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Original Article

Gene polymorphism of LXRα is associated with the pathogenesis of ischemic stroke in Han population in northern China

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Abstract: In this study, whether the gene polymorphism of LXRα is associated with the pathogenesis of ischemic stroke in Han population in northern China is under investigated. A case-control study was applied. 413 patients with ischemic stroke (IS) in Han population in northern China were selected. Another 477 cases of healthy individuals served as controls. Single nucleotide extension method (Multiplex SNaPshot) was used to detect the polymorphism of LXRα gene sites (rs2167079, rs2279238). The results demonstrated that the frequency of rs2279238 TT genotype and T allele of LXRα gene was significantly higher in the IS group (P = 0.006, P = 0.013). After adjustment for gender, age, hypertension, diabetes, smoking, drinking and other traditional risk factors, the frequency of TT genotype and T allele of rs2279238 was still relevant with the incidence of ischemic stroke (OR = 1.65, 95% CI: 1.02-2.68, P = 0.042; OR = 1.76, 95% CI: 1.29-2.45, P = 0.013). In the subgroup with artery atherosclerosis, the frequency of rs2279238 TT genotype and T allele of LXRα was also higher in the IS group (P = 0.001 and P = 0.005, respectively). After adjustment for the above confounding factors, TT genotype and T allele were still associated with the pathogenesis of artery atherosclerotic ischemic stroke (OR = 2.35, 95% CI: 1.18-4.68, P = 0.015; OR = 1.89, 95% CI: 1.32-2.68, P = 0.005). Therefore, our study shows that the polymorphism of LXRα gene rs2279238 is associated with the pathogenesis of artery atherosclerotic ischemic stroke in Han population in northern China.

Keywords: Liver X receptors (LXRα) gene, ischemic stroke, single nucleotide polymorphisms

Introduction

Stroke is a disease with high mortality, morbidity and recurrence rate, and is a major cause of disability and death worldwide [1]. The report of the Global Burden of Disease 2013 published by Lancet this year demonstrated that stroke remains the first cause of death in China [2]. There are 1.5-2 million new stroke cases in Chinese each year, of which 70% were ischemic stroke (IS) [3]. It has been shown that ischemic stroke is a multifactorial disease, of which both genetic and environmental factors participate in the procedure of pathogenesis [4]. The genetic rate of ischemic stroke is 37.9%. Although its exact hereditary mechanism is still unclear, it has been proved that the variation of gene such as HDAC9, PITX2, ZFHX3, etc. is associated with the pathogenesis of ischemic stroke [5-7].

Liver X receptors (LXR) is a member of transcription factors in the nuclear receptor superfamily, including two homologous subtypes, LXRα and LXRβ [8]. LXRα is highly expressed in liver, as well as in other lipid metabolism closely related tissues, such as macrophages, small intestine, adipose tissue, adrenal gland, kidney and lung [9-11]. Human LXRα gene is located in 11p11.2, containing 9 exons and 10 introns, consisted by 1759 bases [9].

Studies have shown that LXRα receptor plays an important role in the regulation of lipid metabolism and inflammation [12]. LXRα receptor is activated when the concentration of hydroxyl, an intracellular cholesterol sterol metabolite, increased [13]. Through adjusting transcription of transporter protein ABCAl and ABCGl, apolipoprotein E (ApoE) of its target gene ATP-binding cassette (ABC), LXRα receptor regulates cholesterol efflux of macrophage, promotes reverse transport of cholesterol, and further regulates metabolism and cholesterol metabolism by promoting bile secretion and

