		448 controls	
			231 cases		204 cases		111 cases		94 cases	

Ischemic stroke patients from Lund Stroke Register (LSR) and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS). NIHSS = NIH stroke scale. Multiple logistic regression models are controlling for covariates age, gender, diabetes mellitus, hypertension and current smoking when analyzing the entire sample as well as separate study groups and age groups. When analyzing males and females separately the covariate gender is omitted from these multivariable models. Pathogenetic ischemic stroke subtype: LVD = Large vessel disease; SVD = Small vessel disease; CE = Cardioembolic stroke. N/A = not applicable due to absence of sampling units with NIHSS ≥ 16.

The *SIGMAR1* region on chromosome 9p13 displays two polymorphisms that have a strong influence in CNS disease, namely rs1799729 (GC-241-240TT) and rs1800866 (Gln2Pro) that show LD forming haplotypes GC-Q and TT-P [39-41]. The rs1799729 is found in the proximal promoter region while rs1800866 is present in the first exon. Only rs1800866 was analysed in our study but these two SNPs are closely related (nearly in complete

LD with  $r^2 = 0.98$ ) and have been reported to be associated with neuroprotection and risk of AD [41], and also risk of depression and alcoholism [38,40]. The polymorphism Gln2Pro is located in the amino acid sequence motif MQWAVGRR [53] at the N-terminal part of the protein, which is an endoplasmatic binding region. Hence, a mutation could affect trafficking of SIGMAR1 associated processes, which have been implicated in rodent models

**Table 5 Analysis of association between outcome after stroke (shown by modified Rankin Scale, mRS) and ten APOD and SIGMAR1 SNPs**

SNP	Deceased patients <i>are not</i> included in mRS:						Deceased patients <i>are</i> included in mRS:					
	Ordinal score:		Dichotomous indicator for dependence of support:				Ordinal score:		Dichotomous indicator for dependence of support:			
	Estimated Spearman's Rho	P-value	Simple logistic regression		Multiple logistic regression		Estimated Spearman's Rho	P-value	Simple logistic regression		Multiple logistic regression	
			OR (95% CI)	P-value	OR (95% CI)	P-value			OR (95% CI)	P-value	OR (95% CI)	P-value
<b>SIGMAR1:</b>												
rs11559048	0.045	0.081	3.35 (0.80-14.1)	0.099	2.75 (0.49-15.3)	0.249	0.026	0.286	2.64 (0.63-11.1)	0.185	2.29 (0.38-13.7)	0.364
rs1800866	0.011	0.655	1.13 (0.93-1.37)	0.231	1.10 (0.88-1.36)	0.404	0.004	0.872	1.08 (0.90-1.30)	0.402	1.06 (0.85-1.31)	0.612
rs12001648	-0.012	0.631	0.94 (0.67-1.32)	0.712	0.77 (0.53-1.11)	0.165	-0.001	0.963	0.98 (0.72-1.34)	0.892	0.75 (0.53-1.07)	0.115
rs7036351	0.006	0.817	1.10 (0.89-1.33)	0.390	1.03 (0.83-1.29)	0.781	-0.007	0.772	1.04 (0.86-1.25)	0.722	0.97 (0.79-1.21)	0.810
rs3808873	0.021	0.436	1.08 (0.90-1.29)	0.392	1.11 (0.91-1.35)	0.290	0.012	0.636	1.05 (0.89-1.24)	0.598	1.07 (0.89-1.30)	0.457
<b>APOD:</b>												
rs76929107	0.002	0.947	0.98 (0.54-1.78)	0.943	0.95 (0.49-1.82)	0.868	-0.013	0.582	0.87 (0.49-1.54)	0.623	0.92 (0.48-1.73)	0.790
rs5952	0.016	0.520	1.98 (0.28-14.1)	0.494	2.90 (0.32-26.7)	0.346	0.025	0.306	2.35 (0.39-14.1)	0.350	3.09 (0.35-27.5)	0.312
rs34697430	-0.019	0.460	0.91 (0.78-1.06)	0.221	0.91 (0.77-1.07)	0.243	0.002	0.946	0.96 (0.83-1.10)	0.514	0.92 (0.79-1.08)	0.295
rs7659	0.002	0.929	1.03 (0.88-1.22)	0.684	0.98 (0.82-1.17)	0.832	-0.005	0.846	1.02 (0.87-1.18)	0.842	0.96 (0.81-1.14)	0.665
rs823510	0.012	0.653	1.01 (0.85-1.21)	0.882	1.02 (0.84-1.23)	0.871	-0.008	0.742	0.97 (0.82-1.14)	0.696	0.97 (0.81-1.17)	0.764

