

## Original Article

# GFA Taq I polymorphism and cleft lip with or without cleft palate (CL/P) risk

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**Abstract:** The transforming growth factor alpha (TGFA) Taq I polymorphism has been indicated to be correlated with cleft lip with or without cleft palate (CL/P) susceptibility, but study results are still debatable. Thus, a meta-analysis was conducted. We conducted a comprehensive search of Embase, Ovid, Web of Science, the Cochrane database, PubMed, the Chinese Biomedical Literature Database (CBM-disc, 1979-2014), the database of National Knowledge Infrastructure (CNKI, 1979-2014) and the full paper database of Chinese Science and Technology of Chongqing (VIP, 1989-2014) to identify suitable studies. There were 18 studies suitable for this meta-analysis, involving a total of 3135 cases and 3575 controls. Significantly increased CL/P risk was observed (OR = 1.49; 95% CI 1.17-1.89;  $P = 0.001$ ). In subgroup analyses stratified by ethnicity, there was evidence in the Caucasian population for an association between this polymorphism and CL/P risk (OR = 1.52; 95% CI 1.14-2.02;  $P = 0.004$ ). However, no significant association was found between this his polymorphism and CL/P risk in African and Hispanic populations. According to a specific CL/P type, increased clip lip and palate risk and clip palate risk were found (OR = 1.38; 95% CI 1.10-1.73;  $P = 0.005$ ; OR = 1.29; 95% CI 1.01-1.66;  $P = 0.042$ ). In conclusion, the present meta-analysis found that the TGFA Taq I polymorphism may be associated with CL/P susceptibility.

**Keywords:** Transforming growth factor alpha, genetics, cleft lip with or without cleft palate, meta-analysis

## Introduction

Cleft lip with or without cleft palate (CL/P) affects about 1 in 700 live births, but the rate varies widely between geographical areas and different ethnic groups [1]. It is also affected by environmental and socioeconomic conditions. Generally, prevalence is high-est among Asian and Native Americans, often up to 1/500. In Europeans the prevalence is about 1/1000, and the lowest rates are among Africans (about 1/2500) [2]. It has been known that CL/P has a strong genetic etiology, and many researchers have attempted to unravel the genetic puzzle underlying CL/P [3, 4].

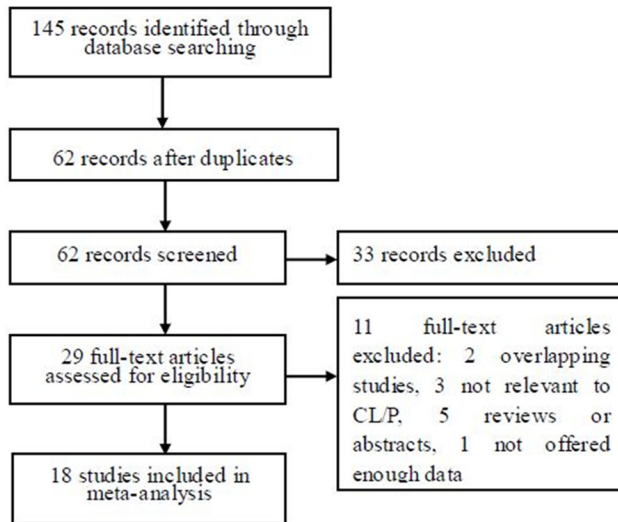
Transforming growth factor alpha (TGFA) belongs to a large family of proteins that regulate cell proliferation, differentiation, migration and apoptosis. Ardinger et al. first reported an association between the Taq I variant in TGFA and CL/P [5]. TGFA has since been extensively investigated for linkage, association and gene-environment interactions with inconsistent

results [6-22]. Meta-analysis is a useful method for investigating associations between genetic factors and diseases, because a quantitative approach is used to combine the results from different studies on the same topic, thereby providing more reliable conclusions. Thus, we performed a meta-analysis to clarify the association of TGFA Taq I polymorphism with CL/P risk.

## Materials and methods

### Publication search

We conducted a comprehensive search of Embase, Ovid, Web of Science, the Cochrane database, PubMed, the Chinese Biomedical Literature Database (CBM-disc, 1979-2014), the database of National Knowledge Infrastructure (CNKI, 1979-2014) and the full paper database of Chinese Science and Technology of Chongqing (VIP, 1989-2014) to identify suitable studies published before Jun, 2014. The following keywords were used for searching: ("Cleft lip



**Figure 1.** Flow of study identification, inclusion, and exclusion.

with or without cleft palate” OR “Cleft lip” OR “cleft palate”) AND (“polymorphism\*” OR “variant\*”) AND (“transforming growth factor alpha” OR “TGFA”). The most complete and recent results were used when there were multiple publications from the same study group. The references of reviews and retrieved articles were also searched simultaneously to find additional eligible studies.