conversion to fatty acids and other ways [9]. Besides, LXRα receptor is also able to regulate immune response by inhibiting macrophage inflammatory response [14]. Plasma cholesterol level and inflammatory processes play important roles in the formation of atherosclerotic plaques, thus LXRα receptor has been extensively studied as a new therapeutic target in the prevention of atherosclerosis and other diseases. The correlation study of LXRα gene and heart or brain vascular disease has become a hot spot in related fields [12]. Animal experiments had proven that the formation of atherosclerotic lesions was weakened by LXRα agonist in rats, which also acted as a protective effect in an animal model of ischemic stroke neuronal injury [15, 16]. The model of LXRα knockout mice was more susceptible to atherosclerosis further provides evidence for the correlation of LXRα and ischemic stroke pathogenesis [17]. A Denmark Copenhagen City Heart Study (N = 10281) and a Copenhagen General Population Study (N = 51429) based on large population sample indicated that the polymorphism of rs6189605 and rs12221497, which locate in the LXRα gene promoter region, can predict the risk of ischemic heart disease, myocardial infarction, ischemic stroke and other ischemic vascular disease [18]. Zhou et al. [19] recently found that the polymorphism of LXRα gene rs12221497 increased the risk of coronary heart disease in Han population.

There is no study reporting that the polymorphism of LXRα gene correlates with ischemic stroke of Han population in northern China. Therefore, in this study, a case-control study on polymorphism sites of LXRα gene rs2167079 and rs2279238 was performed to examine the gene polymorphism of LXRα and hereditary predisposition of IS of Han population in northern China, which provides molecular epidemiological evidence for understanding the underlying genetic mechanisms of pathogenesis of ischemic stroke.

Materials and methods

Subjects

A total of 413 acute ischemic stroke patients (249 males, 164 females) treated in neurology department of Shenyang 202 Hospital of People's Liberation Army between May 2011 and December 2014 were chosen for the IS group. All patients had acute, burst, lasting more than 24 hours of focal neurologic deficits, confirmed as ischemic stroke via the clinical, head MRI, and/or head CT diagnosis, with the exclusion of transient ischemic attack, hemorrhagic infarction, cerebral hemorrhage and subarachnoid hemorrhage, excluding infarction caused by other reasons such as cardiac disease, arterial inflammation, tumor, drugs, trauma, blood disease, vascular malformation or aneurysm, etc., and with the exclusion of liver diseases, kidney diseases, thyroid diseases, etc. Diagnosis was done by professional neurologists. A total of 477 healthy people (279 males and 198 females) under physical examination from Shenyang People's Liberation Army 202 Hospital at the same period were selected as control group, age and gender of which matched the IS group. All subjects had no cerebrovascular diseases and other neurological diseases, liver diseases, kidney diseases, blood diseases, tumor, peripheral vascular diseases, autoimmune diseases, etc. via the examination of medical history, physical examination and clinical examination. All subjects were Han population in Liaoning Province of northern China, and there was no blood relationship between the individuals in this study. This study was approved by the ethics committee of 202 Hospital. All subjects had signed informed consent. Relevant information of all subjects, including age, gender, height, weight, blood pressure, cholesterol, fasting blood sugar, medical history, smoking history, drinking history, etc. was collected through questionnaires, physical examination and laboratory tests, as seen in **Table 1**. The patients were divided into Large-artery atherosclerosis (LAA) group and Small-artery occlusion (SAO) also called lacunar infarction group according to the TOAST classification criteria [20].

Genotyping

3 ml blood was drawn from each subject, was then used for extraction of genomic DNA using DNA extraction kit (Wizard Genomic DNA purification kit; Promega, USA) after anticoagulation by EDTA. The concentration and purity of extracted DNA was measured using the UV spectrophotometer. DNA samples were stored at -20°C.

Rs2167079 and rs2279238 of gene LXRα were selected as genetic marker sites using NCBI's dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) by browsing human genome data.

Table 1. The comparison of general clinical and serum data in IS group and control group

Clinical data	IS group (n = 413)	Control group (n = 477)	*P* value
Age composition	63.47±11.57	62.68±6.32	0.196
Gender (Male/Female)	249/164	279/198	0.638
Body mass index (kg/m^2)	24.28±3.85	24.01±3.72	0.509
Systolic pressure (mmHg)	143.63±18.35	132.04±14.46	<0.001
Diastolic pressure (mmHg)	83.87±10.32	78.76±8.82	<0.001
FBG (mmol/L)	6.41±2.60	5.85±1.71	<0.001
TC (mg/dl)	5.15±0.86	4.99±1.11	0.024
TG (mmol/L)	2.02±3.49	1.63±1.16	0.024
HDL-C (mmol/L)	1.31±0.32	1.33±0.21	0.149
LDL-C (mmol/L)	2.95±0.70	2.95±0.64	0.967
Hypertension (n%)	261 (63.5%)	174 (36.5%)	<0.001
Diabetes (n%)	102 (24.7%)	78 (16.4%)	0.020
Smoking history (n%)	172 (41.6%)	101 (21.2%)	<0.001
Drinking history (n%)	122 (29.5%)	63 (13.2%)	<0.001

