Association Between *ABCB1* Polymorphisms and Outcomes of Clopidogrel Treatment in Patients With Minor Stroke or Transient Ischemic Attack

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IMPORTANCE f *ABCB1* ff f
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lopidogrel bisulfate combined with aspirin is a recommended treatment for patients with acute minor ischemic stroke or transient ischemic attack (TIA).[1-3] However, despite clopidogrel use, a substantial number of patients experience recurrent stroke, which may be explained at least in part by inadequate platelet inhibition.[4,5]

Clopidogrel is a prodrug that requires intestinal absorption and biotransformation to active metabolites by hepatic cytochrome P450 enzymes (CYP450). Previous studies showed that reduced function of *CYP2C19* (OMIM 124020), a gene encoding CYP450, was associated with increased adverse cardiovascular events in patients with coronary artery disease[6,7] or stroke[8-10] treated with clopidogrel. In addition, polymorphisms of the genes regulating intestinal absorption of clopidogrel, such as the gene *ABCB1* (OMIM 171050) encoding the P-glycoprotein multidrug-resistant-1 efflux transporter, might also affect clinical outcomes.[11] Several,[7,12,13] but not all,[14,15] previous studies showed an association of *ABCB1* 3435C>T polymorphisms with reduced efficacy of clopidogrel treatment in coronary artery disease. Recent studies did not detect this association in patients with ischemic stroke.[16,17] However, the sample sizes of these studies were relatively small. Therefore, the association between *ABCB1* polymorphisms and the efficacy of clopidogrel treatment in patients with stroke or TIA remains unclear.

The previous genetic secondary analysis of the Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events (CHANCE) trial showed reduced efficacy of dual therapy of clopidogrel and aspirin in carriers of the *CYP2C19* loss-of-function alleles.[10] In the present study, we further estimate the efficacy and safety of dual therapy of clopidogrel and aspirin compared with aspirin alone according to *ABCB1* genotypes in the context of *CYP2C19* status among patients in the trial.

Study Participants

Details on the rationale, design, and results of the CHANCE trial (NCT00979589) have been published previously.[1,18,19] The trial protocol is given in Supplement 1. In brief, CHANCE was a randomized, double-blind, controlled clinical trial conducted at 114 hospitals in China between October 1, 2009, and July 30, 2012, that compared the efficacy of clopidogrel bisulfate (loading dose of 300 mg followed by 75 mg daily for 90 days) plus aspirin (loading dose of 75-300 mg followed by 75 mg daily for 21 days) with aspirin alone (loading dose of 75-300 mg followed by 75 mg daily for 90 days) among 5170 patients within 24 hours after onset of a minor ischemic stroke (National Institutes of Health Stroke Scale ≤3) or high-risk TIA (ABCD [age, blood pressure, clinical features, duration of TIA, and presence or absence of diabetes]^2≥4). The protocol of the CHANCE trial was approved by the ethics committee of Beijing Tiantan Hospital and all participating centers. Each participant or his or her representative provided written informed consent before being entered into the study. Data were analyzed on March 20, 2018.

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Seventy-three sites with experience collecting samples for genetic studies agreed to participate in the prespecified genetic secondary analysis. All patients at these sites for whom a separate written informed consent was obtained participated in this genetic secondary analysis.

Genotyping

Details on genotyping technology were published previously.[10] Two single-nucleotide polymorphisms (SNPs) of the *ABCB1* gene (-154T>C, rs4148727 and 3435C>T, rs1045642) were genotyped in 3010 participants. Participants were classified as homozygous for the T allele (TT), heterozygous (TC), or homozygous for the C allele (CC) for *ABCB1* -154T>C SNP and homozygous for the C allele (CC), heterozygous (CT), or homozygous for the T allele (TT) for *ABCB1* 3435C>T SNP.

Because genetic variations in *CYP2C19* (*CYP2C19*2* [681G>A, rs4244285] and *CYP2C19*3* [636G>A, rs4986893]) were associated with new stroke among clopidogrel-treated patients with minor stroke or TIA,[10] we also evaluated the influence of *ABCB1* polymorphism in the context of *CYP2C19* status to understand the independent contribution of *ABCB1* polymorphism. Patients with at least 1 loss-of-function allele (*2 or *3) were classified as *CYP2C19* loss-of-function allele carriers.[10]

Genotyping of the 4 SNPs was centralized and performed using the Sequenom MassARRAY iPLEX platform (Sequenom). Genotyping success rate was greater than 94.3% among all samples genotyped for each of the 4 SNPs. The 2836 individuals with complete information for each of the 4 SNPs were included in the current analyses.