#### Study selection

We first performed an initial screening of titles or abstracts to find potentially appropriate articles. A second screening was based on full-text review to identify those containing useful data on the topic of interest for inclusion in the meta-analysis. Studies were considered eligible if they met the following criteria: (1) publications assessed the relationship between TGFA Taq I polymorphism with CL/P risk; (2) used a cohort or case-control studies design; (3) had an appropriate description of TGFA Taq I polymorphism in cases and controls; (4) reported an odds ratio (OR) with 95% confidence interval (CI) or other available data for calculating OR (95% CI).

#### Data extraction

Two investigators extracted the data independently, and a third investigator reviewed the result. The following information was extracted from each study: first author, ethnicity, type of CL/P, gender, the number of patients and con-

trols in the study, genotype information. If any data essential to the analysis were not available from a study, best efforts were made to contact the authors to fill in the missing data.

#### Statistical analysis

The strength of the association between the TGFA Taq I polymorphism with CL/P risk was measured by the OR with 95% CI. The significance of the pooled OR was determined by Z test and a P value of less than 0.05 was considered significant. Then, we examined the association between TGFA Taq I polymorphism with CL/P risk on the genetic comparison model (C2 genotype vs. C1 genotype). The random-effects model, using the DerSimonian-Laird method, was conducted to pool the results when heterogeneity between studies existed on the basis of Q-test P-value which was less than 0.1. Besides, the  $I^2$  statistic was calculated to assess the between-study heterogeneity, and heterogeneity was deemed as apparent when the  $I^2$  statistic value was greater than 50%. Furthermore, several subgroup meta-analyses were performed in an attempt to assess the association between the TGFA Taq I polymorphism with CL/P risk based on the ethnicity and type of CL/P. To validate the credibility of outcomes in this meta-analysis, cumulative meta-analysis and sensitivity analysis were performed. Publication bias was investigated using a funnel plot, in which the standard error of logOR of each study was plotted against its logOR. An asymmetric plot suggested the existence of possible publication bias. In addition, funnel-plot asymmetry was formally assessed by the method of Egger’s linear regression test. All analyses were performed using Stata software, version 12.0 (Stata Corp, College Station, TX). All P values were two-sided.

## Results

#### Eligible studies

Our search in electronic databases identified 145 records. One hundred and sixteen records were excluded after reviewing titles and abstracts and we gained 29 useful studies. After full-text reviewing, we excluded 11 studies and listed the reasons for their exclusion in **Figure 1**. There were 18 studies suitable for

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**Table 1.** Characteristics of the case-control studies

First author	Ethnicity	Type of clip	Gender	Case (n)	Control (n)	HWE	TGFA C2/C1 allele number	
							Case	Control
Beaty 1	Caucasian	Mixed	Mixed	128	135	Yes	15/241	22/248
Beaty 2	African	Mixed	Mixed	24	135	Yes	7/71	22/248
Tanabe	Asian	CL/P	Mixed	28	73	Yes	7/49	17/129
Jara	Hispanic	CL/P	Mixed	39	51	Yes	6/72	8/94
Sassani 1	Caucasian	CL/P	Mixed	81	84	Yes	28/134	15/153
Sassani 2	Asian	CL/P	Mixed	6	6	Yes	2/10	2/10
Sassani 3	African	CL/P	Mixed	10	7	Yes	7/13	3/11
Ardinger	Caucasian	CL/P	Mixed	78	98	Yes	21/135	10/186
Shiang	Caucasian	CP	Mixed	43	170	Yes	17/69	29/311
Hwang	Caucasian	Mixed	Mixed	183	284	Yes	43/323	46/522
Hecht	Caucasian	CL/P	Mixed	12	13	Yes	1/23	4/22
Chenevix	Caucasian	CL/P	Mixed	113	117	Yes	36/198	21/205
Holder	Caucasian	CL/P	Mixed	60	60	Yes	24/86	5/115
Chenevix	Caucasian	CL/P	Mixed	96	100	Yes	33/159	11/189
Stoll	Caucasian	Mixed	Mixed	155	99	Yes	20/291	14/184
Christensen	Caucasian	Mixed	Mixed	256	457	Yes	64/448	124/790
Beaty 1	Caucasian	Mixed	Mixed	130	87	Yes	18/242	8/166
Beaty 2	African	Mixed	Mixed	24	45	Yes	3/45	2/88
Bertoja	Hispanic	CL/P	Mixed	140	142	Yes	27/253	21/263
Passos-Bueno	Hispanic	CL/P	Mixed	536	385	Yes	53/1019	41/729
Lidral 1	Caucasian	Mixed	Mixed	244	251	Yes	52/436	53/449
Lidral 2	Caucasian	Mixed	Mixed	749	776	Yes	118/1380	116/1436