Ischemic stroke patients from Lund Stroke Register and the Sahlgrenska Academy Study on Ischemic Stroke.

mRS = modified Rankin Scale. Ordinal score comprises distinguishable categories 0–2, 3, 4, 5 and, if deceased patients are included, also category 6. Correspondingly, dichotomous indicator comprises categories 0–2 as control subjects and category 3 or above as cases.

of stroke [37]. However; we could not find any association between the *SIGMAR1* polymorphisms and stroke risk, severity or recovery. The significance of the weak association of rs12001648 needs further investigation.

Although rigorous statistical analysis did not provide clear evidence of an association between the *APOD* SNPs and stroke risk, severity or outcome, the possible genetic influence of polymorphism rs7659 is interesting and potentially relevant. Rs7659 is located in the 3'UTR of *APOD*, and a functional variant in this area might influence the transcription of the gene or mRNA splicing. Indeed, this SNP appears to be positioned at a putative binding site for the human splicing factor SR SC35 [54]. Also, it is previously shown that rs7659 may be associated with early onset AD within the subgroup of patients lacking the APOE $\epsilon$ 4 allele [28] and with long term clinical outcome in schizophrenics [54]. Moreover, the *APOD* gene is localized on chromosome 3q2.2-qter in close proximity to the 3q25-26 region linked to AD [24]. Hence, taking into consideration the association between rs7659 to other CNS disease and our finding of a possible association of rs7659 with stroke risk and stroke severity, particularly among the elderly, this strongly encourages further studies of rs7659.

Possible occurrence of false positive *P*-values was suppressed by Bonferroni correction. False negative results cannot be detected since we do not know the infinite population behind our predetermined study sample. By performing a post hoc power analysis including stroke severity (from NIHSS case-control calculations) we found rather modest statistical powers (between 5% and 41%), indicating a weak incentive for replicative studies to find an association between the selected SNPs and stroke severity (and even outcome, defined by the mRS nomenclature).

## Conclusion

In this first attempt to study if stroke repair mechanisms linked to certain regions within the *APOD* and *SIGMAR1* genes may also affect recovery from stroke and severity of stroke, we performed a candidate gene study including twelve SNPs from these two genetic regions.

Our data suggest that the rs7659 SNP within the *APOD* gene could be associated with risk for stroke and stroke severity at stroke onset. This mutation may decrease the levels of APOD and thereby diminish its protective cell signalling and antioxidant action. However, these associations showed only modest statistical significance, suggesting that our study may be underpowered despite the large sample size.

## Competing interests

The authors report no conflict of interest.

## Authors' contributions

Arne Lindgren and Håkan Lövkvist had the overall responsibility for this study, including research design, data analysis, results, discussion, and manuscript preparation. Arne Lindgren, Katarina Jood and Christina were involved in clinical samples and materials collection. Ann-Cathrin Jönsson contributed in data analysis. Holger Luthman selected the SNPs for analysis and discussed the results. Tadeusz Wieloch conceived the idea from experimental studies and discussed the results. All authors were involved in the research design, drafting the manuscript and have read and approved the final manuscript.

## Acknowledgements

This study was supported by grants from the Swedish Research Council (K2008-65X-14605-06-03, K2011-65X-14605-09-6, K2010-61X-20378-04-3, 2011-2684, 2011-2652), the Swedish State (ALFGBG-148861), the Swedish Heart and Lung Foundation (20100256), the Yngve Land Foundation, the Crafoord Foundation, the King Gustaf V and Queen Victoria's Foundation, the Swedish Stroke Association, Lund University, Region Skåne, the EOS Freemason Foundation, the Tore Nilsson Foundation, the Swedish Brain Fund and the Lars Hierta Foundation. Lund University and the Sahlgrenska Academy are members of the International Stroke Genetics Consortium. Biobank services were provided by Region Skåne Competence Centre (RSKC Malmö), and Labmedicin Skåne, University and Regional Laboratories Region Skåne, Sweden. We thank Riksstroke for providing information on 3 month follow-up status for patients in Lund Stroke Register.

