Meta-Analysis

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SLC6A4 gene L/S polymorphism and susceptibility to pulmonary arterial hypertension: a meta-analysis

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Abstract

Objective: To investigate the association between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension (PAH).

Methods: The relevant literature was retrieved from the PubMed® database and the data were extracted. STATA® version 12.0 software was used to calculate pooled odds ratios (ORs) and 95% confidence intervals (CI).

Results: Eight case–control studies qualified for inclusion in the meta-analysis. These studies included 1215 cases and 936 control subjects. There was no significant association between the *SLC6A4* gene L/S polymorphism and PAH risk in the total population (LL versus SS: OR 1.83, 95% CI 0.95, 3.51; LS versus SS: OR 1.37, 95% CI 0.93, 2.02; dominant model: OR 1.38, 95% CI 0.97, 1.97; recessive model: OR 1.54, 95% CI 0.84, 2.83). Subgroup analysis based on study quality scores and Hardy–Weinberg equilibrium also showed no significant association.

Conclusion: The findings of this meta-analysis suggest that the *SLC6A4* gene L/S polymorphism is unlikely to be related to PAH risk. Well-designed studies with more participants will be required to validate these results.

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Introduction

Pulmonary arterial hypertension (PAH), a relatively rare but lethal disease, is characterized by obliterative pulmonary vascular remodelling, which causes progressively enhanced pulmonary vascular resistance that leads to right heart failure. Although clear progress has been made in the modern treatment of this disease, the mortality for heritable and idiopathic PAH remains at approximately 10% annually.[1,2] To some extent, the high mortality rate is in part due to the low efficacy of the approved therapy on pulmonary vascular pathology, including endothelial cell proliferation, inflammation and hyperplasia of fibroblasts and vascular smooth muscle cells.[3] In addition, the substantially varied therapeutic responses among patients emphasize the insufficient understanding of the causes of PAH.

The critical effects of serotonin (5-hydroxytryptamine [5-HT]) has been recently noted in pulmonary vascular remodelling.[4] Patients receiving appetite suppressants that block the 5-HT transporter (5-HTT) have been shown to have an elevated PAH risk.[4] 5-HTT enables the reuptake of excess 5-HT from the synaptic cleft, which is vitally important in regulating 5-HT synaptic function.[5] However, there is controversy over the mechanism underlying the effects of 5-HT on pulmonary vasculature.[6] The solute carrier family 6 member 4 (*SLC6A4*) gene is localized on chromosome 17q11.2-17q12 and it encodes 5-HTT.[4] Polymorphisms of this gene can cause changes in 5-HT concentrations, including two polymorphisms (variable number tandem repeat [VNTR] and 5-HTTLPR), named the *SLC6A4* gene L/S polymorphism.[7] Cells with the 'L/L' 5-HTTLPR genotype have been reported to uptake more serotonin than those with the 'S/L' or 'S/S' genotypes.[4] That is to say, the S allele indicates lower uptake activity.

The pathogenesis of PAH is multifactorial and complex, with a largely unclear mechanism.[8] The relationship between the *SLC6A4* gene L/S polymorphism and PAH risk has been reported by various studies, however, with controversial outcomes.[8–10] Case–control studies with relatively limited sample sizes might not be the best option for comprehensively elucidating the complex relationship due to their inadequate statistical power. Undertaking a meta-analysis is helpful for analysing complex data from multiple case–control studies. Herein, this current meta-analysis aimed to investigate the relationship between the *SLC6A4* gene L/S polymorphism and PAH risk by collecting all currently relevant and accessible articles.

Materials and methods

Publication search strategy

The electronic database PubMed® was searched from January 2000 to January 2020 to identify relevant studies using a combination of keywords and subject terms as follows: "serotonin transporter" or "5-HTT", "polymorphism" and

"pulmonary arterial hypertension". The reference lists of included studies were also manually examined to identify other potentially eligible studies. For literature with overlapping data, studies with the largest number of cases were selected. This current meta-analysis was undertaken in accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) checklist.

Inclusion and exclusion criteria

The eligibility inclusion criteria were as follows: (i) case–control studies evaluating the correlation between the *SLC6A4* gene L/S polymorphism and PAH risk; (ii) patients were clinically diagnosed with PAH; (iii) populations with accessible odds ratio (OR) with 95% confidence interval (CI) or adequate data to calculate OR with 95% CI. Studies were eliminated if they had no control or usable information.

