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ANGIOTENSIN-CONVERTING ENZYME INSERTION/DELETION GENE POLYMORPHISM AND HENOC-SCHONLEIN PURPURA NEPHRITIS RISK IN CHILDREN: A META-ANALYSIS

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It has been demonstrated in many studies that angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is related to Henoch-Schonlein purpura nephritis (HSPN) risk in children. However, this conclusion remains controversial.

In this study, we systemically retrieved relevant studies in electronic databases such as PUBMED, CNKI, and EMBASE followed by calculation of odds ratios (OR) with 95% confidence intervals (CI). In addition, meta-package in STATA version 12.0 was used. Angiotensin-converting enzyme I/D polymorphism was related to HSPN susceptibility in children (D vs. I: OR 1.47, 95% CI: 1.13–1.93; DD vs. II: OR 2.29, 95% CI: 1.29–4.07; DI vs. II: OR 1.10, 95% CI: 0.82–1.48; dominant model: OR 1.44, 95% CI: 1.09–1.89; recessive model: OR 2.26, 95% CI: 1.67–3.06).

In addition, subgroup analysis stratified according to ethnicity indicated a significant relationship between this polymorphism and HSPN susceptibility among Asians and Caucasians. The data extracted from HaploReg indicated that ACE I/D polymorphism was not in linkage disequilibrium with other variants in the ACE gene.

The research shows that ACE I/D polymorphism is related to HSPN susceptibility in children.

Key words: ACE, HSPN, meta-analysis.

Introduction

Henoch-Schonlein purpura (HSP) has been considered among the commonest systematic vasculitis forms, and its annual incidence rate is approximately 10 cases per 100,000 [1]. People of all age groups can have HSP, and most of them are aged below 10 years [2]. Purpuric rashes are the most representative expression, whereas HSP may also cause joint, gut, or kidney impairment to varying degrees. Henoch-Schonlein purpura nephritis (HSPN) belongs to a relatively severe complication, featuring microscopic or gross haematuria in the absence or presence of proteinuria [3]. Research shows that around 30% patients

suffering nephrotic-range proteinuria and HSPN, particularly nephrotic proteinuria, may develop end-stage renal disease or death, although HSP has been considered a self-limiting disorder having favourable prognosis [4]. No efficient HSPN prevention solution has been proposed yet. Therefore, it is urgent to seek a sensitive biomarker of HSPN onset. Genetic factors are probably related to HSPN onset.

Angiotensin-converting enzyme (ACE), one of the zinc metallopeptidases, can degrade bradykinin (vasodilator) and transform angiotensin I into angiotensin II (vasoconstrictor), thus regulating cardiovascular homeostasis and blood pressure (BP) [5]. About 90% of physiological transformation of angio-

tensin I into angiotensin II occurs within the lung. Angiotensin-converting enzyme of peripheral blood has been considered equivalent to lung enzyme and in direct proportion to blood oxygen concentration, indicating the close relationship between serum ACE activity and enzyme level in pulmonary tissue [6].

Human ACE gene is located in chromosome 17q23, with numerous polymorphisms being recognized. An intron 16 insertion/deletion (I/D, rs4646994) polymorphism is detected in the ACE gene, featuring with/without 287bp Alu repetitive sequence [7]. Homozygotes of D allele exhibit maximum plasma ACE levels, closely followed by heterozygotes (ID), while homozygotes of I allele exhibit the minimum levels [8].

Many investigations are made to explore the relationship between ACE I/D polymorphism and HSPN in children. Inconsistencies in these findings result from insufficient statistical ability due to small sample sizes, as well as eco-geographical heterogeneity. Meta-analysis is a statistical approach that can eliminate individual research constraints. Thus, a meta-analysis was conducted to obtain a more accurate estimation of the association of ACE I/D polymorphism with HSPN risk in children.

Material and methods

Search strategy for identification of studies

Related research was collected from electronic databases (PUBMED, CNKI, and EMBASE) using the keywords “angiotensin-converting enzyme/ACE”, “Henoch-Schonlein purpura nephritis/HSPN”, “genetic polymorphism”, and “single nucleotide polymorphism”. Subsequently, the resulting research articles were manually screened based on original references. For repetitive data, the one containing the most comprehensive information was chosen.