## Author details

<sup>1</sup>Department of Clinical Sciences Lund, Neurology, Lund University, Lund, Sweden. <sup>2</sup>Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden. <sup>3</sup>Department of Health Sciences, Lund University, Lund, Sweden. <sup>4</sup>Department of Clinical Sciences Malmö, Medical Genetics, Lund University, Malmö, Sweden. <sup>5</sup>Department of Clinical Neuroscience and Rehabilitation, The Sahlgrenska Academy at University of Gothenburg, Institute of Neuroscience and Physiology, Gothenburg, Sweden. <sup>6</sup>Department of Neurosurgery, Laboratory for Experimental Brain Research, Lund University, Lund, Sweden. <sup>7</sup>R&D Centre Skåne, Skåne University Hospital, SE-221 85 Lund, Sweden.

Received: 20 May 2014 Accepted: 23 September 2014

Published online: 28 September 2014

## References

1. Luengo-Fernandez R, Paul NL, Gray AM, Pendlebury ST, Bull LM, Welch SJ, Cuthbertson FC, Rothwell PM: **Population-based study of disability and institutionalization after transient ischemic attack and stroke: 10-year results of the Oxford Vascular Study.** *Stroke* 2013, **44**(10):2854-2861.
2. McNaughton H, Feigin V, Kerse N, Barber PA, Weatherall M, Bennett D, Carter K, Hackett M, Anderson C: **Ethnicity and functional outcome after stroke.** *Stroke* 2011, **42**(4):960-964.
3. Feigin VL, Barker-Collo S, Parag V, Senior H, Lawes CM, Ratnasabapathy Y, Glen E: **Auckland Stroke Outcomes Study. Part 1: Gender, stroke types, ethnicity, and functional outcomes 5 years poststroke.** *Neurology* 2010, **75**(18):1597-1607.
4. Rensink M, Schuurmans M, Lindeman E, Hafsteinsdottir T: **Task-oriented training in rehabilitation after stroke: systematic review.** *J Adv Nurs* 2009, **65**(4):737-754.
5. Brogårdh C, Lexell J: **Effects of cardiorespiratory fitness and muscle-resistance training after stroke.** *PM R* 2012, **4**(11):901-907.
6. Goldstein LB, Bushnell CD, Adams RJ, Appel LJ, Braun LT, Chaturvedi S, Creager MA, Culebras A, Eckel RH, Hart RG, Hinchey JA, Howard VJ, Jauch EC, Levine SR, Meschia JF, Moore WS, Nixon JV, Pearson TA: **Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association.** *Stroke* 2011, **42**(2):517-584.
7. Endres M, Engelhardt B, Koistinaho J, Lindvall O, Meairs S, Mohr JP, Planas A, Rothwell N, Schwabinger M, Schwab ME, Vivien D, Wieloch T, Dirnagl U: **Improving outcome after stroke: overcoming the translational roadblock.** *Cerebrovasc Dis* 2008, **25**(3):268-278.
8. Pekna M, Pekny M: **The neurobiology of brain injury.** *Cerebrum* 2012, **2012**:9.



