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# Environmental and Genetic Risk Factors of Congenital Anomalies: an Umbrella Review of Systematic Reviews and Meta-Analyses

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## ABSTRACT

**Background:** The prevalence of congenital anomalies in newborns in South Korea was 272.9 per 100,000 in 2005, and 314.7 per 100,000 in 2006. In other studies, the prevalence of congenital anomalies in South Korea was equivalent to 286.9 per 10,000 livebirths in 2006, while it was estimated 446.3 per 10,000 births during the period from 2008 to 2014. Several systematic reviews and meta-analyses analyzing the factors contributing to congenital anomalies have been reported, but comprehensive umbrella reviews are lacking.

**Methods:** We searched PubMed, Google Scholar, Cochrane, and EMBASE databases up to July 1, 2019, for systematic reviews and meta-analyses that investigated the effects of environmental and genetic factors on any type of congenital anomalies. We categorized 8 subgroups of congenital anomalies classified according to the 10th revision of the International Statistical Classification of Diseases (ICD-10). Two researchers independently searched the literature, retrieved the data, and evaluated the quality of each study.

**Results:** We reviewed 66 systematic reviews and meta-analyses that investigated the association between non-genetic or genetic risk factors and congenital anomalies. Overall, 269 associations and 128 associations were considered for environmental and genetic risk factors, respectively. Congenital anomalies based on congenital heart diseases, cleft lip and palate, and others were associated with environmental risk factors based on maternal exposure to environmental exposures (air pollution, toxic chemicals), parental smoking, maternal history (infectious diseases during pregnancy, pregestational and gestational diabetes mellitus, and gestational diabetes mellitus), maternal obesity, maternal drug intake, pregnancy through artificial reproductive technologies, and socioeconomic factors. The association of maternal alcohol or coffee consumption with congenital anomalies was not significant, and maternal folic acid supplementation had a preventive effect on congenital heart defects. Genes or genetic loci associated with congenital anomalies included *MTHFR*, *MTRR* and *MTR*, *GATA4*, *NKX2-5*, *SRD5A2*, *CFTR*, and 1p22 and 20q12 anomalies.

**Conclusion:** This study provides a wide perspective on the distribution of environmental and genetic risk factors of congenital anomalies, thus suggesting future studies and providing health policy implications.

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**Disclosure**

The authors have no potential conflicts of interest to disclose.

**Author Contributions**

Conceptualization: Lee KS, Choi YJ. Data curation: Lee KS, Choi YJ. Formal analysis: Lee H,<sup>1</sup> Cho J, Lee H.<sup>2</sup> Funding acquisition: Hong YC. Investigation: Lee KS, Choi YJ, Lee HJ, Cho J, Lee H.<sup>2</sup> Methodology: Choi YJ. Software: Lee KS, Choi YJ. Validation: Lee KS. Visualization: Park SJ. Writing - original draft: Lee KS, Choi YJ, Lee H,<sup>1</sup> Cho J, Lee H.<sup>2</sup> Writing - review & editing: Hong YC, Park JS.

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**Keywords:** Congenital Disease; Genetic; Meta-Analysis; Non-Genetic; Risk Factor

**INTRODUCTION**

The prevalence of congenital anomalies in newborns in South Korea was 272.9 per 100,000 in 2005, and 314.7 per 100,000 in 2006.<sup>1</sup> In other studies, the prevalence of congenital anomalies in South Korea was equivalent to 286.9 per 10,000 livebirths in 2006,<sup>2</sup> while it was estimated 446.3 per 10,000 births during the period from 2008 to 2014.<sup>3</sup> According to the 2010 Global Burden of Diseases, 510,400 deaths worldwide in 2010 were attributed to congenital anomalies. This equated to 1% of all deaths (6% of neonatal and postnatal infant deaths) and ranked 23rd as all causes of death.<sup>4</sup> The European Surveillance of Congenital Anomalies (EUROCAT), a network of population-based registers of congenital anomalies in Europe covering 1.5 million births annually in 22 countries, reported a prevalence of congenital anomalies of 23.9 per 1,000 births during 2003–2007.<sup>5</sup> Among them, 80% were livebirths, 17.6% underwent termination of pregnancy for fetal anomaly (TOPFA), and the remainder were stillbirths or died in the first week of birth. According to EUROCAT, during 2003–2007, while chromosomal anomalies accounted for 3.6 per 1,000 births, congenital heart defects (CHD) were the most common non-chromosomal anomalies (6.5 per 1,000 births), followed by limb defects (3.8 per 1,000 births), urinary system anomalies (3.1 per 1,000 births), and nervous system anomalies (2.3 per 1,000 births).<sup>5</sup> In 11 EUROCAT countries, the average infant mortality with congenital anomalies was 1.1 per 1,000 births, and the average TOPFA prevalence was 4.6 per 1,000 births, which was more than 4 times the infant mortality related to congenital anomalies.<sup>4</sup>