Clinical Outcomes

The definitions of the outcomes in the current analyses were identical to those in the trial.[1] The primary outcome was a new stroke (ischemic or hemorrhagic) during the 90-day follow-up period. Secondary outcomes were new vascular events (composite of ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death) and ischemic stroke at 90 days. The primary safety outcome was any bleeding. Safety outcome subtypes, including severe, moderate, and mild bleeding, defined according to the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) randomized trial criteria,[20] were also

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examined. All reported efficacy and safety events were verified by a central adjudication committee that was blinded to the study group assignments.

Statistical Analysis

Continuous variables were presented as medians with interquartile ranges and categorical variables as percentages. Baseline characteristics between patients with and without genetic data were compared by Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables. Baseline characteristics for patients with and without *ABCB1* -154 TC/CC or 3435 CT/TT genotype stratified by treatment allocation were described separately. The linkage disequilibrium block and haplotype structure were measured by D′ among the 2 *ABCB1* SNPs. Hardy-Weinberg equilibrium was evaluated with a χ^2 test.

Differences in the outcome end points during the 90-day follow-up period were assessed using a Cox proportional hazards regression model, and hazard ratios (HRs) with 95% CIs were reported. When there were multiple events of the same type, the time to the first event was used in the model. Data from patients who had no event during the study were censored at termination of the trial or nonvascular death. For each model, the proportional hazards assumption was assessed by testing the interaction of treatment by time in the model. Whether the treatment effect differed in certain genotype categories was examined by testing the interactions of treatment by *ABCB1* genotype and treatment by *CYP2C19* loss-of-function allele carrier status in a multivariable Cox model. This model also included treatment group, *ABCB1* genotype, *CYP2C19* loss-of-function allele carrier status, and interaction of *ABCB1* genotype by *CYP2C19* loss-of-function allele carrier status. To exclude the influence of *CYP2C19* polymorphism, we further assessed the association of *ABCB1* polymorphism with the treatment effect among carriers and noncarriers of *CYP2C19* loss-of-function allele.

All tests were 2-sided, and $P < .05$ was considered to be statistically significant. The linkage disequilibrium block and haplotype structure were estimated using the *genetics* package version 3.5.1 in R (R Development Core Team). All other analyses were conducted with SAS, version 9.4 (SAS Institute Inc).

Results

Study Patients

A total of 3010 patients participated in the genetic substudy, of whom 2836 were successfully genotyped for all 4 SNPs (eFigure 1 in Supplement 2). Compared with the 2334 individuals without genetic data, patients included in this genetic study were less likely to have a history of ischemic stroke but more likely to have a history of congestive heart failure and a diagnosis of minor stroke rather than TIA and to be taking concomitant antihypertensive agents (eTable 1 in Supplement 2). The baseline characteristics between the clopidogrel plus aspirin and aspirin alone groups were well balanced in this genetic substudy (eTable 2 in the Supplement 2).

Among the 2836 patients with genetic data, the median (interquartile range) age was 61.8 (54.4-71.1) years, 1887 patients (66.5%) were male, 2077 (73.2%) presented with minor stroke, and 759 (26.8%) presented with TIA. In total, 2266 (79.9%) were TT homozygotes, 507 (17.9%) were TC heterozygotes, and 63 (2.2%) were CC homozygotes for *ABCB1* -154T>C. For *ABCB1*, 3435C>T, 985 (34.7%) were CC homozygotes, 1424 (50.2%) were CT heterozygotes, and 427 (15.1%) were TT homozygotes. A total of 2146 patients (75.7%) were carriers of *ABCB1* -154 TC/CC or 3435 CT/TT genotype (ie, carriers of minor allele of *ABCB1* -154T>C or *ABCB1* 3435C>T). Baseline characteristics for patients with and without *ABCB1* -154 TC/CC or 3435 CT/TT genotype stratified by treatment allocation are presented in **Table 1**.

The linkage disequilibrium block in the 2 *ABCB1* SNPs was not constructed, and we did not show the haplotype in the 2 *ABCB1* SNPs (D′ = 0.67). The 2 *CYP2C19* genetic variants were found to be in Hardy-Weinberg equilibrium (*CYP2C19**2, $P = .87$; *CYP2C19**3, $P = .36$), whereas the 2 *ABCB1* genetic variants were found to be deviated from Hardy-Weinberg equilibrium (*ABCB1* -154T>C, $P = .001$; *ABCB1 3435C>T*, $P = .02$).