9. Wieloch T, Nikolich K: **Mechanisms of neural plasticity following brain injury.** *Curr Opin Neurobiol* 2006, **16**(3):258–264.
10. Cramer SC, Procaccio V: **Correlation between genetic polymorphisms and stroke recovery: analysis of the GAIN Americas and GAIN International Studies.** *Eur J Neurol* 2012, **19**(5):718–724.
11. Lövkvist H, Smith JG, Luthman H, Höglund P, Norrving B, Kristoffersson U, Jönsson AC, Lindgren AG: **Ischaemic stroke in hypertensive patients is associated with variations in the PDE4D genome region.** *Eur J Hum Genet* 2008, **16**(9):1117–1125.
12. Smith JG, Melander O, Lövkvist H, Hedblad B, Engström G, Nilsson P, Carlsson J, Berglund G, Norrving B, Lindgren A: **Common genetic variants on chromosome 9p21 confers risk of ischemic stroke: a large-scale genetic association study.** *Circ Cardiovasc Genet* 2009, **2**(2):159–164.
13. Williams FM, Carter AM, Hysi AM, Surdulescu G, Hodgkiss D, Soranzo N, Traylor M, Bevan S, Dichgans M, Rothwell PM: **Ischemic stroke is associated with ABO locus: The Euroclot study.** *Ann Neurol* 2012, **73**(1):16–31.
14. Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, Burgess AI, Pirinen M, Jackson CA, Traylor M, Strange A, Su Z, Band G, Syme PD, Malik R, Pera J, Norrving B, Lemmens R, Freeman C, Schanz R, James T, Poole D, Murphy L, Segal H, Cortellini L, Cheng YC, Woo D, Nalls MA, Müller-Myhok B, Meisinger C, Seedorf U, Ross-Adams H, et al: **Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke.** *Nat Genet* 2012, **44**(3):328–333.
15. Gschwendtner A, Bevan S, Cole JW, Plourde A, Matarin M, Ross-Adams H, Meitinger T, Wichmann E, Mitchell BD, Furie K, Slowik A, Rich SS, Syme PD, MacLeod MJ, Meschia JF, Rosand J, Kittner SJ, Markus HS, Müller-Myhok B, Dichgans M: **Sequence variants on chromosome 9p21.3 confer risk for atherosclerotic stroke.** *Ann Neurol* 2009, **65**(5):531–539.
16. Holliday EG, Maguire JM, Evans TJ, Koblar SA, Jannes J, Sturm JW, Hankey GJ, Baker R, Golledge J, Parsons MW, Malik R, McEvoy M, Biros E, Lewis MD, Lincz LF, Peel R, Oldmeadow C, Smith W, Moscato P, Barlera S, Bevan S, Bis JC, Boerwinkle E, Boncoraglio GB, Brott TG, Brown RD Jr, Cheng YC, Cole JW, Cotlarciuc I, Devan WJ, et al: **Common variants at 6p21.1 are associated with large artery atherosclerotic stroke.** *Nat Genet* 2012, **44**(10):1147–1151.
17. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, Fornage M, Ikram MA, Malik R, Bevan S, Thorsteinsdottir U, Nalls MA, Longstreth W, Wiggins KL, Yadav S, Parati EA, Destefano AL, Worrall BB, Kittner SJ, Khan MS, Reiner AP, Helgadottir A, Achterberg S, Fernandez-Cadenas I, Abboud S, Schmidt R, Walters M, Chen WM, Ringelstein EB, O'Donnell M, et al: **Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies.** *Lancet Neurol* 2012, **11**(11):951–962.
18. Gretarsdottir S, Thorleifsson G, Manolescu A, Styrkarsdottir U, Helgadottir A, Gschwendtner A, Kostulas K, Kühlenbäumer G, Bevan S, Jonsdottir T, Bjarnason H, Saemundsdottir J, Palsson S, Arnar DO, Holm H, Thorgeirsson G, Valdimarsson EM, Sveinbjörnsdottir S, Gieger C, Berger K, Wichmann HE, Hillert J, Markus H, Gulcher JR, Ringelstein EB, Kong A, Dichgans M, Gudbjartsson DF, Thorsteinsdottir U, Stefansson K: **Risk variants for atrial fibrillation on chromosome 4q25 associate with ischemic stroke.** *Ann Neurol* 2008, **64**(4):402–409.
19. Gudbjartsson DF, Holm H, Gretarsdottir S, Thorleifsson G, Walters GB, Thorgeirsson G, Gulcher J, Mathiesen EB, Njølstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Kucera G, Stubblefield T, Carter S, Roden D, Ng MC, Baum L, So WY, Wong KS, Chan JC, Gieger C, Wichmann HE, Gschwendtner A, Dichgans M, Kühlenbäumer G, Berger K, Ringelstein EB, et al: **A sequence variant in ZFX3 on 16q22 associates with atrial fibrillation and ischemic stroke.** *Nat Genet* 2009, **41**(8):876–878.
20. Biffi A, Anderson CD, Jagiella JM, Schmidt H, Kissela B, Hansen BM, Jimenez-Conde J, Pires CR, Ayres AM, Schwab K, Cortellini L, Pera J, Urbanik A, Romero JM, Rost NS, Goldstein JN, Viswanathan A, Pichler A, Enzinger C, Rabionet R, Norrving B, Tirschwell DL, Selim M, Brown DL, Silliman SL, Worrall BB, Meschia JF, Kidwell CS, Broderick JP, Greenberg SM, et al: **APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study.** *Lancet Neurol* 2011, **10**(8):702–709.
21. Maguire J, Thakkinian A, Levi C, Lincz L, Bisset L, Sturm J, Scott R, Whyte S, Attia J: **Impact of COX-2 rs5275 and rs20417 and GP1IIa rs5918 polymorphisms on 90-day ischemic stroke functional outcome: a novel finding.** *J Stroke Cerebrovasc Dis* 2011, **20**(2):134–144.
22. Pearson-Fuhrhop KM, Minton B, Acevedo D, Shahbaba B, Cramer SC: **Genetic variation in the human brain dopamine system influences motor learning and its modulation by L-Dopa.** *PLoS One* 2013, **8**(4):e61197.