Inclusion criteria

Eligible research articles were screened with the following inclusion criteria:

- assessment of ACE gene I/D polymorphism as well as HSPN risk,
- case-control research,
- genotype data can be assessed.

Exclusion criteria:

- research not related to HSPN,
- reviews,
- studies without accessible information,
- repeated research.

Data extraction

Two researchers read the related articles to extract the data. If any controversy occurred, a third investigator assessed the articles. Information to be

searched were first author, publication year, area, number of cases and controls, genotype frequencies of cases and controls, and evidence for Hardy-Weinberg equilibrium (HWE) of controls.

Statistical analysis

The genotyping distribution of the HWE test of control group was established by Fisher's exact test. Odds ratios (OR) and relevant 95% confidence intervals (CI) (D vs. I), heterozygote comparison (DI vs. II), homozygote comparison (DD vs. II), recessive model (II + DI vs. DD), and dominant model (DD + DI vs. II) were utilized to assess the correlation strength of HSPN risk with ACE I/D polymorphism. Heterogeneity among studies was analysed through the I^2 test. $I^2 > 50\%$ stood for heterogeneity, and so we selected the random effects model; otherwise, we utilized the fixed effects model. This study also carried out sensitivity analysis by omitting individual studies one by one. An individual study was judged to be excessively sensitive when the point estimate in omitted analysis exceeded 95% CIs from combined analyses. For the assessment of underlying publication bias, we visually inspected Begg's funnel plot. This study adopted STATA (version 12.0; Stata Corporation, College Station, TX) in statistical analysis.

Functional predictions

Linkage disequilibrium analysis was made by employing the website of HaploReg v4.1 (<http://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) to predict the function of ACE I/D polymorphism.

Trial sequential analysis

Meta-analysis might be affected by the increased risk of random errors and repeated significance testing. Trial sequential analysis (TSA) can increase the robustness of the conclusions by estimating the amount of the required information size (RIS) and the threshold for statistical significance. During the analysis, the significance levels for type I and type II errors were set to 5% and 20%, respectively, and relative risk reduction was set at 20%. When the cumulative Z-curve crosses the TSA boundary or enters the insignificance area, it demonstrates a sufficient level of evidence, and no further study is necessary. TSA software (version 0.9.5.10 β) was used for data processing [9].

Ethics

This work was supported by the Hubei Paediatric Alliance Medical Research Project (HPAM-RP202117). Informed consent was obtained from each participant included in the study.

Results

Study characteristics

A total of 206 studies were searched based on the retrieval strategy. Following the research inclusion criteria, 8 case-control papers satisfied the inclusive criteria, while the remaining 198 were removed. Figure 1 lists the research selection flow chart [10–16]. The 8 chosen studies contained a total of 415 patients and 760 normal subjects. The publication year for the selected studies was in the range 1998–2013. The HWE test was done to explore genotype distribution among the controls; as could be observed, all 8 papers were within the HWE ($p > 0.05$) except for Ozkaya *et al.* Stratified by ethnicity, there were 6 Asian studies and 2 Caucasian studies. Basic features of the chosen studies are shown in Table I.

Meta-analysis outcomes

The primary results in this study were the relation between ACE I/D polymorphism and HSPN susceptibility, as observed from Table II. According to the combined meta-analysis findings, ACE I/D polymorphism was significantly correlated with HSPN susceptibility (D vs. I: OR 1.47, 95% CI: 1.13–1.93; DD vs. II: OR 2.29, 95% CI: 1.29–4.07; DI vs. II: OR 1.10, 95% CI: 0.82–1.48; dominant model: OR 1.44, 95% CI: 1.09–1.89; recessive model: OR 2.26, 95% CI: 1.67–3.06) (Fig. 2). In addition, subgroup analysis stratified according to ethnicity indicated a significant relationship between this polymorphism and HSPN susceptibility among Asians and Caucasians. Furthermore, sensitivity analysis was also performed by deleting individual studies each time. Finally, pooled results could be hardly modified when every individual study was removed, which was indicative of their robustness (Fig. 3).