Efficacy Outcomes

Clopidogrel plus aspirin compared with aspirin was associated with a reduced rate of new stroke in patients with *ABCB1* -154 TT and 3435 CC genotype (HR, 0.43; 95% CI, 0.26-0.71; $P < .001$) but not in those with *ABCB1* -154 TC/CC or 3435 CT/TT genotype (HR, 0.78; 95% CI, 0.60-1.03; $P = .08$) ($P = .04$ for interaction) (**Table 2**). Cumulative risk of new stroke among patients with or without *ABCB1* -154 TC/CC or 3435 CT/TT genotype by treatment assignment is shown in **Figure 1**A. Separate analyses according to *CYP2C19* loss-of-function allele carrier status showed a combined association of *ABCB1* and *CYP2C19* polymorphisms with new stroke at 3 months (HR, 0.28; 95% CI, 0.12-0.63; $P = .002$ in patients with *ABCB1* -154 TT and 3435 CC genotype and without CYP2C19 loss-of-function allele) (**Figure 2**). Similar results were observed for the outcomes of composite event ($P = .04$ for interaction) and ischemic stroke (

group irrespective of *ABCB1* 3435C>T polymorphism (rate among CC homozygotes, 7.9% vs 12.5%; HR, 0.62; 95% CI, 0.41-0.92; *P* = .02; rate among CT heterozygotes, 8.8% vs 11.7%; HR, 0.75; 95% CI, 0.54-1.04; *P* = .08; rate among TT homozygotes, 7.1% vs 11.3%; HR, 0.62; 95% CI, 0.33-1.17; *P* = .14; *P* = .78 for interaction) (eFigure 2 in Supplement 2). The *ABCB1* 3435C>T genotype was not associated with modified efficacy of clopidogrel plus aspirin treatment in carriers or in noncarriers of *CYP2C19* loss-of-function allele (*P* = .70 for interaction in carriers; and

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populations[24,25] and higher than that in persons of European (66.5%) and African (52.5%) descent.[25] The *ABCB1* -154 TC/CC or 3435 CT/TT genotype may be associated with higher P-glycoprotein expression and thus an enhanced intestinal efflux, possibly of clopidogrel.[11,21] This study provided evidence that besides *CYP2C19*, the genetic polymorphism of *ABCB1* encoding the P-glycoprotein, which plays an important role in intestinal absorption of clopidogrel, should also be considered when prescribing clopidogrel for patients with minor ischemic stroke and TIA. Genetic testing may allow clinicians to personalize antiplatelet therapy; however, its cost-effectiveness needs further investigation. Varying the dose of clopidogrel or shifting to new antiplatelet agents (eg, prasugrel) based on genetic results may be another alternative but also needs to be further evaluated.[10] Future research may focus on the cost-effectiveness of genetic testing in clinical practice and evaluation of efficacy of alternatives for those with *AB*

Table 3. Association of Clopidogrel Plus Aspirin vs Aspirin Alone With Clinical Outcome Stratified by *ABCB1* -154T>C Genotypes

Outcome	*ABCB1*-154 TC/CC No. (%) Total (n = 570)	Aspirin (n = 276)	Clopidogrel Plus Aspirin (n = 294)	HR (95% CI)	*P* Value	*ABCB1*-154 TT No. (%) Total (n = 2266)	Aspirin (n = 1146)	Clopidogrel Plus Aspirin (n = 1120)	HR (95% CI)	*P* Value	*P* Value for Interaction
Stroke	51 (8.9)	22 (8.0)	29 (9.9)	1.25 (0.72-2.17)	.44	234 (10.3)	147 (12.8)	87 (7.8)	0.59 (0.46-0.77)	<.001	.02
Composite event[a]	52 (9.1)	23 (8.3)	29 (9.9)	1.19 (0.69-2.06)	.53	235 (10.4)	148 (12.9)	87 (7.8)	0.59 (0.45-0.77)	<.001	.02
Ischemic stroke	51 (8.9)	22 (8.0)	29 (9.9)	1.25 (0.72-2.17)	.44	229 (10.1)	145 (12.6)	84 (7.5)	0.58 (0.44-0.76)	<.001	.02
Bleeding[b]											
Severe	0	0	0	NE	NA	0	0	0	NE	NA	NA
Moderate	0	0	0	NE	NA	1 (0.0)	0	1 (0.1)	NE	NA	NA
Mild	3 (0.5)	0	3 (1.0)	NE	NA	23 (1.0)	9 (0.8)	14 (1.3)	1.54 (0.67-3.55)	.31	.99
Any bleeding	8 (1.4)	3 (1.1)	5 (1.7)	1.58 (0.38-6.59)	.53	44 (1.9)	18 (1.6)	26 (2.3)	1.39 (0.76-2.55)	.29	.76

nisms were not available in the CHANCE trial, it was impossible to assess the influence of stroke mechanisms on the pharmacogenetic effect of *ABCB1* in this study. Third, the event rates for bleeding were low in this population, which may limit statistical power to detect the association with the safety outcome. Fourth, caution is needed when explaining the results because the 2 *ABCB1* genetic variants were deviated from Hardy-Weinberg equilibrium.

