The ClC-Kb^{T481S} Chloride Channel Gene Polymorphism, Ischaemic Stroke and Hypertension

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Abstract: Stroke is a polygenic disorder. Previous genetic studies focused on candidate genes influencing pathogenic processes, with little emphasis on genes influencing vascular risk factors. Previous research linked the ClC-Kb^{T481S} polymorphism to blood pressure (BP). We therefore undertook an association study to determine the relevance of this polymorphism to stroke, particularly lacunar stroke, given its strong correlation with hypertension. We genotyped DNA from 180 patients with acute ischaemic stroke (44 having lacunar stroke) and 298 age- and gender-matched controls using a sequence-specific polymerase chain reaction method (SS-PCR). We found no association between the ClC-Kb^{T481S} polymorphism and ischaemic stroke (Odds Ratio (OR): 0.87, 95% Confidence Interval (CI): 0.57-1.33). Stratification for stroke subtype did not alter this finding. This polymorphism showed a borderline association with history of hypertension ($p=0.06$) but was not associated with systolic or diastolic BP ($p>0.05$). To our knowledge there are no other studies published on this polymorphism and stroke.

INTRODUCTION

Previous genetic studies on stroke patients considered candidate genes that influence pathogenic processes, with little emphasis on genes that influence vascular risk factors.

The ClC-Kb^{T481S} polymorphism causes a threonine to serine amino acid substitution at position 481 of the renal tubule chloride channel ClC-Kb protein, resulting in enhanced activity and renal salt retention [1]. A previous study reported an association with hypertensive BP levels [2]. Therefore, given the strong positive association between BP and stroke [3,4], particularly of lacunar stroke with hypertension [5], we initiated an association study to determine the relevance of this polymorphism to stroke.

Our aim was to determine the relevance of this chloride channel polymorphism to (a) ischaemic stroke, (b) lacunar stroke, (c) BP and (d) hypertension. To our knowledge, this is the first study to investigate the association between this polymorphism and stroke.

MATERIALS AND METHODS

We genotyped DNA from 180 acute ischaemic stroke patients (44 with lacunar stroke) and 298 age- and gender-matched controls, sourced from our earlier prospective case-control study, using the same method [6]. Research ethics approval was obtained. All participants had been assessed for known cerebrovascular risk factors including age, gender, ethnicity, family history of stroke, cigarette smoking, hypercholesterolemia, hypertension, diabetes and atrial fibrillation.

Hypertension was defined as a history of hypertension, on treatment for hypertension, or BP>140mm Hg systolic or >90mm Hg diastolic [6]. Stroke subtype was determined using Oxfordshire Community Stroke Project criteria [7] and brain Computerised Tomography or Magnetic Resonance Imaging.

The two forward allele-specific primers were wild-type (A) CAGGGGCTGTGACCCACA and mutant (T) CAG GGGCTGTGACCCACT, while the sequence of the reverse consensus primer was AGAACAGAGCACAGCTGTGT (from Genbank Accession Number NT_004873.16, accessed 7/10/2004, synthesized by GeneWorks Pty Ltd, South Australia). The detected SNP sequence was confirmed by sequence analysis. The 338 base-pair fragment around the ClC-Kb^{T481S} polymorphism was amplified from the extracted DNA, using the SS-PCR method [8]. Amplified products were examined by gel electrophoresis, with a positive control (DRB1 HLA primer) in each reaction mix, as previously described [6], and negative controls in every run. Only gel results with a positive control band present were accepted; any banding in the negative controls resulted in the entire assay being repeated. Genotypes were determined by visual inspection by a single trained reader (AGM).

STATISTICAL ANALYSIS

Logistic regression was used to determine the risk of ischaemic stroke associated with the ClC-Kb^{T481S} polymorphism for all stroke patients and, following stratification, for stroke subtype [6]. The association between the ClC-Kb^{T481S} polymorphism and BP was determined using a 2–sample t–test, and between the polymorphism and hypertension using Pearson's Chi-square analysis. Hardy-Weinberg equilibrium was assessed.

Using the minor allele population frequency of 12.4% [2], power was calculated using Win Episcope (ver. 2, http://www.clive.ed.ac.uk/winepiscope/). For 95% confi-

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Table 1. Association of the ClC-Kb^{T481S} Gene Polymorphism with Ischaemic and Lacunar Stroke Patients. Values represent the number of subjects (percentage of subjects in parentheses), Odds Ratio (O.R.) with 95% confidence intervals and probability (p) values

ClC-Kb^{T481S} genotype	Controls	Ischaemic Stroke Subjects				Lacunar Stroke Subjects			
	n (%)	n (%)	O.R.	95% C.I.	p	n (%)	O.R.	95% C.I.	p
AA	217 (73)	136 (76)	0.87	0.57 – 1.33	0.51	34 (77)	0.79	0.37 – 1.66	0.53
AT or TT	81 (27)	44 (24)				10 (23)			
Totals	298 (100)	180 (100)				44 (100)			

dence levels and 80% beta, 471 subjects were required to detect a minimum odds ratio of 2.0.