Data extraction

All possible articles were independently reviewed by two investigators (F. Z. & M. Y.), followed by data extraction. Any discrepancies were resolved by discussion with another investigator (Y. H.). The following data were retrieved from each paper: country, number of cases and controls, publication year, genotype frequencies in cases and controls, first author name and Hardy–Weinberg equilibrium (HWE) evidence in control subjects.

Quality score assessment

The quality of the included studies was evaluated independently by two investigators (Y. C. & S. X.) according to a set of predetermined criteria (Table 1) modified from previous research.[11] Any disagreements were resolved by discussion among the two investigators to reach consensus.[11] Scores ranged from 0 (lowest) to 10 (highest) and studies with scores ≥ 6 were classified as high-quality studies and studies with scores < 6 were classified as low-quality studies.

Statistical analyses

The association between the *SLC6A4* gene L/S polymorphism and PAH susceptibility was determined by ORs and corresponding 95% CIs using a homozygote comparison (LL versus SS), a heterozygote comparison (LS versus SS), a dominant model (LL+LS versus SS) and a recessive model (LL versus SS+LS) between groups. The I^2 test was used to assess the potential heterogeneity among the articles. An I^2 of $> 50\%$ suggested the presence of heterogeneity among the studies, so a random-effects model was employed; otherwise, a fixed-effects model was adopted. The stability of the results was determined by a one-way sensitivity analysis. Every individual study in the meta-analysis was sequentially omitted to identify the effects of this specific study on the pooled OR. The diversity among different studies were examined by subgroup analyses stratified by HWE. Moreover, the Begg's analysis was used to determine the underlying publication bias ($P < 0.05$ indicated statistical significance). Statistical analyses were undertaken with STATA® version 12.0 software (STATA Corp., College Station, TX USA). The power of each study was computed as the probability of detecting an association between the *SLC6A4* gene L/S polymorphism and PAH using a significance level of 0.05, assuming an OR of 1.5 (small effect size). Power analysis was performed using the statistical program PS: Power and Sample Size Calculation.[12]

Results

A flow diagram showing the study selection process is presented in Figure 1. Eight case–control studies were finally enrolled

Table 1. Criteria used to assess the quality of the case–control studies included in a meta-analysis to investigate the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension (PAH).

Criteria	Score
Source of cases	
Selected from population disease registry or multiple centre sites	2
Selected from hospital	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based	1
Not described	0
Ascertainment of PAH	
Standard method confirmation	2
Not described	0
Genotyping examination	
Genotyping done under blinded conditions	1
Unblinded or not mentioned	0
Hardy–Weinberg equilibrium in controls	
Hardy–Weinberg equilibrium	3
Hardy–Weinberg disequilibrium	0
Association assessment	
Assessed association between genotypes and PAH with appropriate statistics and examining confounders and effect modifiers	1
Inappropriate statistics used	0

according to the inclusion criteria, involving 1215 cases and 936 controls.[6,8–10,13–16] The genotype frequencies were consistent with the HWE in all studies except three.[9,15,16]All publications were written in English. The general features and the allele and genotype distributions were summarized in Table 2.[6,8–10,13–16] The results of the quality score assessment ranged from 4 to 8. The statistical powers of these eight studies ranged from 12.6% to 48%. None of the studies had a statistical power that exceeded 80%.

The major outcomes in this study are summarized in Table 3. The *SLC6A4* gene L/S polymorphism was not significantly associated with PAH under any genetic models (Figure 2; LL versus SS: OR 1.83, 95% CI 0.95, 3.51; LS versus SS: OR 1.37, 95% CI 0.93, 2.02; dominant model: OR 1.38, 95% CI 0.97, 1.97; recessive model: OR 1.54, 95% CI 0.84, 2.83). In the subgroup analysis where studies were stratified according to their quality scores, there was no significant association observed with high-quality studies. In the subgroup analysis where studies were stratified according to HWE, the heterogeneity was removed for stratification analysis after removing articles deviating from HWE.

The stability of the present outcomes was assessed by a sensitivity analysis by sequentially removing a single study each time. The results demonstrated that no individual study significantly influenced the pooled ORs (Figure 3).