Among patients with minor ischemic stroke or TIA, the *ABCB1* polymorphism was found to be associated with reduced efficacy of clopidogrel plus aspirin treatment compared with aspirin alone in this study. However, further validations are needed in other studies with large sample sizes.

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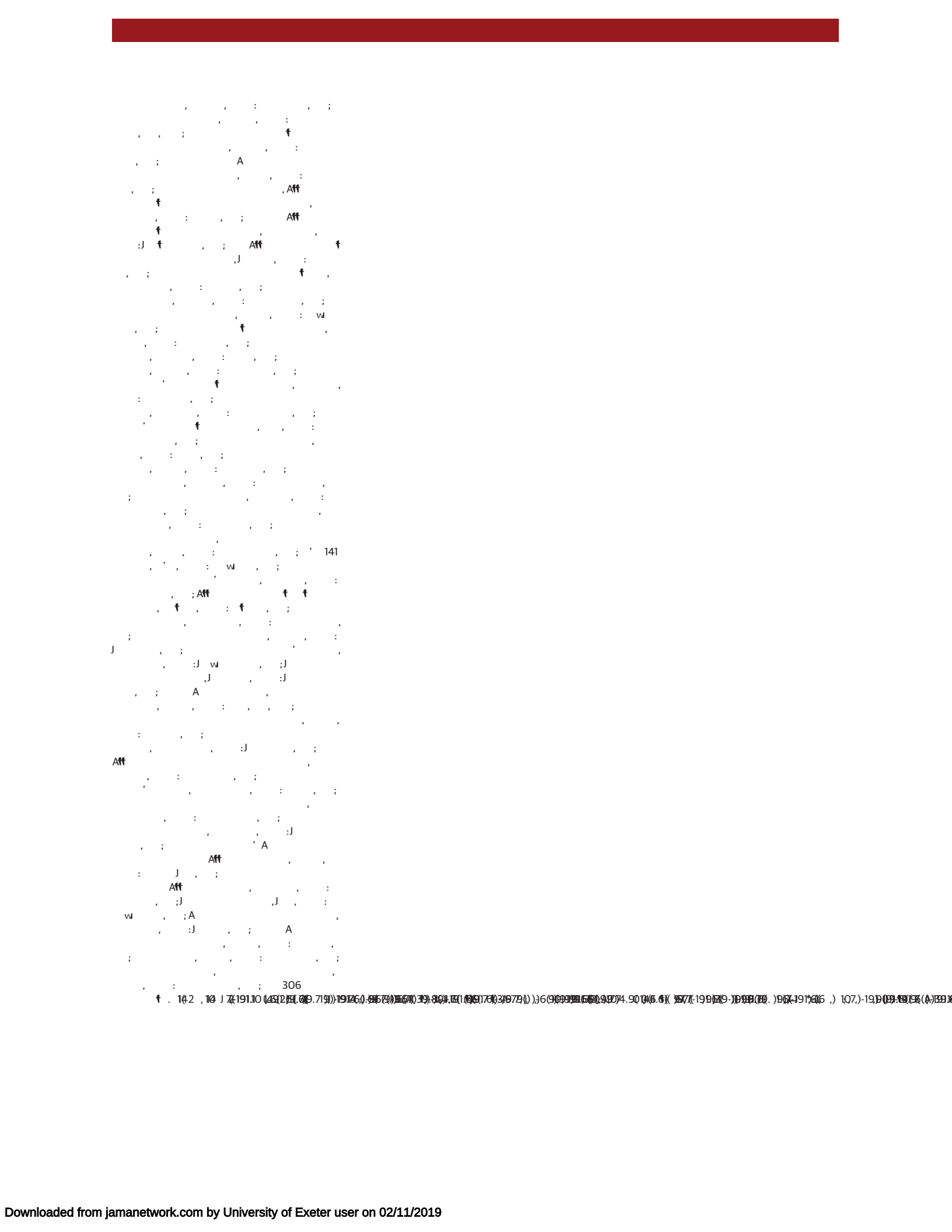

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