Notes: P<0.05 was considered statistically significant between the IS group and control group.

Genotyping was performed using SNaP shot multiple micro sequencing [21].

The genomic DNA was amplified by multiplex PCR reaction, of which the system (20 μL) contained 1x GC buffer I, 3.0 mM Mg^{2+}, 0.3 mM dNTP, 1 U HotStarTaq polymerase (Qiagen Inc.), 1 μL DNA sample and 1 μL multiplex PCR primers. Primer sequences are as follows: rs2279238F: 5'-TGGCTGAGTCAGGGAGAACATGA-3'; rs2279238R: 5'-GGAAGCCCGAGGCCTTGTC-3'; rs2167079F: 5'-GGCCTTTGCCCTTTTAGCTTCA-3'; rs2167079R: 5'-GGCTCTCCTCCAGCTCCTTCTC-3'.

PCR cycling program: 95°C 2 min; 11 cycles (94°C 20 s, 65°C-0.5°C/cycle 40 s, 72°C 1 min 30 s); 24 cycles (94°C 20 s, 59°C 30 s, 72°C 1 min 30 s); 72°C 2 min. Thereafter 1U SAP enzyme (Promega) and 1U Exonuclease I enzyme (Epicentre) were added in a 10 μL PCR product, bathed in 37°C temperature for one hour, and then inactivated in 75°C for 15 minutes. So the PCR product was purified. SNaPshot multiple single base extension reaction was performed after the purification: extending reaction system (10 μL) comprised with 5 μL SNaPshot Multiplex Kit (ABI), 2 μL purified multiplex PCR products, 1 μL extension primer mix and 2 μL ultrapure water. PCR cycling program: 96°C 1 min; 28 cycles (96°C 10 s, 52°C 5 s, 60°C 30 s). 1U SAP enzyme was added in 10 μL extension product, bathed in 37°C temperature for one hour, and then inactivated in 75°C for 15 minutes. 0.5 μL purified extension products was taken to be mixed with 0.5 μL Liz120 SIZE STANDARD and 9 μl Hi-Di, denatured in 95°C for 5 minutes, and then ABI3130XL sequencer was applied. The raw data were collected by GeneMapper 4.0 (Applied Biosystems Co., Ltd., USA) to analyze genotyping type.

Statistical analysis

A database was established using all experimental data in SPSS 16.0 software. Data were expressed as mean ± standard deviation or percentage. Demographic data, risk factors, genotype, allele frequencies were compared using the Student's t-test or Pearson's x^2 test. ANOVA analysis was used to compare the influence of genotype on lipid levels between the two groups. To evaluate the correlation between gene polymorphism and ischemic stroke, odds radio (OR) as well as 95% confidence interval (CI) before and after the adjustment were calculated. *Chi*-squared test was used to verify whether frequency of SNP genotype of each site was in accord with Hardy-Weinberg equilibrium. SHEsis online software was used for the linkage disequilibrium and haplotype analysis of the two SNPs sites of LXRα gene. P<0.05 was considered statistically significant.

Results

Basic information of both groups was collected and compared, as seen in **Table 1**. There was no significant difference in age composition, gender composition, body mass index, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) in the two groups. While other indicators such as hypertension, diabetes, smoking history, drinking history, systolic blood pressure, diastolic blood pressure, fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), were significantly higher in the IS group (P<0.05) (**Table 1**).