A funnel plot and Begg's test was adopted to determine publication bias, which showed

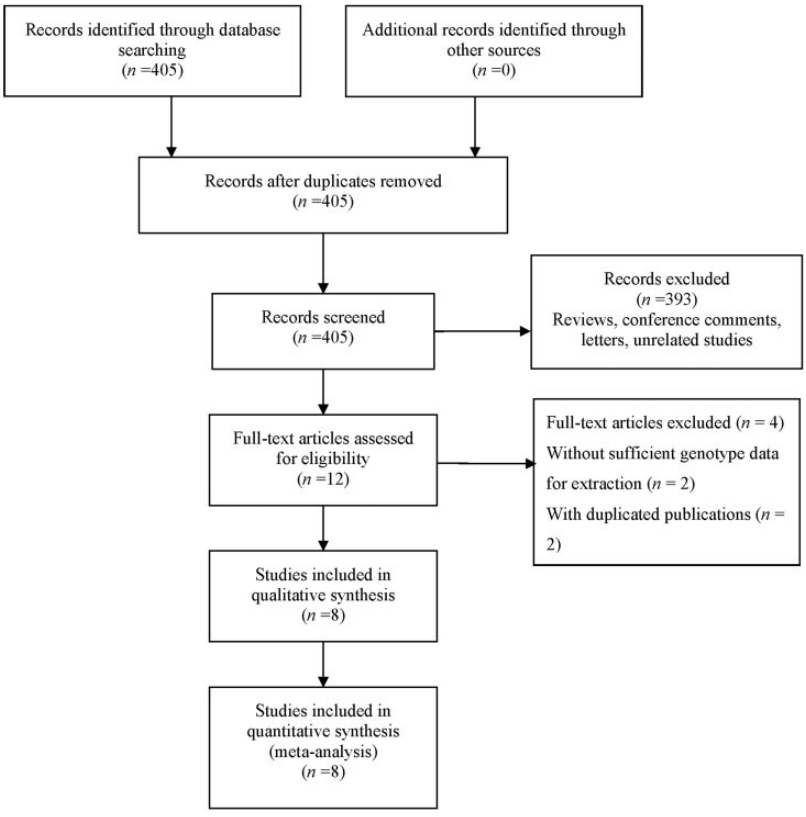

Figure 1. Flow diagram of eligible studies showing the number of citations identified, retrieved and included in the meta-analysis to investigate the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension.

Table 2. Study selection and subject characteristics of studies included in a meta-analysis to investigate the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension.[6,8–10,13–16]

Author	Year	Country	Cases (*n*)	Controls (*n*)	Genotypes of cases SS/SL/LL	Genotypes of controls SS/SL/LL	*P*-value for HWE	Quality scores
Eddahibi et al.[8]	2003	France	103	98	17/54/32	20/50/28	$P = 0.79$	8
Machado et al.[9]	2006	England	528	353	114/244/170	88/157/108	$P = 0.04$	5
Willers et al.[10]	2006	USA	223	125	46/99/78	19/63/43	$P = 0.60$	8
Cao et al.[13]	2009	China	140	140	71/51/18	86/45/9	$P = 0.35$	8
Ulrich et al.[6]	2010	Switzerland	27	22	5/16/6	8/12/2	$P = 0.40$	8
Baloira et al.[14]	2011	Spain	49	50	13/26/10	12/23/15	$P = 0.59$	8
Shivani et al.[15]	2011	India	65	100	16/28/21	57/19/24	$P < 0.01$	4
Ulasli et al.[16]	2013	Turkey	80	48	24/19/37	17/11/20	$P < 0.01$	5

HWE, Hardy–Weinberg equilibrium.

Table 3. Summary odds ratio (OR) and 95% confidence interval (CI) for total and subgroup meta-analysis of the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension.[6,8–10,13–16]

Variables	n[a]	LL versus SS OR (95% CI) Model	LS versus SS OR (95% CI) Model	Dominant model OR (95% CI) Model	Recessive model OR (95% CI) Model
Total	8	1.83 (0.95, 3.51) R	1.37 (0.93, 2.02) R	1.38 (0.97, 1.97) R	1.54 (0.84, 2.83) R
HWE					
Yes	5	1.18 (0.80, 1.74) F	1.10 (0.80, 1.51) F	1.16 (0.87, 1.56) F	1.14 (0.84, 1.55) F
No	3	2.92 (1.31, 6.48) R	1.93 (0.75, 4.94) R	1.79 (0.85, 3.78) R	2.10 (0.83, 5.34) R
Study quality					
High quality	5	1.18 (0.80, 1.74) F	1.10 (0.80, 1.51) F	1.16 (0.87, 1.56) F	1.14 (0.84, 1.55) F
Low quality	3	2.92 (1.31, 6.48) R	1.93 (0.75, 4.94) R	1.79 (0.85, 3.78) R	2.10 (0.83, 5.34) R

[a]Number of comparisons.
R, random-effects model; HWE, Hardy–Weinberg equilibrium; F, fixed-effects model.

that there was no evidence of publication bias in this current study (Figure 4).