CL: clip lip, CL/P: clip lip and palate, CP: clip palate.

**Table 2.** Results of this meta-analysis

	OR (95% CI)	P	I <sup>2</sup> (%)
Overall	1.49 (1.17-1.89)	0.001	62
Hispanic	1.04 (0.75-1.44)	0.816	0
Caucasian	1.52 (1.14-2.02)	0.004	75
African	1.15 (0.50-2.66)	0.741	27
CP	1.38 (1.10-1.73)	0.005	25
CL/P	1.29 (1.01-1.66)	0.042	63

CL/P: clip lip and palate, CP: clip palate.

this meta-analysis finally, involving a total of 3135 cases and 3575 controls. Two of the included studies were conducted on an Asian population, three studies were conducted on an African population, three studies were conducted on a Hispanic population, and the rest studies were conducted on a Caucasian population. Characteristics of eligible studies were summarized in **Table 1**.

### Meta-analysis

The main results of this meta-analysis were presented in **Table 2**. In the overall analyses,

there was an association between the TGFA Taq I polymorphism with CL/P risk (**Figure 2**). Significantly increased CL/P risk was observed (OR = 1.49; 95% CI 1.17-1.89; P = 0.001). In subgroup analyses stratified by ethnicity, there was evidence in the Caucasian population for an association between this polymorphism and CL/P risk (OR = 1.52; 95% CI 1.14-2.02; P = 0.004). However, no significant association was found between this his polymorphism and CL/P risk in African and Hispanic populations. According to a specific CL/P type, increased clip lip and palate risk and clip palate risk were found (OR = 1.38; 95% CI 1.10-1.73; P = 0.005; OR = 1.29; 95% CI 1.01-1.66; P = 0.042).

With regard to the cumulative meta-analysis, the evidence was observed to support a significant association of the TGFA Taq I polymorphism with CL/P risk (**Figure 3**). With regard to the sensitivity analysis, the pooled ORs were not altered qualitatively when any single study was removed, which indicated the results of the present meta-analysis were relatively stable and credible (**Figure 4**).