Table 2. Frequency of genotype and allele of 2 sites of LXRα gene

Genotype	Controls (%)	Patients (%)	OR (95% CI)	P	Adjusted OR (95% CI)	P
Rs2167079						
CC	37 (7.8)	39 (9.4)	1.00	-	1.00	-
CT	193 (40.5)	176 (42.6)	0.87 (0.53-1.42)	0.566	0.73 (0.43-1.24)	0.245
TT	246 (51.7)	198 (47.9)	0.75 (0.51-1.10)	0.138	0.74 (0.48-1.12)	0.737
C allele	267 (28.0)	254 (30.8)	1.00	-		
T allele	685 (72.0)	572 (69.2)	1.14 (0.93-1.40)	0.211		
Rs2279238						
CC	63 (13.2)	39 (9.4)	1.00	-	1.00	-
CT	229 (48.0)	171 (41.4)	1.21 (0.77-1.88)	0.410	1.10 (0.68-1.77)	0.709
TT	185 (38.8)	203 (49.2)	1.77 (1.13-2.77)	0.012	1.65 (1.02-2.68)	0.042
C allele	355 (37.2)	249 (30.1)	1.00	-		
T allele	599 (62.7)	577 (69.9)	1.76 (1.29-2.45)	0.013		

Notes: Having gender, age, hypertension, diabetes, smoking, drinking, serum TC, LDL-C and blood glucose adjusted.

Table 3. The distribution frequency of genotype and allele in LAA and SAO subgroup

	LAA			SAO		
	Patients (%)	Controls (%)	P	Patients (%)	Controls (%)	P
Rs2169079						
CC	22 (10.4)	37 (7.8)	0.553	17 (8.5)	37 (7.8)	0.660
CT	93 (43.9)	193 (40.5)		83 (41.3)	193 (40.5)	
TT	97 (45.8)	246 (51.7)		101 (50.2)	246 (51.7)	
C	137 (32.3)	267 (28.0)	0.300	117 (29.1)	267 (28.0)	0.660
T	287 (67.7)	685 (72.0)		285 (70.9)	685 (72.0)	
Rs2279238						
CC	17 (8.0)	63 (13.2)	0.001	22 (10.9)	63 (13.2)	0.795
CT	82 (38.7)	229 (48.0)		89 (44.3)	229 (48.0)	
TT	113 (53.3)	185 (38.8)		90 (44.8)	185 (38.8)	
C	116 (27.4)	355 (37.2)	0.005	133 (33.1)	355 (37.2)	0.547
T	308 (72.6)	599 (62.7)		269 (66.9)	599 (62.7)	

The distribution of genotype of rs2167079 and rs2279238 were in line with Harding-Weinberg equilibrium (P>0.05), 0.990 and 0.493 in the IS group, 0.92 and 0.55 in the control group, respectively. Indicating there was group representative in the samples we selected in this study. Analysis of frequency of two SNP sites and allele in IS and control group is seen in **Table 2**. Results illustrated that there was no significant difference in frequency distribution of genotype and allele of rs2167079 (P>0.05) in IS and control group, while the frequency of TT genotype and T allele of rs2279238 was significantly higher in the IS group (P = 0.006, P = 0.013). Data of artery atherosclerosis (LAA) and small artery occlusion (SAO) subgroups are seen in **Table 3**. Results showed that the frequency of TT genotype and T allele of rs2279238 was also significantly higher (P = 0.001, P = 0.005) in the LAA subgroup. However, there was no significant difference in the SAO subgroup. The frequency of TT genotype and T allele was still relevant with the incidence of ischemic stroke after adjusting confounding factors such as gender, age, smoking, alcohol consumption, hypertension, history of diabetes, blood lipids, blood glucose levels, etc., thus it could be considered as the pathogenic factor (OR = 1.65, 95% CI: 1.02-2.68, P = 0.042; OR = 1.76, 95% CI: 1.29-2.45, P = 0.013). Analysis of LAA subgroup also exhibited similar results (OR = 2.35, 95% CI: 1.18-4.68, P = 0.015; OR = 1.89, 95% CI: 1.32-2.68, P = 0.005), as seen in **Table 4**.