Publication bias

Publication bias was measured using a funnel plot, indicating a shortage of evidence concerning publication bias during meta-analysis (Fig. 4). These findings suggested low publication bias during the meta-analysis.

Functional predictions

The data extracted from HaploReg indicated that ACE I/D polymorphism was not in linkage disequilibrium with other variants in the ACE gene.

Trial sequential analysis

This study conducted TSA to reduce random errors and to fortify the robustness of our result. According to our results, the cumulative Z-curve did not surpass RIS, and the TSA and RIS thresholds

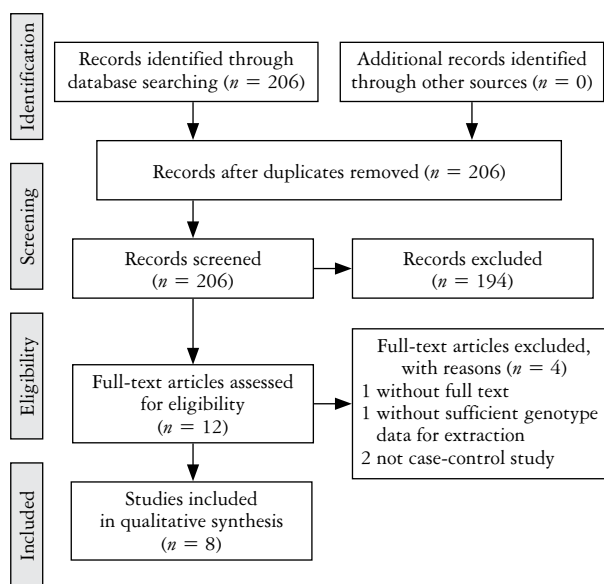


Fig. 1. Flow diagram of included/excluded studies

were not crossed, indicating that the results were unreliable and that more studies should be included (Fig. 5).

Discussions

Angiotensin-converting enzyme I/D polymorphism has a close connection with the risk of many other autoimmune illnesses, including Kawasaki disease and lupus nephropathy in children [17, 18]. Furthermore, ACE I/D polymorphism possibly affects angiotensin II regulation, which may lead to renal injury. A previous meta-analysis showed the ACE D allele was a potential risk factor combating HSPN risk [19]. However, this meta-analysis did not perform subgroup analysis aimed at HSPN. Recently, reports have proven that the ACE I/D polymorphism is associated with HSPN susceptibility in children. However, there are still controversies in the final results. Therefore, deepening the knowledge of this issue has prominent clinical significance because it suggests an effect of ACE I/D polymorphism in predicting HSPN risk. Given this, the present meta-analysis focused on further evaluating the association of ACE I/D polymorphism with HSPN susceptibility in children.

Altogether 8 case-control papers meeting the inclusion criteria were enrolled. Our results indicated that ACE I/D polymorphism was related to HSPN susceptibility in children according to the statistical capacity of studies enrolled by the current meta-analysis. Through stratified analyses based on race, the results suggest that ACE I/D polymorphism was markedly related to HSPN susceptibility across Caucasian and Asian populations in children. Only 2 papers regarding HSPN were enrolled in this meta-analysis among Caucasians, which was a limitation of this