Since prenatal medical intervention for the fetus is limited, and structural anomalies cannot be reversed during fetal development, primary prevention of congenital anomalies based on pre-conceptional care is critical at the population level. Identification of risk factors for congenital anomalies is fundamental for such primary prevention. To understand the causes of congenital anomalies, a more comprehensive literature review is required. A number of systematic reviews and meta-analyses have analyzed the association between environmental and genetic risk factors and various types of congenital anomalies; however, most articles have focused on a specific risk factor and a specific type of congenital anomaly. A few articles have covered risk factors in a comprehensive manner, but have focused on specific types of congenital anomalies, such as hip dysplasia<sup>6,7</sup> or anorectal anomaly,<sup>8</sup> while articles that comprehensively covered congenital anomalies focused on specific categories of risk factors, such as paternal risk factors,<sup>9,10</sup> maternal smoking,<sup>11</sup> secondhand smoking,<sup>12</sup> maternal drug intake,<sup>13,14</sup> maternal influenza,<sup>15</sup> or artificial reproductive technologies.<sup>16,17</sup> To the best of our knowledge, there has been no umbrella review of the available systematic reviews and meta-analyses regarding all reported non-genetic and genetic risk factors of all types of known congenital anomalies. The purpose of this article was to outline the spectrum of risk factors for congenital anomalies as comprehensively as possible, thereby providing not only a summary of currently available evidence, but also a suggestion for further study topics in the future.

## METHODS

### Databases and search strategy

We searched the PubMed, Google Scholar, Cochrane, and Embase databases up to July 1, 2019. Exposures included both environmental and genetic factors. The terms used in the searches were as follows: (“risk factors” [MeSH Terms] OR (“risk” [All Fields] AND “factors” [All Fields]) OR “risk factors” [All Fields] OR (“risk” [All Fields] AND “factor” [All Fields]) OR “risk factor” [All Fields]) AND (“congenital” [Subheading] OR “congenital” [All Fields]) AND (“meta-analysis” [Publication Type] OR “meta-analysis as topic” [MeSH Terms] OR “meta-analysis” [All Fields]).

We also manually searched the bibliography of identified papers for additional articles. The literature search, abstract screening, and full article review were independently performed by 2 researchers. Disagreements in the course of article searching were resolved by discussion until a consensus was reached.

### Selection criteria

The following papers were selected: 1) systematic review and meta-analysis reports that analyzed the association between environmental or genetic risk factors and congenital anomalies, 2) papers written fully in English, and 3) papers that were peer-reviewed. Systematic reviews without meta-analyses were excluded. Studies published only as conference abstracts or case reports were also excluded.

### Assessment of methodological quality

Two researchers independently assessed the methodological quality of the included studies according to AMSTAR2 (Assessing the Methodological Quality of Systematic Reviews, cohort and case control studies, but not case series).<sup>18</sup> We used the tools included in the AMSTAR2 checklist ([https://amstar.ca/Amstar\\_Checklist.php](https://amstar.ca/Amstar_Checklist.php)). Disagreement in the assessment was resolved by discussion until consensus was reached. For certainty of evidence, we used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system.<sup>18</sup> **Supplementary Table 1** shows the completed AMSTAR checklist based on the studies involved.

### Data retrieval

Two researchers independently retrieved information from selected articles, including authors; year of study; risk factors; congenital anomalies as outcomes; the number of studies included in each meta-analysis; the database used for each systematic review; the date of database searching; the total number of study participants included in each study; the risk ratio (RR) or odds ratio (OR) and 95% confidence interval (CI); the methods of meta-analysis (fixed effects vs. random effects model); the methods used for assessing publication bias; and the method of assessment of methodological quality of studies included in each meta-analysis. Disagreement of the data retrieval between the 2 researchers was resolved through discussion.

### Statistical analysis

We categorized 8 subgroups of congenital anomalies according to the 10th revision of the International Statistical Classification of Diseases (ICD-10). We estimated the summary of evidence on the significance of the effect size for the associations according to non-genetic risk factors and genetic risk factors. In addition, we evaluated the evidence of the studies showing significant effects by assessing the beta estimate, its 95% CI, and  $I^2$  ranges,  $P$  values for heterogeneity, and the number of meta-analyses included. We also plotted the range of

ORs or RRs for associations between non-genetic risk factors or genetic risk factors and each subgroup of congenital anomalies.