Table 4. The regression analysis of LAA subgroup

		Adjusted OR	95% CI	*P*
Rs2169079	CC	1	-	-
	CT	0.68	(0.33-1.39)	0.286
	TT	0.63	(0.31-1.26)	0.192
Rs2279238	CC	1.00	-	-
	CT	1.11	(0.56-2.23)	0.761
	TT	2.35	(1.18-4.68)	0.015

Notes: Having gender, age, hypertension, diabetes, smoking, drinking, serum TC, LDL-C and blood glucose adjusted.

Table 5. LXRα gene haplotype analysis in IS group and control group

	Frequency of IS group	Frequency of control group	*P* value	OR (95% CI)
CC	22.7%	27.9%	0.198	1.144 [0.932-1.404]
TC	7.5%	9.4%	0.164	0.786 [0.559-1.104]
TT	61.8%	62.7%	0.706	0.964 [0.795-1.168]

SHEsis online software was used for matching linkage disequilibrium test of two sites. Results showed that linkage effect was weak in rs2167079- rs2279238 sites (D' = 0.897, r^2 = 0.574).

SHEsis software was used to calculate the distribution frequency of possible haplotypes in two sites (rs2167079, rs2279238) of LXRα gene in the IS and control group. Results showed that the frequency of each haplotype exhibited no significant difference (P>0.05), as seen in **Table 5**.

LXRα is closely related to lipid metabolism; therefore we further analyzed the correlation between genotypes of rs2279238 with lipid levels and BMI, as seen in **Table 6**. There was no significant difference of the index between the two groups.

Discussion

In this study, LXRα gene rs2167079 and rs2279238 SNP sites were selected to conduct a case-control study with the Han population of northern Chinese with ischemic stroke. According to the classic TOAST classification, only patients with atherosclerotic aorta or small arteries occlusive were chosen, excluding other types. The former occurred when atherosclerosis leads to large or medium cerebral vascular stenosis or occlusion, while the latter was due to small blood vessels hyalinization, arteriosclerosis or cellulose necrosis caused by high blood pressure or diabetes. There was no significant difference in the distribution frequency of genotype and allele of LXRα gene rs2167079 between IS and control group, while frequency of rs2279238 TT genotype and T allele in the IS group was significantly higher (P = 0.006, P = 0.013). After adjustment for traditional risk factors such as gender, age, hypertension, diabetes, smoking, drinking, etc., frequency of rs2279238 TT genotype and T allele was still relevant with the incidence of ischemic stroke (OR = 1.65, 95% CI: 1.02-2.68, P = 0.042; OR = 1.76, 95% CI: 1.29-2.45, P = 0.013), indicating that rs2279238 TT genotype and T allele were independent risk factors for the pathogenesis of IS in Han population in northern China, the risk of which increased to 1.65 times. After adjusting traditional risk factors, the frequency of TT genotype and T allele in LAA subgroup was still significantly higher, of which the risk increased to 2.35 times (OR = 2.35, 95% CI: 1.18-4.68, P = 0.015; OR = 1.89, 95% CI: 1.32-2.68, P = 0.001). No correlation or differences was found in small artery occlusion subgroup. Therefore, our findings suggested that genetic polymorphism of rs2279238 in Han population in northern China might be genetic risk markers of ischemic stroke, especially large artery atherosclerotic ischemic stroke. Previous animal experiments proved that activation of LXRα receptor exhibited anti-atherosclerotic effect [15-17], but our study on Han population in northern China found that gene polymorphism of rs2279238 increased the risk of IS, especially large artery atherosclerotic IS, which was consistent with Stender et al. [18], who found that carrying LXRα gene-840AA/-115AA increased the risk of IS to 1.7 times, and the risk of ischemic heart disease and myocardial infarction increased to 1.3 and 1.6 times respectively. Recently, a US INVEST-GENES study on patients with hypertension and coronary heart disease showed that rs2279238 increased the risk of primary endpoint events [22], presumably that was relevant with the activation of LXRα, resulting in the produce of triglyceride-rich LDL which lead to atherosis [23]. Rs2279238, which located in the promoter re-