Discussion

Pulmonary arterial hypertension, a severe and lethal disease, is characterized by progressively elevated pulmonary vascular resistance but normal left heart pressure. Despite the unclear pathogenesis, research has demonstrated that specific cellular pathways are involved in the development of PAH lesions.[2] In 2000, bone morphogenetic protein receptor type 2 was reported to be responsible for the pathogenesis of hereditary PAH, which was considered as one of the most significant findings in this field of research.[17] In addition, studies have investigated the potential genes associated with vascular regulation in recent years.[8–10] 5-HT can stimulate the proliferation of smooth muscle cells within the pulmonary vasculature.[4] This current meta-analysis evaluated the relationship between the *SLC6A4* gene L/S polymorphism and the risk of PAH.

To assess the role of the *SLC6A4* gene L/S polymorphism in the susceptibility to PAH, eight case–control studies were included in this meta-analysis, involving 1215 cases and 936 healthy control subjects. There was no significant association between this variant and PAH susceptibility among the total population. The actual effects of any single gene or polymorphism in the 5-HTT system is likely to be less than expected. However, the non-significant relationship between the *SLC6A4* gene L/S polymorphism and PAH does not necessarily eliminate the possibility that other variants or combinations of alleles at multiple loci within the same genes might be related to PAH. Therefore, it is necessary to systematically screen for functional variants within the *SLC6A4* gene and other related genes, followed by functional assays to validate the causal variants and their epistatic interactions in PAH pathogenesis.[18] In addition, significant between-study heterogeneity was displayed among all comparison models in the current meta-analysis. However, after removing articles deviating from HWE, the heterogeneity was removed for stratification analysis, suggesting that studies deviating from HWE were a significant source of the heterogeneity.[19]

This current meta-analysis had several limitations. First, only articles published in English-language journals were included, with unpublished or non-English-language

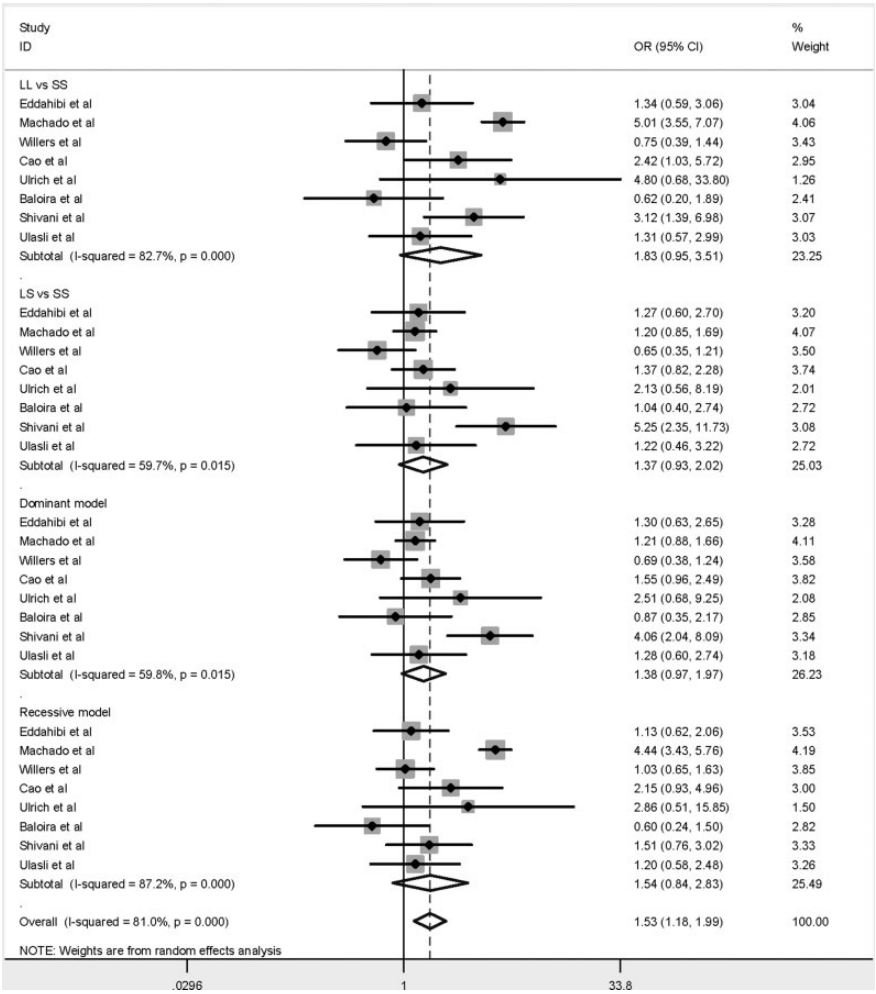

Figure 2. Forest plot of a meta-analysis to investigate the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension. Data are pooled odds ratios (OR) with 95% confidence intervals (CI) determined using a random-effects model. Error bars indicate the 95% CIs.[6,8–10,13–16]