## TGA and CL/P risk

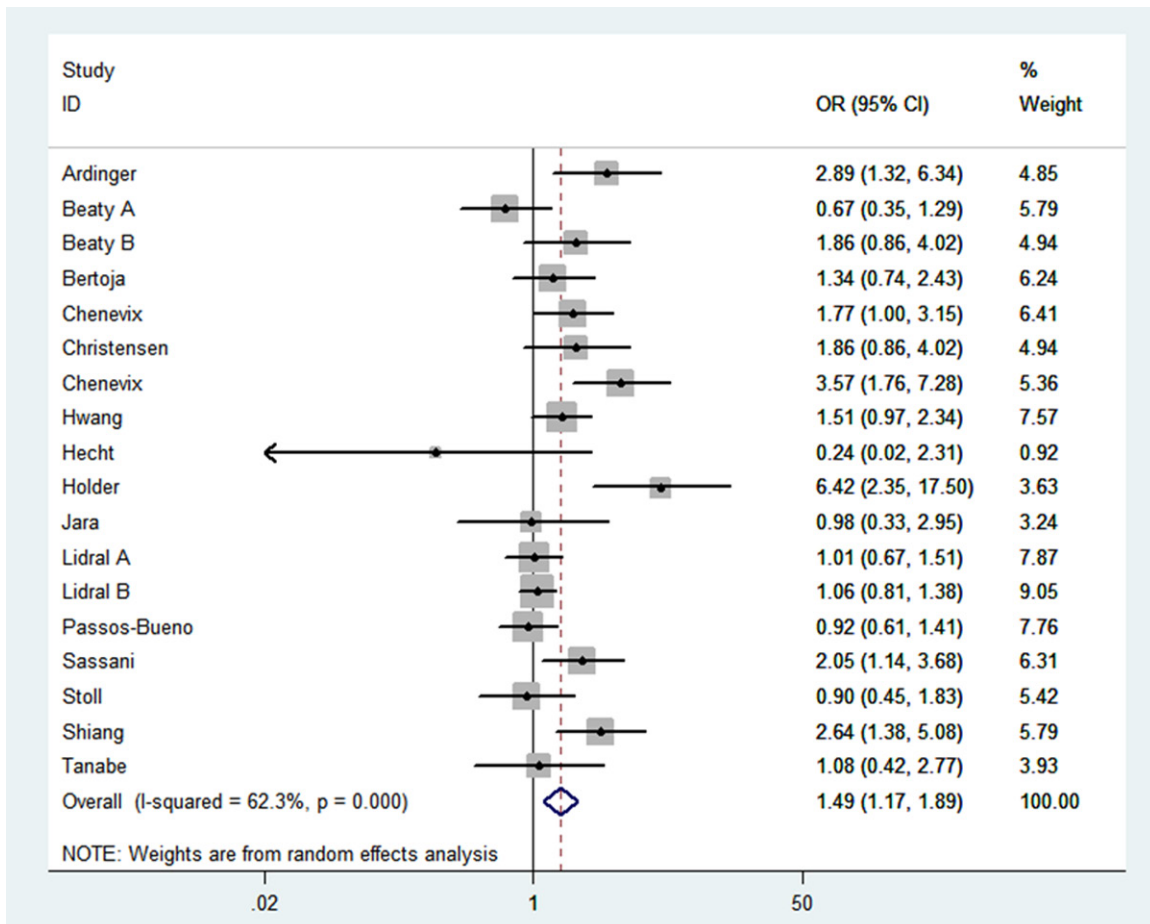


Figure 2. Meta-analysis for the association between the TGFA Taq I polymorphism and CL/P risk.

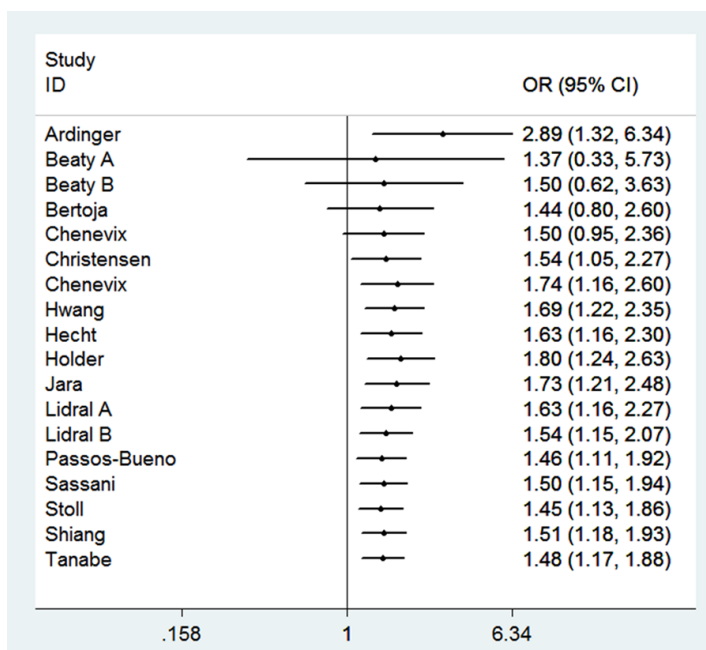


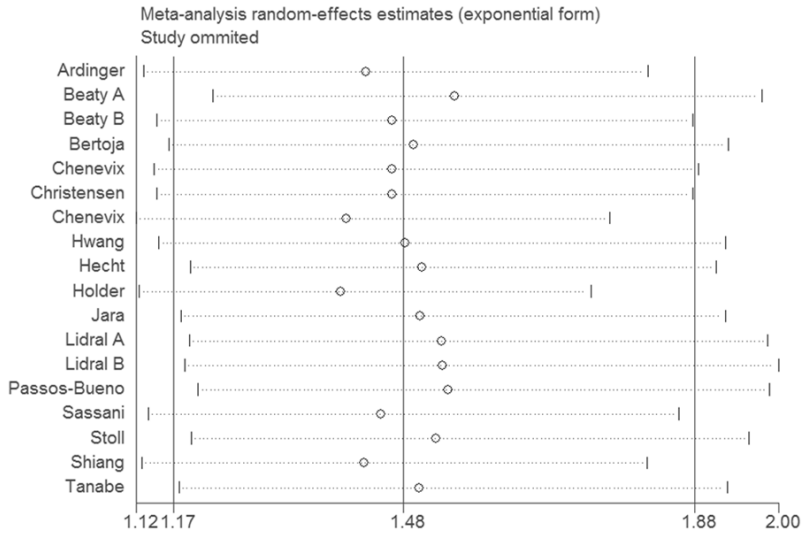
Figure 3. Cumulative meta-analysis for association between the GFA Taq I polymorphism and CL/P risk.

Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures. As shown in **Figure 5**, the shape of the funnel plots seemed symmetrical, suggesting no presence of publication bias. Then, the Egger's test was adopted to provide statistical evidence. The results have shown that no publication bias was evident in this meta-analysis ( $P = 0.156$ ).

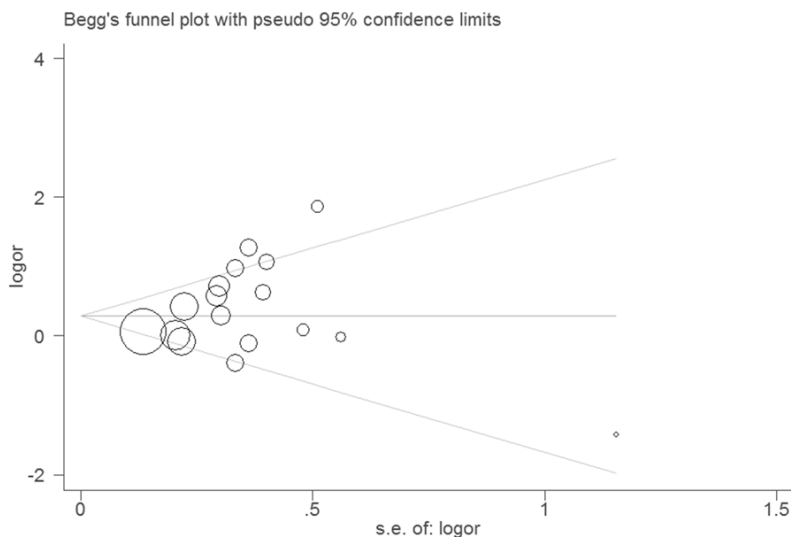
### Discussion

This present meta-analysis investigated the relationship between TGFA Taq I polymorphism and CL/P risk. Eighteen case-control studies with a total of 3135 cases and 3575 controls were eligible. At the overall analysis, the TGFA Taq I polymorphism was significantly

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**Figure 4.** Sensitivity analysis for association between the GFA Taq I polymorphism and CL/P risk.



**Figure 5.** Funnel plot for association between the GFA Taq I polymorphism and CL/P risk.

associated with CL/P risk. In the subgroup analysis by ethnicity, we noted that Caucasians carrying the TGFA Taq I polymorphism had an increased CL/P risk. Moreover, after stratifying based on CL/P type, the significant associations were found in clip lip and palate risk and clip palate risk.

To investigate the stability of the result, we performed cumulative meta-analysis and sensitivity analysis. The cumulative meta-analysis showed a trend of significant association

between this polymorphism and the risk of CL/P. Additionally, sensitivity analysis showed that no single study qualitatively changed the pooled odds ratios, indicating that the results of this meta-analysis are stable.

Expression of the TGFA gene occurs in a wide spectrum of normal tissue from the preimplantation period in mouse embryos to adult life [23]. During craniofacial development, TGFA is expressed at the medial edge epithelium of fusing palatal shelves [24]. In palatal cultures, TGFA promotes synthesis of extracellular matrix and mesenchymal cell migration, thereby ensuring the strength of the fused palate [25].

Certain limitations of the present meta-analysis should be considered when interpreting the results. Selection bias may exist as the studies without sufficient data were excluded. The analysis largely used unadjusted estimates, as not all the included studies were adjusted by the same potential confounders, such as lifestyles and exposures, which may influence the combined results. Although subgroup analysis was per-

formed by ethnicity and the type of CL/P, significant heterogeneity existed in certain subgroups. Owing to the limited original information, potential gene-gene and gene-environment interactions, which have an important impact on CL/P risk, were not evaluated in the study.

In conclusion, the present meta-analysis found that the TGFA Taq I polymorphism may be associated with CL/P susceptibility. Since limited studies were included in the study, larger sample sizes and well-designed case-control stud-

ies are required in the future to confirm this association.

#### Disclosure of conflict of interest

None.

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