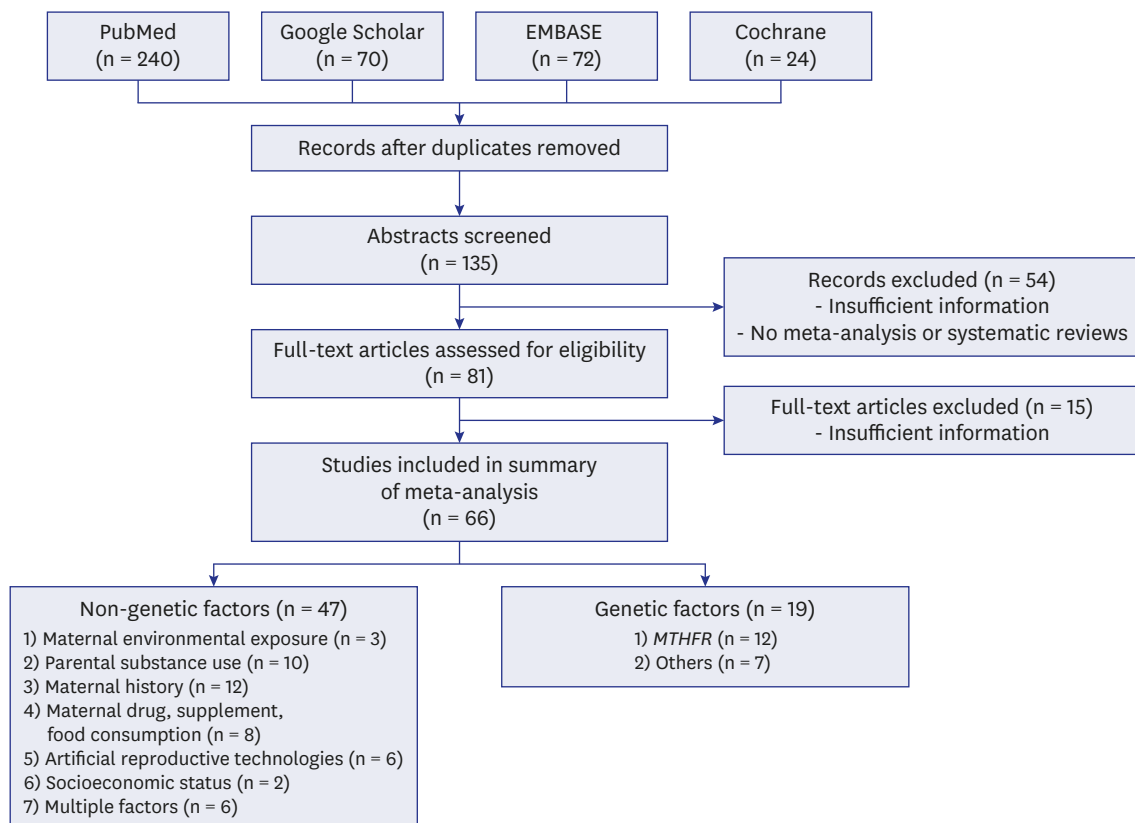
## RESULTS

### Description of eligible meta-analyses

The detailed search procedures are summarized in **Fig. 1**. The search strategy identified 406 references. After excluding duplicated articles, we reviewed the titles and abstracts of all 135 identified studies, and further excluded 54 irrelevant studies. Then, the full texts of the remaining 81 articles were reviewed according to the inclusion and exclusion criteria. After examining these articles in more detail, further 15 studies were excluded due to irrelevance or insufficient data. Finally, 66 studies were included in the systematic review.

### Non-genetic risk factors with evidence of association

**Table 1** provides a summary of the association between non-genetic factors and congenital anomalies. We summarized the evidence of risk factors for congenital anomalies in the meta-analysis showing significant effects in **Table 2** and **Fig. 2**, and describe the details below. We also listed the relevant studies and their characteristics in **Supplementary Table 2**.



**Fig. 1.** Flowchart showing meta-analysis of studies on genetic and non-genetic factors for congenital diseases. *MTHFR* = methylene tetrahydrofolate reductase.