articles being excluded although they might have met the inclusion criteria. Secondly, the OR value was acquired without correction, but OR would normally be corrected by ethnicity, age and other exposure factors possibly related to PAH risk in order to produce accurate outcomes. Thirdly, inter-gene and gene–environment interactions might also influence the accuracy of these current outcomes. A lack of the original data restricted further evaluation of the potential inter-gene and gene–environment interactions.

In conclusion, this current meta-analysis demonstrated that the *SLC6A4* gene L/S polymorphism did not appear to be related to PAH risk. Based on the limitations

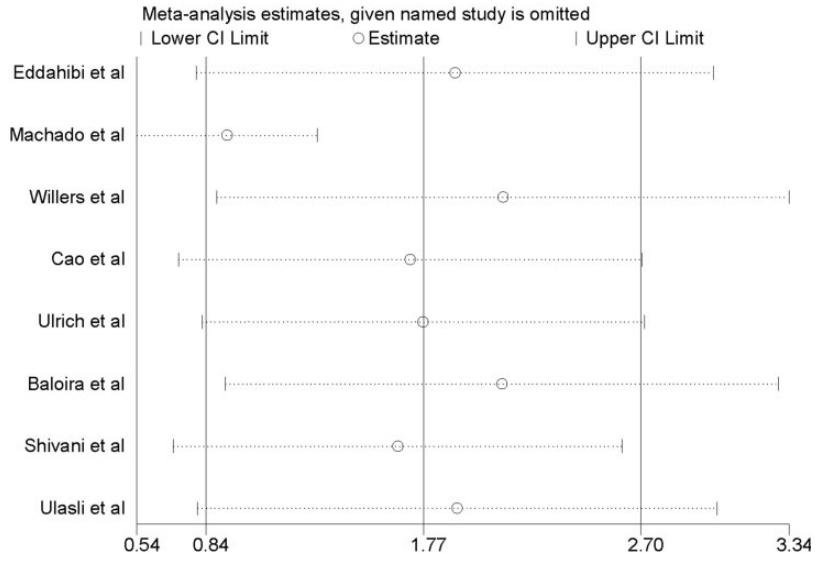

Figure 3. Sensitivity analysis of the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension.

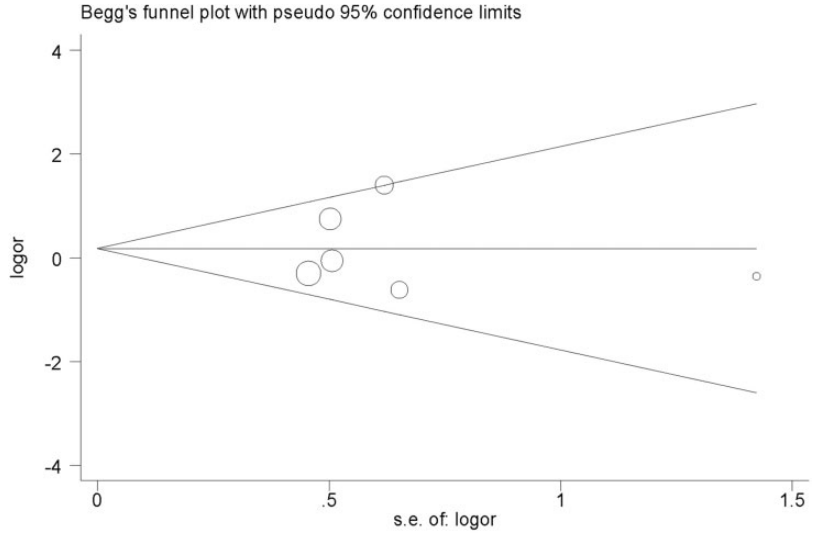

Figure 4. Begg's funnel plot of studies included in a meta-analysis to investigate the relationship between the solute carrier family 6 member 4 (*SLC6A4*) gene L/S polymorphism and pulmonary arterial hypertension to test for publication bias. Area of each circle represents the contribution of the study to the pooled odds ratio (OR).

described earlier, high-quality studies are required to validate these findings.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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