Table I. Characteristics of the included studies for meta-analysis

STUDY	YEAR	RACE	CASES/ CONTROLS	CASES	CONTROLS	CASES	CONTROLS	AGE (YEARS)	AGE (YEARS)	ALLELE FOR CASES		ALLELE FOR CONTROLS		GENOTYPES FOR CASES		GENOTYPES FOR CONTROLS		HWE		
										D	I	D	I	DD	ID	DD	ID		DD	ID
						MALE/FEMALE	MALE/FEMALE													
Yoshioka	1998	Asian	40/79	18/22	41/38	4-15	4-15	4-15	4-15	49	31	101	57	17	15	8	33	35	11	0.73
Chang	2002	Asian	33/28	/	/	/	/	/	/	31	35	28	28	8	15	10	7	14	7	1.00
Zhou	2004	Asian	103/100	62/41	60/40	3-14	/	/	/	92	114	51	149	30	32	41	6	39	55	0.79
Zhang	2004	Asian	38/100	/	/	/	/	/	/	30	46	73	127	7	16	15	10	53	37	0.15
Ozkaya	2006	Caucasian	44/164	73/41	98/66	3.5-16	/	/	/	45	43	155	173	8	29	7	19	117	28	0.00
Cui	2010	Asian	32/100	/	63/37	/	6.4-9.6	6.4-9.6	6.4-9.6	41	23	82	118	13	15	4	13	56	31	0.12
Sinem	2013	Caucasian	64/72	/	/	/	/	/	/	77	51	68	76	27	23	14	16	36	20	0.98
Liu	2010	Asian	61/217	81/61	126/92	2.83-16.33	/	/	/	55	67	144	290	11	33	17	21	102	94	0.38

HWE - Hardy-Weinberg equilibrium

meta-analysis; therefore, a larger number of independent case-control studies among the Caucasians should be conducted for a more comprehensive conclusion. Further, subgroup analysis was also done to remove papers with genotype distribution that deviated from HWE among the controls. At the same time, no modified result was observed, suggesting the statistical significance of meta-analysis results. Trial sequential analysis was used to check the reliability of conclusions in the present study. The cumulative Z-curve of ACE I/D polymorphism did not reach the trial sequential monitoring boundary and RIS line, suggesting that larger sample, multi-ethnic research is required to verify the associations.

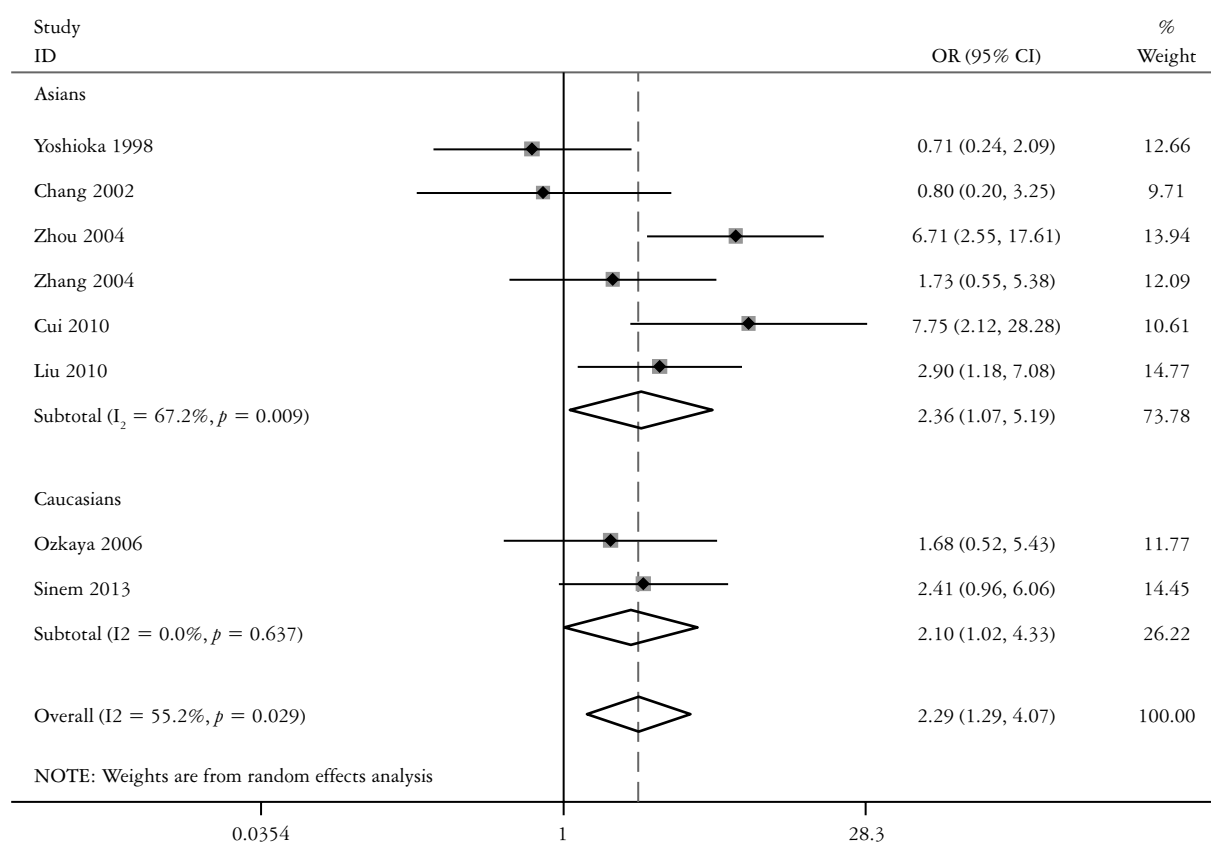
The precise mechanism underlying ACE I/D polymorphism with HSPN in children is not fully illustrated. Angiotensin-converting enzyme I/D polymorphism can be detected within one intron in the ACE gene. It contributes to around 50% changes in the plasma ACE content [20]. Perticone *et al.* [21] indicated the correlation between ACE DD genotype and arterial dysfunction confined to NO pathways. The correlation of ACE genotype and arterial function can be explained in 2 ways: 1. ACE can inactivate bradykinin, and an increase in ACE levels decreases bradykinin bioactivity, which reversely decreases receptor-mediated release of NO; and 2. Another feasible explanation is that angiotensin II promotes superoxide levels by increasing activity in the activities of oxidases nicotinamide adenine dinucleotide phosphate and nicotinamide adenine dinucleotide, thus lowering the bioactivity in NO. In brief, changes in local hormonal equilibrium may result in platelet aggregation, monocyte adhesion, haemostasis, and pro-inflammatory/growth factor release. In theory, for patients having DD genotype, increased ACE activity as well as angiotensin II formation within the vascular bed are likely to induce vascular changes. The locus was not in linkage disequilibrium with other variants located in ACE.