23. Elliott DA, Weickert CS, Garner B: **Apolipoproteins in the brain: implications for neurological and psychiatric disorders.** *Clin Lipidol* 2010, **5**(4):555–573.
24. Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, Terrisse L, Milne R: **Apolipoprotein D.** *Biochim Biophys Acta* 2000, **1482**(1–2):185–198.
25. Ordonez C, Navarro A, Perez C, Martinez E, del Valle E, Tolivia J: **Gender differences in apolipoprotein D expression during aging and in Alzheimer disease.** *Neurobiol Aging* 2012, **33**(2):433 e411–420.
26. Thomas EA, Dean B, Pavey G, Sutcliffe JG: **Increased CNS levels of apolipoprotein D in schizophrenic and bipolar subjects: implications for the pathophysiology of psychiatric disorders.** *Proc Natl Acad Sci U S A* 2001, **98**(7):4066–4071.
27. Belloir B, Kovari E, Surini-Demiri M, Savioz A: **Altered apolipoprotein D expression in the brain of patients with Alzheimer disease.** *J Neurosci Res* 2001, **64**(1):61–69.
28. Shibata N, Nagata T, Shinagawa S, Ohnuma T, Shimazaki H, Komatsu M, Kuerban B, Tomson K, Nakayama K, Yamada H, Arai H: **Genetic association between APOA1 and APOD polymorphisms and Alzheimer's disease in a Japanese population.** *J Neural Transm* 2013, **120**(11):1599–1603.
29. Ordonez C, Navarro A, Perez C, Astudillo A, Martinez E, Tolivia J: **Apolipoprotein D expression in substantia nigra of Parkinson disease.** *Histol Histopathol* 2006, **21**(4):361–366.
30. Navarro A, Alonso A, Garrido P, Gonzalez C, Gonzalez Del Rey C, Ordonez C, Tolivia J: **Increase in placental apolipoprotein D as an adaptation to human gestational diabetes.** *Placenta* 2010, **31**(1):25–31.
31. Rickhag M, Deierborg T, Patel S, Ruscher K, Wieloch T: **Apolipoprotein D is elevated in oligodendrocytes in the peri-infarct region after experimental stroke: influence of enriched environment.** *J Cereb Blood Flow Metab* 2008, **28**(3):551–562.
32. Franz G, Reindl M, Patel SC, Beer R, Unterrichter I, Berger T, Schmutzhard E, Poewe W, Kampfl A: **Increased expression of apolipoprotein D following experimental traumatic brain injury.** *J Neurochem* 1999, **73**(4):1615–1625.
33. Chen Y, Jia L, Wei C, Wang F, Lv H, Jia J: **Association between polymorphisms in the apolipoprotein D gene and sporadic Alzheimer's disease.** *Brain Res* 2008, **1233**:196–202.
34. Ganfornina MD, Do Carmo S, Lora JM, Torres-Schumann S, Vogel M, Allhorn M, Gonzalez C, Bastiani MJ, Rassart E, Sanchez D: **Apolipoprotein D is involved in the mechanisms regulating protection from oxidative stress.** *Aging Cell* 2008, **7**(4):506–515.
35. Bhatia S, Knoch B, Wong J, Kim WS, Else PL, Oakley AJ, Garner B: **Selective reduction of hydroperoxyeicosatetraenoic acids to their hydroxy derivatives by apolipoprotein D: implications for lipid antioxidant activity and Alzheimer's disease.** *Biochemical J* 2012, **442**(3):713–721.
36. Kourrich S, Su TP, Fujimoto M, Bonci A: **The sigma-1 receptor: roles in neuronal plasticity and disease.** *Trends Neurosci* 2012, **35**(12):762–771.
37. Ruscher K, Shamloo M, Rickhag M, Ladunga I, Soriano L, Gisselsson L, Toresson H, Ruslim-Litrus L, Oksenberg D, Urfer R, Johansson BB, Nikolich K, Wieloch T: **The sigma-1 receptor enhances brain plasticity and functional recovery after experimental stroke.** *Brain* 2011, **134**(3):732–746.
38. Kishi T, Yoshimura R, Okochi T, Fukuo Y, Kitajima T, Okumura T, Tsunoka T, Kawashima K, Yamanouchi Y, Kinoshita Y, Umene-Nakano W, Naitoh H, Nakamura J, Ozaki N, Iwata N: **Association analysis of SIGMAR1 with major depressive disorder and SSRI response.** *Neuropharmacology* 2010, **58**(7):1168–1173.
39. Ishiguro H, Ohtsuki T, Toru M, Itokawa M, Aoki J, Shibuya H, Kurumaji A, Okubo Y, Iwawaki A, Ota K, Shimizu H, Hamaguchi H, Arinami T: **Association between polymorphisms in the type 1 sigma receptor gene and schizophrenia.** *Neurosci Lett* 1998, **257**(1):45–48.
40. Miyatake R, Furukawa A, Matsushita S, Higuchi S, Suwaki H: **Functional polymorphisms in the sigma1 receptor gene associated with alcoholism.** *Biol Psychiatry* 2004, **55**(1):85–90.
41. Feher A, Juhasz A, Laszlo A, Kalman J Jr, Pakaski M, Kalman J, Janka Z: **Association between a variant of the sigma-1 receptor gene and Alzheimer's disease.** *Neurosci Lett* 2012, **517**(2):136–139.
42. Hallström B, Jönsson AC, Nerbrand C, Petersen B, Norrving B, Lindgren A: **Lund Stroke Register: hospitalization pattern and yield of different screening methods for first-ever stroke.** *Acta Neurol Scand* 2007, **115**(1):49–54.
43. Jood K, Ladenvall C, Rosengren A, Blomstrand C, Jern C: **Family history in ischemic stroke before 70 years of age: the Sahlgrenska Academy Study on Ischemic Stroke.** *Stroke* 2005, **36**(7):1383–1387.
44. Goldstein LB, Bertels C, Davis JN: **Interrater reliability of the NIH stroke scale.** *Arch Neurol* 1989, **46**(6):660–662.