Table 6. The comparison of effect on various genotypes of rs2279238 on blood lipid levels and BMI in IS group and control group

	IS Group				Control Group				P^b
	CC	CT	TT	P^a	CC	CT	TT	P^a	
TC	5.25±1.03	5.23±0.89	5.06±0.80	0.112	4.97±1.08	5.02±1.13	4.98±1.09	0.717	0.475
TG	1.79±1.20	1.84±1.19	2.20±4.83	0.564	1.63±1.43	1.63±1.06	1.65±1.18	0.981	0.421
LDL-C	2.85±0.75	2.91±0.68	3.00±0.70	0.326	2.97±0.69	2.95±0.65	2.88±0.57	0.394	0.969
HDL-C	1.21±0.29	1.32±0.38	1.32±0.28	0.142	1.36±0.21	1.32±0.22	1.35±0.21	0.37	0.518
BMI	24.93±5.11	23.84±3.41	24.32±3.91	0.214	23.39±6.87	24.09±2.39	24.11±3.56	0.37	0.658

Notes: [a]t test was used to compare the IS and control group, and *P* value represents effects of each genotype on blood lipid levels, [b]ANOVA analysis was used to compare the IS and control group, and *P* value represents effects of each genotype on blood lipid levels. P<0.05 was statistically significant.

gion of exon of SRp55 binding sites that contained shear factor, possibly played a role in affecting LXRα function by regulating transcription or linkage disequilibrium of a certain site with a direct impact on LXRα function.

Genetic polymorphism of LXRα was associated with metabolic abnormalities. A study of 559 cases on Swedish population illustrated that polymorphism of -115C/T (rs2279238) had significant correlation with the elevated BMI, while the wild-type CAAGCC haplotype that carried C allele was associated with a reduced BMI [24]. A study of 732 cases based on French- Canadian population by Robitaille et al. [25] demonstrated that polymorphism of rs12221497, rs61896015 and rs3758674 was associated with plasma total cholesterol levels. Two studies of 1130 cases and 1160 cases of the French population by Legry et al. [26] proved that the mutation of -6G>A in LXRα gene was associated with HDL levels, and could reduce the risk of metabolic syndrome. They also proved that the polymorphism did not change expression of LXRα and transporter protein ABCA1 in macrophages, suggesting that it may affect the efficiency of translation. The birth cohort of genome-wide association analysis of more than 4000 people in northern Finland indicated that polymorphism of rs2167079 and rs7120118 of LXRα gene was correlated with the increased HDL levels [27]. The recent HELENA study on European adolescents (n = 1144) demonstrated that rs11039155 of LXRα gene was associated with elevated levels of HDL-C, while rs12221497 was related with the reduced levels of HDL-C [28]. Our study did not confirm whether polymorphism of rs2279238 gene was associated with BMI or lipid levels. The results were not entirely consistent with other population, that possibly because there were genetic differences between racial backgrounds and environmental factors as well as the study design and statistical methods, etc., indicating LXRα may affect the susceptibility of stroke by lipid-independent pathway.

Taken together, the current research on polymorphism of LXRα gene and IS in domestic or foreign is rare. Our study found that T allele and TT genotype of rs2279238 in LXRA gene could increase the risk of aorta atherosclerosis IS of Han population in northern China, which may be susceptibility genes of large artery atherosclerotic IS. However, there are also some limitations in this study, such as the sample size is small, so are the research sites, and there is no simultaneously measurement of mRNA or protein expression levels of LXRα, etc. Therefore, in the future, research on LXRα should be continued, so as to further clarify the pathogenesis mechanisms and genetic predisposition of IS.

Disclosure of conflict of interest

None.

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References

[1] Bonita R, Mendis S, Truelsen T, Bogousslavsky J, Toole J and Yatsu F. The global stroke initiative. Lancet Neurol 2004; 3: 391-393.

[2] Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic

analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385: 117-171.

[3] Liu M, Wu B, Wang WZ, Lee LM, Zhang SH and Kong LZ. Stroke in China: epidemiology, prevention, and management strategies. Lancet Neurol 2007; 6: 456-464.

[4] Bevan S, Traylor M, Adib-Samii P, Malik R, Paul NL, Jackson C, Farrall M, Rothwell PM, Sudlow C, Dichgans M and Markus HS. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. Stroke 2012; 43: 3161-3167.

[5] 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, Ho WK, Pera J, Lemmens R, Norrving B, Higgins P, Benn M, Sale M, Kuhlenbaumer G, Doney AS, Vicente AM, Delavaran H, Algra A, Davies G, Oliveira SA, Palmer CN, Deary I, Schmidt H, Pandolfo M, Montaner J, Carty C, de Bakker PI, Kostulas K, Ferro JM, van Zuydam NR, Valdimarsson E, Nordestgaard BG, Lindgren A, Thijs V, Slowik A, Saleheen D, Pare G, Berger K, Thorleifsson G, Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi C, Seshadri S, Gschwendtner A, Boncoraglio GB, Sharma P, Bis JC, Gretarsdottir S, Psaty BM, Rothwell PM, Rosand J, Meschia JF, Stefansson K, Dichgans M and Markus HS. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol 2012; 11: 951-962.

[6] Kilarski LL, Achterberg S, Devan WJ, Traylor M, Malik R, Lindgren A, Pare G, Sharma P, Slowik A, Thijs V, Walters M, Worrall BB, Sale MM, Algra A, Kappelle LJ, Wijmenga C, Norrving B, Sandling JK, Ronnblom L, Goris A, Franke A, Sudlow C, Rothwell PM, Levi C, Holliday EG, Fornage M, Psaty B, Gretarsdottir S, Thorsteinsdottir U, Seshadri S, Mitchell BD, Kittner S, Clarke R, Hopewell JC, Bis JC, Boncoraglio GB, Meschia J, Ikram MA, Hansen BM, Montaner J, Thorleifsson G, Stefanson K, Rosand J, de Bakker PI, Farrall M, Dichgans M, Markus HS and Bevan S. Meta-analysis in more than 17,900 cases of ischemic stroke reveals a novel association at 12q24.12. Neurology 2014; 83: 678-685.

[7] 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, Muller-Myhsok B, Meisinger C, Seedorf U, Ross-Adams H, Boonen S, Wloch-Kopec D, Valant V, Slark J, Furie K, Delavaran H, Langford C, Deloukas P, Edkins S, Hunt S, Gray E, Dronov S, Peltonen L, Gretarsdottir S, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boncoraglio GB, Parati EA, Attia J, Holliday E, Levi C, Franzosi MG, Goel A, Helgadottir A, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Worrall BB, Kittner SJ, Mitchell BD, Kissela B, Meschia JF, Thijs V, Lindgren A, Macleod MJ, Slowik A, Walters M, Rosand J, Sharma P, Farrall M, Sudlow CL, Rothwell PM, Dichgans M, Donnelly P and Markus HS. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet 2012; 44: 328-333.

[8] Geyeregger R, Zeyda M and Stulnig TM. Liver X receptors in cardiovascular and metabolic disease. Cell Mol Life Sci 2006; 63: 524-539.

[9] Beaven SW and Tontonoz P. Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia. Annu Rev Med 2006; 57: 313-329.

[10] Joseph SB and Tontonoz P. LXRs: new therapeutic targets in atherosclerosis? Curr Opin Pharmacol 2003; 3: 192-197.

[11] Tontonoz P and Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. Mol Endocrinol 2003; 17: 985-993.

[12] Calkin AC and Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. Arterioscler Thromb Vasc Biol 2010; 30: 1513-1518.

[13] Naik SU, Wang X, Da Silva JS, Jaye M, Macphee CH, Reilly MP, Billheimer JT, Rothblat GH and Rader DJ. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. Circulation 2006; 113: 90-97.

[14] Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, Hogenesch J, O'Connell R M, Cheng G, Saez E, Miller JF and Tontonoz P. LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 2004; 119: 299-309.

[15] Verschuren L, de Vries-van der Weij J, Zadelaar S, Kleemann R and Kooistra T. LXR agonist suppresses atherosclerotic lesion growth and promotes lesion regression in apoE*3Leiden mice: time course and mechanisms. J Lipid Res 2009; 50: 301-311.

[16] Morales JR, Ballesteros I, Deniz JM, Hurtado O, Vivancos J, Nombela F, Lizasoain I, Castrillo A and Moro MA. Activation of liver X recep-

tors promotes neuroprotection and reduces brain inflammation in experimental stroke. Circulation 2008; 118: 1450-1459.
[17] Schuster GU, Parini P, Wang L, Alberti S, Steffensen KR, Hansson GK, Angelin B and Gustafsson JA. Accumulation of foam cells in liver X receptor-deficient mice. Circulation 2002; 106: 1147-1153.
[18] Stender S, Frikke-Schmidt R, Anestis A, Kardassis D, Sethi AA, Nordestgaard BG and Tybjaerg-Hansen A. Genetic variation in liver X receptor alpha and risk of ischemic vascular disease in the general population. Arterioscler Thromb Vasc Biol 2011; 31: 2990-2996.
[19] Zhou YF, Zhang J, Li ZX, Miao JL, Yin QX, Li JJ, Zhang XY, Li YY and Luo HL. Association of liver X receptor alpha (LXRalpha) gene polymorphism and coronary heart disease, serum lipids and glucose levels. Lipids Health Dis 2014; 13: 34.
[20] Toda N, Ayajiki K and Okamura T. Cerebral blood flow regulation by nitric oxide: recent advances. Pharmacol Rev 2009; 61: 62-97.
[21] Tsutsui M. Neuronal nitric oxide synthase as a novel anti-atherogenic factor. J Atheroscler Thromb 2004; 11: 41-48.
[22] Price ET, Pacanowski MA, Martin MA, Cooper-DeHoff RM, Pepine CJ, Zineh I and Johnson JA. Liver X receptor alpha gene polymorphisms and variable cardiovascular outcomes in patients treated with antihypertensive therapy: results from the INVEST-GENES study. Pharmacogenet Genomics 2011; 21: 333-340.
[23] Okazaki H, Goldstein JL, Brown MS and Liang G. LXR-SREBP-1c-phospholipid transfer protein axis controls very low density lipoprotein (VLDL) particle size. J Biol Chem 2010; 285: 6801-6810.
[24] Dahlman I, Nilsson M, Jiao H, Hoffstedt J, Lindgren CM, Humphreys K, Kere J, Gustafsson JA, Arner P and Dahlman-Wright K. Liver X receptor gene polymorphisms and adipose tissue expression levels in obesity. Pharmacogenet Genomics 2006; 16: 881-889.
[25] Robitaille J, Houde A, Lemieux S, Gaudet D, Perusse L and Vohl MC. The lipoprotein/lipid profile is modulated by a gene-diet interaction effect between polymorphisms in the liver X receptor-alpha and dietary cholesterol intake in French-Canadians. Br J Nutr 2007; 97: 11-18.
[26] Legry V, Cottel D, Ferrieres J, Chinetti G, Deroide T, Staels B, Amouyel P and Meirhaeghe A. Association between liver X receptor alpha gene polymorphisms and risk of metabolic syndrome in French populations. Int J Obes (Lond) 2008; 32: 421-428.
[27] Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, Jones CG, Zaitlen NA, Varilo T, Kaakinen M, Sovio U, Ruokonen A, Laitinen J, Jakkula E, Coin L, Hoggart C, Collins A, Turunen H, Gabriel S, Elliot P, McCarthy MI, Daly MJ, Jarvelin MR, Freimer NB and Peltonen L. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 2009; 41: 35-46.
[28] Legry V, Bokor S, Beghin L, Galfo M, Gonzalez-Gross M, Molnar D, Moreno LA, Amouyel P, Dallongeville J and Meirhaeghe A. Associations between common genetic polymorphisms in the liver X receptor alpha and its target genes with the serum HDL-cholesterol concentration in adolescents of the HELENA Study. Atherosclerosis 2011; 216: 166-169.