There are also several limitations in the current meta-analysis. Firstly, heterogeneity may affect the explanation for meta-analysis results. Although the likelihood had been minimized through a prudent screening on related publications, as well as strict inclusion criteria and data extraction and analysis procedures, remarkable heterogeneity was inevitably found through comparisons between studies. Such heterogeneity is caused by the different control selection criteria, lifestyle factors, or age groups. Furthermore, only published articles were enrolled in the meta-analysis. There was some publication bias, suggesting that insignificant or negative results are not available yet. Thirdly, during subgroup analyses, various ethnic groups might be mixed with other populations, thus causing heterogeneity. Ultimately, the predictive value of a single gene test in a complex

Table II. Summary of different comparative results

PARAMETERS	N	OR (95% CI)				
		D vs. I	DD vs. II	DI vs. II	DOMINANT MODEL	RECESSIVE MODE
Total	8	1.47 (1.13–1.93)	2.29 (1.29–4.07)	1.10 (0.82–1.48)	1.44 (1.09–1.89)	2.26 (1.67–3.06)
Race						
Asian	6	1.49 (1.04–2.13)	2.36 (1.07–5.19)	1.14 (0.82–1.60)	1.49 (1.10–2.04)	2.25 (1.22–4.15)
Caucasian	2	2.10 (1.02–4.33)	2.10 (1.02–4.33)	0.95 (0.51–1.78)	1.24 (0.68–2.25)	2.18 (1.24–3.86)
HWE						
Yes	7	1.52 (1.13–2.06)	2.38 (1.24–4.55)	1.11 (0.81–1.52)	1.48 (1.11–1.97)	2.30 (1.39–3.80)
No	1	/	/	/	/	/

CI – confidence interval, N – number, OR – odds ratio


Fig. 2. Forest plot for meta-analysis of the association between the angiotensin-converting enzyme insertion/deletion polymorphism and Henoch-Schonlein purpura nephritis risk with DD vs. II

disease is highly limited for diagnostic or preventive purposes and thus cannot be recommended. Based on data of simulation studies and other complex diseases, the use of genetic profiling that incorporates multiple genetic risk factors holds promise for clinical application. The results of genome-wide association studies will be crucial in establishing this genetic risk profile for HSPN. In the future, HSPN prediction may be possible, based on a prediction model that incorporates genes, personal factors, and environmental risk factors.