45. Multicenter trial of hemodilution in ischemic stroke—background and study protocol. Scandinavian Stroke Study Group. *Stroke* 1985, **16**(5):885–890.
46. Gray LJ, Ali M, Lyden PD, Bath PM: Interconversion of the National Institutes of Health Stroke Scale and Scandinavian Stroke Scale in acute stroke. *J Stroke Cerebrovasc Dis* 2009, **18**(6):466–468.
47. Adams HP Jr, Bendixen BH, Leira E, Chang KC, Davis PH, Woolson RF, Clarke WR, Hansen MD: Antithrombotic treatment of ischemic stroke among patients with occlusion or severe stenosis of the internal carotid artery: A report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). *Neurology* 1999, **53**(1):122–125.
48. Eriksson M, Appelros P, Norrving B, Terent A, Stegmayr B: Assessment of functional outcome in a national quality register for acute stroke: can simple self-reported items be transformed into the modified Rankin Scale? *Stroke* 2007, **38**(4):1384–1386.
49. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J: Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988, **19**(5):604–607.
50. Olsson S, Jood K, Blomstrand C, Jern C: Genetic variation on chromosome 9p21 shows association with the ischaemic stroke subtype large-vessel disease in a Swedish sample aged  $\leq 70$ . *Eur J Neurol* 2011, **18**(2):365–367.
51. Starby H, Delavaran H, Andsberg G, Lövkvist H, Norrving B, Lindgren A: Multiplicity of risk factors in ischemic stroke patients: relations to age, sex, and subtype - a study of 2,505 patients from the Lund Stroke Register. *Neuroepidemiology* 2014, **42**(3):161–168.
52. Flossmann E, Schulz UG, Rothwell PM: Systematic review of methods and results of studies of the genetic epidemiology of ischemic stroke. *Stroke* 2004, **35**(1):212–227.
53. Schutze MP, Peterson PA, Jackson MR: An N-terminal double-arginine motif maintains type II membrane proteins in the endoplasmic reticulum. *EMBO J* 1994, **13**(7):1696–1705.
54. Hansen T, Hemmingsen RP, Wang AG, Olsen L, Timm S, Soeby K, Jakobsen KD, Fenger M, Parnas J, Rasmussen HB, Werge T: Apolipoprotein D is associated with long-term outcome in patients with schizophrenia. *Pharmacogenomics J* 2006, **6**(2):120–125.

doi:10.1186/s12883-014-0191-2

**Cite this article as:** Lövkvist et al.: Variations in apolipoprotein D and sigma non-opioid intracellular receptor 1 genes with relation to risk, severity and outcome of ischemic stroke. *BMC Neurology* 2014 **14**:191.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit

