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Deiodinases, organic anion transporter polypeptide polymorphisms and ischemic stroke outcomes

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ABSTRACT

Background: Ischemic stroke is a major cause of premature death and chronic disability worldwide, and individual variation in functional outcome is strongly influenced by genetic factors. Neuroendocrine signaling by the hypothalamic – hypophyseal – thyroid axis is a critical regulator of post-stroke pathogenesis, suggesting that allelic variants in thyroid hormone (TH) signaling can influence stroke outcome.

Aim: To examine associations between acute ischemic stroke (AIS) outcome and allelic variants of the TH metabolizing enzymes deiodinase type 1–3 (DIO1–3) and membrane transporting organic anion polypeptide C1 (OATP1C1).

Methods: Eligible AIS patients from Lithuania (n = 248) were genotyped for ten DIO1–3 and OATP1C1 single nucleotide polymorphisms (SNPs): DIO1 rs12095080-A/G, rs11206244-C/T, and rs2235544-A/C; DIO2 rs225014-T/C and rs225015-G/A; DIO3 rs945006-T/G; OATP1C1 rs974453-G/A, rs10444412-T/C, rs10770704-C/T, and rs1515777-A/G. Functional outcome was evaluated one year after index AIS using the modified Rankin Scale. Analyses were adjusted for important confounders, including serum free triiodothyronine.

Results: After adjustment for potential confounders, the major allelic (wild-type) DIO3 genotype rs945006-TT was associated with better 1-year AIS functional outcome (odds ratio [OR] = 0.25; 95% confidence interval [CI]: 0.08–0.74; p = .013), while the wild-type OATP1C1 genotype rs10770704-CC was associated with poorer outcome (OR = 2.00, 95%CI: 1.04–3.86; p = .038).

Conclusion: Allelic variants in thyroid axis genes may prove useful for prognosis and treatment guidance.

1. Introduction

Stroke is the most frequent cause of serious long-term disability in many industrialized countries such as the United States [1], and ischemic stroke, which accounts for about 87% of all strokes, has continued to increase globally in both incidence and prevalence over the past several decades [2]. During and following ischemic stroke events, the extent of brain tissue injury is determined by early cellular necrosis in the ischemic core (infarct), more delayed and selective neuronal apoptosis in the surrounding region (penumbra), and by various longer-term inflammatory and neuroplastic processes [3]. Ischemic stroke also induces other systemic pathophysiological responses, including alterations in endocrine function [4]. The neuroendocrine system, composed of hypothalamus, hypophysis, and thyroid (the thyroid axis), also reacts rapidly to acute ischemic stroke (AIS). Serum levels of thyroid stimulating hormone (TSH) produced in the hypophysis, thyroid prohormone thyroxine (T4) produced in the thyroid, and the active thyroid hormone triiodothyronine (T3) produced from T4 primarily in peripheral organs reflect stroke severity [5], with worse post-ischemic stroke outcome usually associated with low T3, high T4, and normal or slightly reduced TSH. These hormonal alterations are not associated with primary thyroid or hypophysis disease and so are usually referred to collectively as non-thyroidal illness syndrome (NTIS) [6]. It is thought that NTIS as part of the acute response to stroke is triggered by caloric insufficiency, oxidative stress, and cytokines acting on factors determining the tissue bioavailability of thyroid hormones (TH) [7].

The thyroid axis normally maintains TH levels within a narrow range, and individual variations in these levels are influenced by a

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variety of genetic and environmental factors [8,9]. On the other hand, active local homoestatic mechanisms involving transmembrane transport, deiodination by deiodinases (DIOs), and TH receptor-mediated gene transcription modulate TH levels within target cells despite stable serum TH [10]. For example, it was shown that intraneuronal bioavailability of T3 is reduced under hypoxic conditions due to local tissue induction of the deiodinating enzyme deiodinase 3 (DIO3) [11].

The TH membrane transporters are members of several transporter protein families, and include the monocarboxylate transporters MCT8 and MCT10 as well as the organic anion transporters OATP1 and OATP3, of which MCT8 and OATP1C1 are the most important for brain TH homeostasis [12]. Indeed, MCT8 and OATP1C1 gene mutations are associated with severe neurologic dysfunction. Mutations in the MCT8 gene cause Allan-Herndon-Dudley syndrome, which is characterized by a severe neurologic phenotype due to brain hypothyroidism and peripheral hyperthyroidism as a result of high serum T3 [13]. In addition, a more recently described OATP1C1 mutation has been associated with severe brain hypometabolism and juvenile neurodegeneration with normal serum TH concentrations [14]. Furthermore, associations were found between single nucleotide polymorphisms (SNPs) in the OATP1C1 gene, namely rs10444412 and rs10770704, and fatigue with depression in hypothyroid patients [15].

The DIOs initiate (DIO1 and DIO2) or terminate (DIO3) thyroid hormone action, thus modulating the biological effects of TH [16]. No mutations inactivating DIOs have been identified in humans, but associations have been reported between DIO genetic polymorphisms and TH serum concentrations, affective disorders, and other morbidities [17]. The most intensively investigated DIO SNP is DIO2 rs225014 [18]. The clinical relevance of this SNP is suggested by its association with the response to combination therapy for hypothyroidism [19]. Moreover, rs225014 has demonstrated potential as a biomarker for Alzheimer's disease development in certain populations [20]. Other DIO2 SNP rs225015 was associated with with early-onset Type 2 diabetes in the case–control study with Pima Indians [21]. In addition, the DIO1 SNP rs11206244 was associated with lifetime risk of major depression in Caucasian females [22] and with the clinical effects of the antidepressant sertraline in depressed patients co-treated with T3 [23]. It was shown that DIO1 rs2235544, rs12095080, rs2235544 [17] and OATP1C1 rs1515777 [24] SNPs influence serum TH levels in humans. One meta-analysis also suggested a possible protective association of the minor allele variant of DIO3 rs945006 (G) with osteoarthritis onset [25], although no associations with neurological function has been reported.

Given these associations and the known contributions of TH signaling to stroke pathogenesis, we speculated that some of these genetic alterations would influence stroke outcome. We therefore compared the genotypes of 10 SNPs in DIO1–3 and OATP1C1 genes among a large cohort of AIS patients.

2. Methods

2.1. Ethics

The study protocol was approved by the Regional Biomedical Research Ethics Committee (Permission Numbers: P1-BE-2-11/2013, P2-BE-2-11/2013) and is described in detail elsewhere [26]. Written informed consent was obtained from all patients directly or from relatives in cases where the patient was unable to sign the consent form due to paralysis or vision impairment.

2.2. Study design

On admission, the following baseline demographic and clinical data were recorded: age, sex, stroke severity, level of disability before stroke, arterial blood pressure, use of antithrombotics (i.e., antiaggregants, anticoagulants), history of arterial hypertension (AH), atrial flutter (AF), diabetes mellitus (DM), previous ischemic stroke and/or transient ischemic attack (PISTIA), peripheral artery disease, myocardial infarction (MI), smoking behavior, and intravenous thrombolysis treatment. Stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS). Disability before index stroke was evaluated by the modified Rankin Scale (mRS) through interview with the patient or caregiver in case of dysphasia. Arterial hypertension was defined as systolic blood pressure $\geq$ 130 mmHg, diastolic pressure $\geq$ 80 mmHg, or current antihypertensive drug administration. Diabetes mellitus was diagnosed by a fasting plasma glucose level $\geq$ 7.0 mmol/L, plasma glucose $\geq$11.1 mmol/L 2 h following 75 g oral glucose, or current administration of hypoglycemic agents for pre-existing diabetes (Type 1 or Type 2). Patients were considered active smokers if they had evidence of current active smoking or had smoked at least 100 cigarettes in their lifetime but had been smoke-free for the last four weeks.

Blood specimens were collected from all patients on admission and again when possible at the end of the first week. Patient outcomes were evaluated by mRS one year after AIS based on telephone interviews with the participants' family. A mRS score $>$ 2 was defined as poor AIS 1-year outcome and mRS $\leq$ 2 as good outcome.

2.3. Study population

Consecutive patients presenting with AIS and admitted to the departments of neurology at Klaipeda University Hospital or the Hospital of Lithuanian University of Health Sciences Kauno Klinikos over six-month periods during 2013 and 2014 years, respectively, or to Klaipeda Seamen's Hospital over a one-year period starting September 2016 were considered for enrolment.

We calculated a minimum sample size of 205 patients (degree of variability 0.5; confidence interval (CI) of 95%; absolute precision of 5%) according to Yamane [27] after a finite population correction under the assumption of 1250 combined annual ischemic stroke cases at the three study centers.

Patients were accepted into to the study if presenting within 48 h of AIS onset and if older than 18 and younger than 80 years. Acute ischemic stroke was defined according to World Health Organization criteria [28] and confirmed by brain computed tomography (CT). In cases with possible stroke mimics (epilepsy, tumor, migraine aura, peripheral neuropathy, demyelinating disease, posterior reversible encephalopathy syndrome, or encephalitis), brain magnetic resonance tomography was also performed. Patients were exluded for the following reasons: known thyroidopathy, severe infection, malignancy, liver or renal insufficiency, thyroid axis hormone profile on admission outside biochemical euthyroidism, subclinical hypo- or hyperthyroidism, low-T3 syndrome, current administration of thyroid hormone, antithyroid drugs, steroids, or amiodarone, and iodinated contrast agent exposure within the previous 2 weeks (Fig. 1). A total of 248 patients were included in the study.

2.4. Measures

2.4.1. Assessment of thyroid axis hormones

Venous blood specimens were collected on admission to hospital and within 48 h after AIS onset. Serum TSH, free T3 (FT3), and free T4 (FT4) levels were measured on admission and repeated for eligible patients upon discharge from the participating neurology departments. Patients who refused to give consent for blood withdrawal (n = 22), who used amiodarone for arrhythmia, received iodinated contrast agents (n = 3), who died (n = 16), developed severe illnesses (n = 7), or who could not provide the blood sample for other reasons (n = 27) were excluded from the second blood sampling. In total, second blood samples were obtained for 173 patients (69.8%).

The serum was separated from blood by centrifugation at 3000g and then frozen at -70 °C. Serum levels of TSH, FT3, and FT4 were analyzed using an electrochemiluminescence immunoassay (Advia Centaur

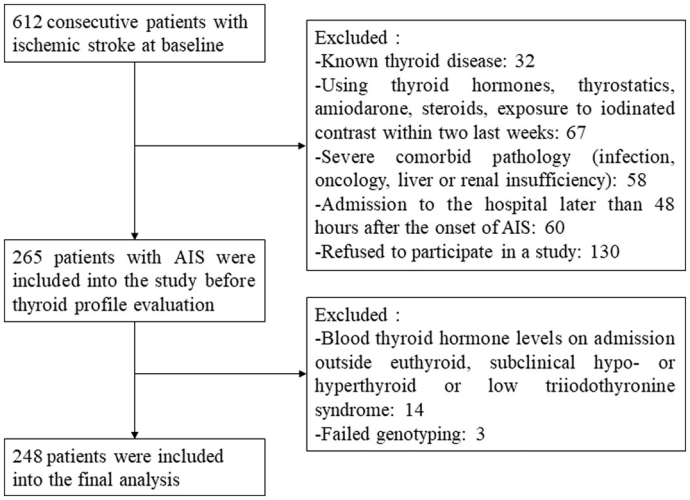

Fig. 1. Study recruitment profile.

XP 2016; Siemens Osakeyhtio). The normal (biochemically euthyroid) range for TSH was defined as 0.55–4.78 mIU/L, FT3 as 3.50–6.50 pmol/L, and FT4 as 11.50–22.70 pmol/L. Subclinical hypothyroidism was defined as serum TSH > 4.780 mIU/L with normal levels of FT4 and FT3, subclinical hyperthyroidism as TSH < 0.550 mIU/L with normal FT4 and FT3, and low-T3 syndrome as FT3 < 3.50 pmol/L with normal levels of TSH and FT4.

2.4.2. Genotype analyses

Blood samples were frozen and stored at −70 °C. In this study, we evaluated 10 SNPs for thyroid axis-related genes selected according to a previously published study [24]: DIO1 rs12095080-A/G, rs11206244-C/T, and rs2235544-A/C; DIO2 rs225014-T/C and rs225015-G/A; DIO3 rs945006-T/G; OATP1C1 rs974453-G/A, rs10444412-T/C, rs10770704-C/T, and rs1515777-A/G. SNPs were selected on the basis of previously established their possible influence on various morbidities and physiological states, as mentioned earlier, except for OATP1C1 rs974453 with yet unknown associations. We selected the minor allele according to the 1000 Genomes study (European population) and Genetic variation in the Estonian population from the National Centre for Biotechnology Information dbSNP (https://www.ncbi.nlm.nih.gov/snp/). Genotyping was performed according to the manufacturer's instructions using the following commercially available SNP assays and TaqMan®Universal Master Mix II, no UNG (all from Applied Biosystems, Foster City, USA): C_31601225_10 (rs12095080), C_334342_20 (rs11206244), C_15952583_10 (rs2235544), C_15819951_10 (rs225014), C_568127_10 (rs225015), C_7565113_10 (rs945006), C_1710074_20 (rs974453), C_1710122_10 (rs10444412), C_31106584_10 (rs10770704), and C_9579217_10 (rs1515777). All reactions were conducted using the ABI 7900HT real-time PCR Thermocycler (Applied Biosystems). Samples were measured in duplicate and nuclease-free water (Ambion, Austin, TX, USA) was used as the no-template control.

2.5. Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) or as median (interquartile range) according to data distribution. The change in FT3 (ΔFT3) is defined as baseline value minus value at discharge. Continuous variables were compared using Student's *t*-test or Mann−Whitney *U* test. Group differences in categorical variables were analyzed using the chi-square test.

All evaluated SNPs were tested for Hardy−Weinberg equilibrium (HWE) by chi-square tests before inclusion in the association statistics (threshold $p > .01$). The three genotype groups were compared according to dominant (wild-type homozygous + heterozygous versus minor allele homozygous), recessive (wild-type homozygous versus heterozygous + minor allele homozygous), and additive (minor allele homozygous versus heterozygote versus wild type homozygous) models [29]. The distributions of minor alleles and of the dominant and recessive genotype models were compared between good and poor outcome groups using the chi-square test. The distributions of the additive genotype model were compared between good and poor outcome groups using the Cochran−Armitage test of trend. For the additive model, dummy variables were produced because model variables have three category levels. Significant predictors for AIS outcome (genetic and non-genetic confounders such as age, NIHSS, mRS before AIS ≤ 2, diastolic blood pressure on admission, PISTIA, previous MI and FT3 serum levels on admission) were included in the multivariate regression model. Results are reported as odds ratio (OR) and 95% confidence interval (CI). All statistical tests were two-tailed and $p < .05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 23) for Windows.

3. Results

3.1. Patients characteristics

Table 1 summarizes baseline clinical and laboratory data according to outcome group (poor or good). Median age of the entire cohort was 69 (62–75) years and there was slight male predominance (58.9%). The median NIHSS score was 7 (4–13) and the vast majority of patients (94.4%) had a premorbid mRS ≤ 2. Among all patients, 76 (30.6%) were treated by intravenous thrombolysis.

Patients with poor outcome at 1 year after AIS were significantly older (all $p = .011$), had more severe stroke according to NIHSS ($p < .001$), were more often disabled before the index AIS ($p = .001$), and exhibited higher diastolic blood pressure on admission ($p = .010$), more frequent previous PISTIA ($p = .011$), more frequent MI ($p = .041$), and lower serum FT3 ($p = .020$) compared to the good outcome group. The groups did not differ significantly in other evaluated demographic and clinical features (including serum TSH and FT4) on admission (Table 1).

Patients who donated a second blood sample one week post-admission were significantly less likely to receive intravenous thrombolysis then those who did not (23.1% vs. 48.0%, $p < .001$). There was no difference in serum ΔFT3 between good and poor AIS outcome groups ($p = .566$).

Table 1
Baseline characteristics of participants.

Baseline characteristics	All AIS patients	Outcomes 1 year after AIS according to modified Rankin Scale		*p*
		≤2	> 2	
n	248	95	153	
Demographic characteristics				
Age, years median (IQR)	69 (62–75)	65.5 (59–74)	70 (63–76)	**0.011**
Men, n (%)	146 (58.9)	58 (61.1)	88 (57.5)	0.598
National Institutes of Health Stroke Scale, median (IQR)	7 (4–13)	4 (3–8)	11 (6–16.5)	< **0.001**
Modified Rankin Scale before AIS ≤ 2, n (%)	234 (94.4)	95 (100)	139 (90.8)	**0.001**
Specific treatment of AIS				
AIS treatment with intravenous thrombolysis, n (%)	76 (30.6)	32 (33.7)	44 (28.8)	0.249
Blood pressure on admission				
Diastolic arterial blood pressure, median (IQR)	90 (80–100)	90 (80–95)	90 (80.5–100)	**0.010**
Systolic arterial blood pressure, median (IQR)	158 (14–171.75)	155 (140–166)	159 (145–180)	0.051
Antithrombotic treatment before admission				
Antiaggregants, n (%)	73 (29.4)	29 (30.5)	44 (28.8)	0.776
Anticoaguliants, n (%)	27 (10.9)	10 (10.5)	17 (11.1)	1.000
Vascular risk factors				
Arterial hypertension, n (%)	185 (74.6)	64 (67.4)	121 (79.1)	0.051
Atrial fibrillation, n (%)	99 (39.9)	33 (34.7)	66 (43.1)	0.230
Smoking, n (%)	59 (23.8)	21 (22.1)	38 (24.8)	0.649
Diabetes mellitus, n (%)	40 (16.1)	12 (12.6)	28 (18.3)	0.288
Previous ischemic stroke and/or transient ischemic attack, n (%)	52 (21)	12 (12.6)	40 (26.1)	**0.011**
Peripheral artery disease, n (%)	11 (4.4)	3 (3.2)	8 (5.2)	0.539
Previous myocardial infarction, n (%)	22 (8.9)	13 (13.7)	9 (5.9)	**0.041**
Laboratory findings on admission				
Free triiodothyronine (pmol/L), mean ± SD	4.28 ± 0.72	4.42 ± 0.62	4.20 ± 0.76	**0.020**
Free thyroxine (pmol/L), mean ± SD	16.15 ± 2.52	15.87 ± 2.39	16.33 ± 2.59	0.166
Ratio of free triiodothyronine and free thyroxine, mean ± SD	0.27 ± 0.06	0.28 ± 0.52	0.26 ± 0.56	**0.003**
Thyroid stimulating hormone (mIU/L), median (IQR)	1.15 (0.70–1.88)	1.22 (0.81–1.94)	1.03 (0.65–1.87)	0.052
Laboratory findings on discharge				
n	173	64	109	
ΔFT3, median (IQR)	−0.12 (−0.52–0.36)	−0.18 (−0.47–0.22)	−0.10 (−0.56–0.41)	0.566

AIS, acute ischemic stroke; IQR, interquartile range; SD standard deviation; ΔFT3, free triiodothyronine at baseline minus values on discharge. Bold notes significant difference between groups.

3.2. Association of SNPs with functional outcome at the end of the first year after AIS

All genotypes (Table 2) were in Hardy–Weinberg equilibrium ($p > .05$). Minor allele frequencies (MAFs) in the poor and good outcome groups are shown in Table 3. The DIO3 rs945006 SNP minor allele (G) was significantly more frequent in the poor outcome group than the good outcome group (0.08 vs. 0.03, $p = .025$), while the OATP1C1 rs10770704 minor allele (T) was less frequent in the poor outcome group (0.38 vs. 0.49, $p = .016$).

Wild-type DIO3 rs945006 genotype (TT) was associated with lower odds of poor outcome one year after index AIS, even after adjustment for important confounders (OR = 0.25; 95% CI: 0.08–0.74; $p = .013$), compared to genotypes with at least one minor G allele (recessive model), suggesting a protective role (Table 4). The same results were found when applying the additive model. In contrast, the wild-type OATP1C1 rs10770704 genotype (CC) was associated with increased odds for poor 1-year outcome after adjustment for important confounders (OR = 2.00, 95% CI: 1.04–3.86; $p = .038$) versus other genotypes using the recessive model. A similar effect was found when applying the additive model (OR = 1.60, 95% CI: 1.04–2.45, $p = .031$).

4. Discussion

Many physiological and genetic factors are associated with AIS outcome, including secondary stroke, concomitant diseases, and the efficacy of post-stroke rehabilitation, so prediction accuracy in individual cases is still poor [30]. Genetic variants are among the most promising factors for outcome prediction in individual cases [31], but relatively few have been identified. These include polymorphisms of the Pals1-associated tight junction gene [32] and heme oxygenase [33]. Stroke outcome involves numerous pathogenic processes acting over different time frames, so it is likely that multiple prognostic genes must be identified. We contribute to this effort by identifying two allelic variants in thyroid axis genes significantly associated with AIS outcome.

Our study investigated 10 SNPs within genes that contribute to intracellular TH bioavailability, including three DIO1, two DIO2, one DIO3, and four OATP1C1 SNPs. Analyses identified novel associations of DIO3 rs945006 and OATP1C1 rs10770704 with 1-year outcome, although the underlying mechanisms are a matter of speculation. Thyroid hormones modulate numerous aspects of neural function [34], including inflammatory cell behavior [35], neuroplasticity [36], adult neurogenesis [37], and apoptosis [38], processes implicated in AIS stroke lesion formation or recovery. Therefore, genetic factors influencing TH metabolism and bioavailability (e.g., DIOs and TH cellular membrane transporters) are likely to impact multiple stroke-related processes that contribute to functional outcome. Numerous associations have been identified between non-coding genetic variants and diseases [39], and both DIO3 rs945006 and OATP1C1 rs10770704 are located in non-coding regions. It is possible that, like other disease associations, these SNPs affect basal transcription or transcriptional responses to various external stimuli.

According to the human protein atlas (https://www.proteinatlas.org), DIO3 is expressed at different levels in cortex, caudate, hippocampus, and hypothalamus. Its main function is to inactivate T3 and protect neural cells against excessive intracellular concentrations. Mouse mutants lacking active DIO3 show sensory, behavioral, and cognitive dysfunction [40]. The DIO3 SNP rs945006 is located in the 3′ untranslated region (3′-UTR) of the mRNA, where it may exert a post-transcriptional influence on gene expression through interaction with microRNAs and various translational regulatory proteins. Studies have

Table 2
The genotype frequency distribution of DIO1–3 and OATP1C1 SNPs in all AIS patients and in groups with poor and good AIS outcome.

Polymorphism ID	Variation	All AIS patients	Outcomes 1 year after AIS according to modified Rankin Scale		*p*
			≤2	> 2	
		248	95	153	
DIO1 rs12095080	Minor allele homozygous GG	1 (0.4)	1 (1.1)	0	0.480
	Heterozygous AG	40 (16.1)	12 (12.6)	28 (18.3)	
	Wild type homozygous AA	207 (83.5)	82 (86.3)	125 (81.7)	
DIO1 rs11206244	Minor allele homozygous TT	37 (14.9)	13 (13.7)	24 (15.7)	0.817
	Heterozygous CT	120 (48.4)	45 (47.4)	75 (49.0)	
	Wild type homozygous CC	91 (36.7)	37 (38.9)	54 (35.3)	
DIO1 rs2235544	Minor allele homozygous CC	51 (20.6)	19 (20.0)	32 (20.9)	0.666
	Heterozygous AC	122 (49.2)	50 (52.6)	72 (47.1)	
	Wild type homozygous AA	75 (30.2)	26 (27.4)	49 (32.0)	
DIO2 rs225014	Minor allele homozygous CC	17 (6.9)	5 (5.3)	12 (7.8)	0.115
	Heterozygous CT	105 (42.3)	48 (50.5)	57 (37.3)	
	Wild type homozygous TT	126 (50.8)	42 (44.2)	84 (54.9)	
DIO2 rs225015	Minor allele homozygous AA	18 (7.3)	7 (7.4)	11 (7.2)	0.221
	Heterozygous AG	101 (40.7)	45 (47.4)	56 (36.6)	
	Wild type homozygous GG	129 (52.0)	43 (45.3)	86 (56.2)	
DIO3 rs945006	Minor allele homozygous GG	1 (0.4)	0	1 (0.7)	**0.025**
	Heterozygous GT	29 (11.7)	6 (6.3)	23 (15.0)	
	Wild type homozygous TT	218 (87.9)	89 (93.7)	129 (84.3)	
OATP1C1 rs974453	Minor allele homozygous AA	9 (3.6)	5 (5.3)	4 (2.6)	0.190
	Heterozygous AG	69 (27.8)	21 (22.1)	48 (31.4)	
	Wild type homozygous GG	170 (68.5)	69 (72.6)	101 (66.0)	
OATP1C1 rs10444412	Minor allele homozygous CC	56 (22.6)	24 (25.3)	32 (920.9)	0.574
	Heterozygous CT	117 (30.2)	41 (43.2)	76 (49.7)	
	Wild type homozygous TT	75 (30.2)	30 (931.6)	45 (29.4)	
OATP1C1 rs10770704	Minor allele homozygous TT	50 (20.2)	23 (24.2)	27 (17.6)	**0.044**
	Heterozygous CT	109 (44.0)	47 (49.5)	62 (40.5)	
	Wild type homozygous CC	89 (35.9)	25 (26.3)	64 (41.8)	
OATP1C1 rs1515777	Minor allele homozygous GG	41 (16.5)	19 (20.0)	22 (14.4)	0.420
	Heterozygous GA	123 (49.6)	43 (45.3)	80 (52.3)	
	Wild type homozygous AA	84 (33.9)	33 (34.7)	51 (33.3)	

AIS, acute ischemic stroke; DIO1, deiodinase 1; DIO2, deiodinase 2; DIO3, deiodinase 3; OATP1C1, organic anion transporting polypeptide C1; SNP, single nucleotide polymorphism. Bold notes significant difference between groups.

established associations between several 3′-UTR SNPs and neurodegenerative disorders [41,42]. We suggest that 3′-UTR SNPs can affect miRNA activity and consequently DIO3 protein expression. The association between poor AIS outcome and the rs945006 minor allele may arise through direct effects on T3 bioavailability. Alternatively, there may be other functional SNPs in linkage disequilibrium with rs945006, or this SNP may act through genomic imprinting [40].

The membrane transporter OATP1C1 is responsible for delivering serum T4 to the brain [43]. OATP1C1 rs10770704 SNP could impact neurologic phenotype, at least in certain circumstances, as this SNP has been associated with affective symptoms in hypothyroid patients [15]. This OATP1C1 SNP is located within an intron of immature mRNA. It is known that introns participate in regulation of gene expression [44] and a recent meta-analysis of genome-wide association studies identified an association between recovery from ischemic stroke and an intronic variant in the PTCH1 gene [45]. We speculate that the minor allele of rs10770704 could act to protect neurons from post-stroke pathogenesis by affecting intracellular TH levels.

An important question arising from our analysis is why the established major allele of OATP1C1 rs10770704 is associated with poor AIS outcome, as it is usually the minor allele that is associated with disease [46] due to negative selection pressure. However, for some

Table 3
Minor allele frequency of genotyped loci for DIO1–3 and OATP1C1 SNPs in AIS patients with poor and good outcome groups.

Polymorphism ID	Functional consequence	Variation	Study MAF			*p*
			All	Modified Rankin Scale 1 year after AIS		
				≤2	> 2	
DIO1 rs12095080	Non coding transcript variant	A > G	0.085	0.07	0.16	0.488
DIO1 rs11206244	Non coding transcript variant	C > T	0.391	0.37	0.40	0.530
DIO1 rs2235544	Intron variant	A > C	0.452	0.46	0.44	0.684
DIO2 rs225014	Missense variant	T > C	0.280	0.31	0.27	0.328
DIO2 rs225015	3 prime UTR variant	G > A	0.276	0.20	0.26	0.178
DIO3 rs945006	3 prime UTR variant	T > G	0.063	0.03	0.08	**0.025**
OATP1C1 rs974453	Intron variant	G > A	0.175	0.16	0.18	0.572
OATP1C1 rs10444412	Non coding transcript variant	T > C	0.462	0.47	0.46	0.813
OATP1C1 rs10770704	Intron variant	C > T	0.42	0.49	0.38	**0.016**
OATP1C1 rs1515777	Intron variant	A > G	0.413	0.43	0.41	0.643

AIS, acute ischemic stroke; DIO1, deiodinase 1; DIO2, deiodinase 2; DIO3, deiodinase 3; MAF, minor allele frequency; OATP1C1, organic anion transporting polypeptide C1; SNP, single nucleotide polymorphism; UTR, untranslated region. Bold notes significant difference between groups.

Table 4
Genotype distribution and association with 1-year AIS outcome for DIO1–3 and OATP1C1 SNPs.

SNP	Genotype model		Modified Rankin Scale ≤ 2 n = 95 (38.3%)	Modified Rankin Scale > 2 n = 153 (61.7%)	p	Multivariate logistic regression, OR (95% CI)	p
DIO1 rs12095080	Dominant	GG	1 (1.1)	0 (0.0)	0.383	–	–
		AA + AG	94 (98.9)	153 (100)			
	Recessive	AA	82 (86.3)	125 (81.7)	0.383	0.59 (0.26–1.34)	0.206
		AG + GG	13 (13.7)	28 (18.3)			
	Additive	GG/AG/AA	1 (1.1)/12(12.6)/94 (98.9)	0 (0.0)/ 28(18.3)/125 (81.7)	0.479	0.66 (0.31–1.44)	0.300
DIO1 rs11206244	Dominant	TT	13 (13.7)	24 (15.7)	0.717	1.05 (0.44–2.47)	0.918
		CC + CT	82 (86.3)	129 (84.3)			
	Recessive	CC	37 (38.9)	54 (35.3)	0.589	1.23 (0.66–2.31)	0.518
		CT+ TT	58 (61.1)	99 (64.7)			
	Additive	TT/CT/CC	13 (13.7)/45 (47.4)/37 (38.9)	24 (15.7)/75 (49.0)/54 (35.3)	0.527	1.12 (0.72–1.74)	0.614
DIO1 rs2235544	Dominant	CC	19 (20.0)	32 (20.9)	0.862	0.62 (0.29–1.33)	0.222
		AA + AC	76 (80.0)	121 (79.1)			
	Recessive	AA	26 (27.4)	49 (32.0)	0.479	0.94 (0.481–1.86)	0.941
		AC + CC	69 (72.6)	104 (68.0)			
	Additive	CC/AC AA	19 (20.0)/50 (52.6)/26 (27.4)	32 (20.9)/72 (47.1)/49 (32.0)	0.686	1.20 (0.78–1.86)	0.409
DIO2 rs225014	Dominant	CC	5 (5.3)	12 (7.8)	0.606	0.84 (0.22–3.21)	0.837
		TT + TC	90 (94.7)	141 (92.2)			
	Recessive	TT	42 (44.2)	84 (54.9)	0.102	1.48 (0.80–2.74)	0.214
		TC + CC	53 (55.8)	69 (45.1)			
	Additive	CC /TC/ TT	5(5.3)/48(50.5)/42 (44.2)	12 (7.8)/ 57(34.5)/84 (54.9)	0.339	1.28 (0.76–2.13)	0.350
DIO2 rs225015	Dominant	AA	7 (7.4)	11 (7.2)	1.000	1.86 (0.53–6.06)	0.336
		GG + GA	88 (92.6)	142 (92.8)			
	Recessive	GG	43 (45.3)	86 (56.2)	0.117	1.57 (0.84–2.91)	0.155
		GA + AA	52 (54.7)	67 (43.8)			
	Additive	AA/GA/GG	7 (7.4)/45(47.4)/ 43 (45.3)	11 (7.2)/56 (36.6)/86 (56.2)	0.174	1.49 (0.90–2.47)	0.123
DIO3 rs945006	Dominant	GG	0 (0.0)	1 (0.7)	1.000	–	–
		TT + TG	95 (100)	152 (99.3)			
	Recessive	TT	89 (93.7)	129 (84.3)	**0.029**	**0.25 (0.08–0.74)**	**0.013**
		TG + GG	6 (6.3)	24 (15.7)			
	Additive	GG /TG/TT	0 (0.0)/6(6.3)/89 (93.7)	1 (0.7)/23(15)/129 (84.3)	**0.025**	**0.25 (0.08–0.74)**	**0.013**
		AC + CC	69 (72.6)	104 (68.0)			
	Additive	CC/AC AA	19 (20.0)/50 (52.6)/26 (27.4)	32 (20.9)/72 (47.1)/49 (32.0)	0.686	1.20 (0.78–1.86)	0.409
OATP1C1 rs974453	Dominant	AA	5 (5.3)	4 (2.6)	0.310	4.05 (0.72–22.66)	0.112
		GG + GA	90 (94.7)	149 (97.4)			
	Recessive	GG	69 (72.6)	101 (66.0)	0.325	0.89 (0.46–1.74)	07355
		GA + AA	26 (27.4)	52 (34.0)			
	Additive	AA/GA/GG	5 (5.3)/21 (22.1)/69 (72.6)	4 (2.6)/48 (31.4)/101 (66.0)	0.579	1.08 (0.61–1.92)	0.782
OATP1C1 rs10444412	Dominant	CC	24 (25.3)	32 (20.9)	0.438	1.19 (0.58–2.45)	0.636
		TT + TC	71 (74.7)	121 (79.1)			
	Recessive	TT	30 (31.6)	45 (29.4)	0.777	0.86 (0.44–1.02)	0.653
		TC + CC	65 (68.4)	108 (70.6)			
	Additive	CC/TC/TT	24 (25.3)/41 (43.2)/30 (31.6)	32 (20.9)/76 (49.7)/45 (29.4)	0.817	1.00 (0.65–1.53)	0.996
OATP1C1 rs10770704	Dominant	TT	23(24.2)	26 (17.1)	0.210	1.77 (0.83–3.77)	0.139
		CC + CT	72 (75.8)	126 (82.9)			
	Recessive	CC	25 (26.3)	64 (41.8)	**0.013**	**2.00 (1.04–3.86)**	**0.038**
		CT + TT	70 (73.7)	89 (58.2)			
	Additive	TT/CT/CC	23(24.2)/47 (49.5)/25 (26.3)	26 (17.1)/62 (40.5)/64 (41.8)	**0.021**	**1.60 (1.04–2.45)**	**0.031**
OATP1C1 rs1515777	Dominant	GG	19 (20.0)	22 (14.4)	0.292	0.73 (0.32–1.64)	0.443
		AA + AG	76 (80.0)	131 (85.6)			
	Recessive	AA	33 (34.7)	51 (33.3)	0.890	0.882 (0.46–1.69)	0.705
		AG + GG	62 (65.3)	102 (66.7)			
	Additive	GG/AG/AA	19 (20.0)/43 (45.3)/33 (34.7)	22 (14.4)/80 (52.3)/51 (33.3)	0.639	1.04 (0.66–1.62)	0.871

Multivariate analysis was adjusted to patients age, National Institutes of Health Stroke Scale, disability according to modified Rankin Scale ≤2 before AIS, diastolic blood pressure on admission, earlier experienced cerebral ischemic event, previous myocardial infarction and free triiodothyronine serum levels on admission.
AIS, acute ischemic stroke; DIO1, deiodinase 1; DIO2, deiodinase 2; DIO3, deiodinase 3; CI, confidence interval; OATP1C1, organic anion transporting polypeptide C1; OR, odds ratio; SNP, single nucleotide polymorphism. Bold notes significant difference between groups.

environment- or lifestyle-dependent disorders such as Alzheimer's disease and Parkinson's disease, a previously neutral major allele with recent environmental changes could became a risk allele that has not yet been substantially affected by natural selection [46,47]. According to this notion, one-year ischemic stroke outcome could also depend on various environment/lifestyle-dependent conditions influenced by "risky" major alleles.

4.1. Limitations

Our study has several limitations. First, blood samples for hormone assessment were collected on admission regardless of time of day, so diurnal variation could influence group differences [48]. Second, no radiologic markers, such as stroke volume and leukoaraiosis, were used to assess severity [49]. Third, post-stroke rehabilitation programs [50] were not considered as confounders. Finally, a second assessment of the serum TH was possible only for some surviving patients.

5. Conclusions

In conclusion, we report for the first time significant associations of 1-year AIS functional outcome with SNPs DIO3 rs945006 and OATP1C1 rs10770704 independent of serum thyroid axis hormone levels. The wild-type DIO3 rs945006 polymorphism decreased while OATP1C1 rs10770704 increased the odds of poor outcome one year after AIS compared to genotypes with at least one minor allele. These SNPs may

be biomarkers for predicting AIS outcome thus providing aid for discharge planning, patient management and cost of health delivery at the individual level independently of serum TSH and TH levels.

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Declaration of Competing Interest

None.

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