RESULTS

Analysis was performed on 180 patients and 298 controls from whom DNA was available. Allelic frequencies were in Hardy-Weinberg equilibrium (Chi-square<3.841, p>0.05). Full study demographics and risk factor characteristics described previously showed no significant difference between patients and controls for vascular risk factors [6]. Control allelic frequencies (14.1%) were reported previously [9]. Data for the whole study group (n=478) as well as the lacunar stroke subset of patients is presented in Table **1**. The odds ratio confidence intervals encompass unity; hence the data offer no evidence that the odds of stroke are influenced by this chloride channel genotype. Adjustment for the cerebrovascular risk factors cited above did not alter the outcome.

We found that the ClC-Kb^{T481S} polymorphism demonstrated a borderline association with the sub-group that had a history of hypertension (Table **2**; p=0.06), but no association with the larger above-defined hypertension group. We also found no association with either systolic BP or diastolic BP (Table **3**; p>0.05).

Table 2. Association of the ClC-Kb^{T481S} Gene Polymorphism with the 'History of Hypertension' Sub-Group.

ClC-Kb^{T481S} genotype	Nil History of Hypertension	History of Hypertension	
	n (%)	n (%)	p
AA	175 (70)	178 (78)	0.06
AT or TT	74 (30)	51 (22)	
Totals	249 (100)	229 (100)	

Table 3. Association of the ClC-Kb^{T481S} Gene Polymorphism with Mean BP (mm of Mercury)

ClC-Kb^{T481S} genotype	Mean Blood Pressures, mmHg (SD)		
	Systolic	Diastolic	p
AA	148 (27)	78 (12)	0.69
AT or TT	147 (24)	77 (12)	0.69

DISCUSSION

Our novel association study sought to determine the relevance of the ClC-Kb^{T481S} polymorphism to stroke. We also sought to clarify any link between this polymorphism and either current BP or history of hypertension in view of the varied findings of other studies [2,10-12]. Our study found no association between the ClC-Kb^{T481S} polymorphism and ischaemic stroke or, following stratification, lacunar stroke. We also found that this polymorphism is not a predictor of BP or hypertension, although there may be some trend towards hypertension, demonstrated by the borderline association with the sub-group that had a history of hypertension (Table **2**, p = 0.06).

The lack of association with BP or hypertension contrasts with previous positive findings in Jeck's paper on a smaller sample of 220 subjects in Tübingen, Germany [2]. They reported a significant association of the T allele with higher BP (~6.0 and ~4.2 mm Hg systolic and diastolic, respectively) and an allelic frequency of 12.4% in whites in their study [2,13]. We found a similar allelic frequency (14.1%) in our controls [9]. However, the small size of their study population leaves room for uncertainty [13]. Our findings agree with a recent study of larger sample size and add further evidence that this polymorphism is not a predictor of BP [12].

Jeck also reported a higher prevalence of hypertensive BP levels and postulated that this polymorphism predisposed to the development of essential hypertension [2]. Several more recent studies with larger sample groups have not found a link with hypertension [10-12]. Our data appears to confirm this observation. However, the borderline probability value of the association with hypertension (Table **2**, p=0.06) may reflect the earlier observation by Jeck's group of a 'predisposition' to hypertension [2]. This may be worthy of further investigation with a larger population group. Nevertheless, the overall lack of association with BP or hypertension would provide a plausible biological explanation for the lack of association with stroke that we observed.

With respect to sample size, we were sufficiently powered to detect an association with BP, hypertension or stroke, although not for individual stroke subtypes. A limitation to our study is the lack of exclusion of some confounders, e.g. diuretics. Since the aim of the original study was unrelated to diuretic use, this data was not collected. Other limitations in our cohort have been addressed previously [6].

CONCLUSIONS

In this first study seeking a link between the ClC-Kb^{T481S} gene polymorphism and stroke, we found no association

between this polymorphism and increased risk of ischaemic stroke. The ClC-Kb^{T481S} polymorphism is also not a predictor of BP. However, there may be a weak association with hypertension. We suggest that if an association with lacunar stroke is to be explored further, studies of larger sample size are required. Such studies may also clarify whether there is a link with hypertension. Furthermore, other genetic influences may play a role and therefore warrant further investigation. To our knowledge there are no other studies published on this polymorphism and stroke.

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