Conclusions

The present meta-analysis suggests that the ACE D allele might increase the risk of HSPN in children. In future research, more rigorous patient screening criteria should be adopted, with well-matched controls and more samples. In addition, attention should also be paid to gene-gene and gene-environment interactions.

The authors declare no conflict of interest.

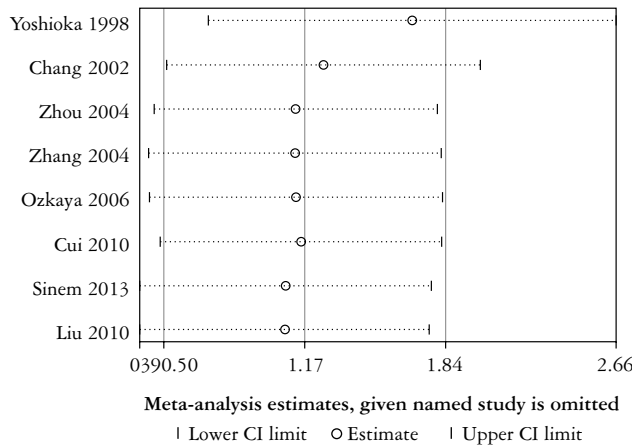


Fig. 3. Sensitivity analysis of the association between the angiotensin-converting enzyme insertion/deletion polymorphism and Henoch-Schonlein purpura nephritis risk with DD vs. II

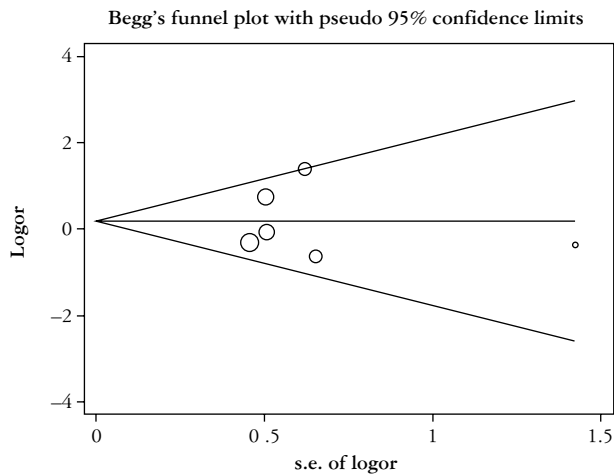


Fig. 4. Begg's funnel plot analysis to detect potential publication bias for angiotensin-converting enzyme insertion/deletion polymorphism

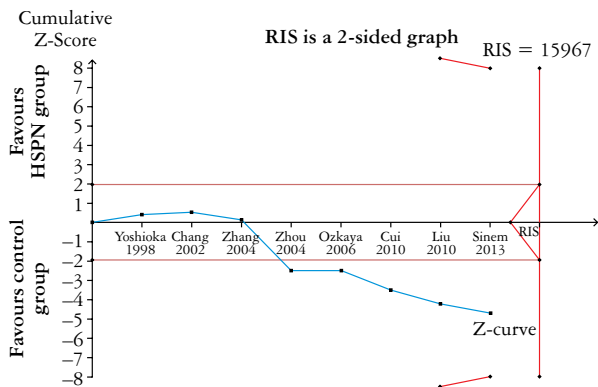


Fig. 5. Trial sequential analysis for angiotensin-converting enzyme insertion/deletion polymorphism

HSPN – Henoch-Schonlein purpura nephritis, RIS – required information size
 Liu 2010 We calculated α -spending adjusted required information size by using $\alpha = 0.05$ (2-sided), power = 80%. The cumulative Z-curve – blue, conventional boundary – deep red, Trial sequential analysis boundary – red.

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