**Table 1.** The summary of evidence on significance with association between non-genetic risk factors for congenital diseases

Risk factors	#	Subgroup	Any congenital malformations	Congenital heart defect	Cleft lip/palate	Neural tube defect	Digestive tract defect	Urogenital tract defect	Respiratory tract defect	Musculoskeletal defect	Eye, ear, face, and neck defect
Maternal environmental exposure	2	Air pollution		NO <sub>2</sub> ↑ (1/1) <sup>a</sup> SO <sub>2</sub> , PM <sub>10</sub> , CO, O <sub>3</sub> → (1/1) PM <sub>2.5</sub> → (1/1)	SO <sub>2</sub> , PM <sub>10</sub> , CO, O <sub>3</sub> → (1/1)						
Parental substance use	7	Toxic chemicals	↑ (1/1)								
	7	Parental smoking	↑ (2/2)	↑ (5/5)	↑ (1/1)	↑ (1/2)	↑ (2/2)	↑ (1/3)	→ (2/2)	↑ (1/2)	↑ (1/2)
	3	Maternal alcohol consumption		→ (2/2)	→ (1/1)						
Maternal history	3	Maternal infectious diseases (influenza, other viral infection, and fever)	↑ (1/1)	↑ (3/3)	↑ (1/1)	↑ (1/1)	↑ (1/1)				
	4	Pregestational and gestational diabetes	↑ (2/2)	↑ (2/2)							
	2	Maternal obesity		↑ (2/2)							
	3	Other maternal history	→ (2/2)	→ (1/1)							
			Prenatal exercise	Maternal parity							
			Polycystic ovary syndrome								
Maternal drug, supplement, food consumption	5	Maternal drug intake (beta-blocker, valproic acid, fluoxetine)	↑ (2/3)	↑ (4/5)	↑ (2/2)	↑ (2/3)	→ (1/1)	↑ (1/2)	→ (1/1)	↑ (1/2)	→ (1/1)
	2	Maternal supplementation (folic acid)		↓ (2/2)							
	1	Maternal diet (coffee)				→ (1/1)					
Artificial reproductive technologies	6		↑ (5/6)	↑ (2/2)		↑ (1/1)	↑ (1/1)	↑ (1/2)		↑ (1/1)	↑ (1/1)
Low socioeconomic status	2			↑ (1/2)	↑ (1/1)	→ (1/1)					
Multiple factors	6		Maternal age, preterm delivery, baby gender, birth weight, maternal history ↑ (2/2)	Maternal age, paternal smoking, paternal drinking, toxicant ↑ (2/2)	Paternal smoking ↑ (1/1) Paternal age → (1/1)	Paternal age → (1/1) Paternal smoking ↑ (1/1)	Paternal age → (1/1)	Maternal weight, maternal history ↑ (1/1)		Breech delivery, gender, parental history, delivery mode ↑ (2/2)	

↑ = positive pooled estimate association, → = no effect, ↓ = negative pooled estimate association, # = the numbers of meta-analysis studies.

<sup>a</sup>(numbers of meta-analyses studies with positive/negative/no effect pooled estimate/total numbers of meta-analysis).

### Maternal environmental exposure

#### 1) Air pollution

We identified 2 meta-analysis studies reporting the association between air pollution and congenital anomalies. Chen et al.<sup>19</sup> showed that nitrate dioxide (NO<sub>2</sub>) concentration was significantly associated with coarctation of the aorta based on 2 case-control studies and 2 cohort studies (pooled OR, 1.20; 95% CI, 1.02–1.41 per 10 ppb). However, there was no evidence of an association of SO<sub>2</sub>, PM<sub>10</sub>, CO, and O<sub>3</sub> with congenital anomalies. Hall and Robinson<sup>20</sup> showed that there was no significant association between PM<sub>2.5</sub> and congenital heart defects.

#### 2) Toxic chemicals

We identified 1 systematic review that meta-analyzed the association between toxic chemicals

**Table 2.** Results of effectiveness of congenital anomalies in the significant studies of meta-analysis included for non-genetic risk factors

Study	Study year	Exposure	Outcome	Pooled OR or RR (95% CI)	$P_{\text{heterogeneity}}$	$I^2$ (%)	# of studies
Chen et al. <sup>19</sup>	2014	Air pollution	Coarctation of the aorta	1.20 (1.02–1.41)	0.560	0.0%	4
Spinder et al. <sup>21</sup>	2019	Toxic chemicals	Congenital anomalies	1.31 (1.06–1.63)	0.760	0.0%	5
Salmasi et al. <sup>22</sup>	2010	Parental smoking	Congenital anomaly	1.18 (1.04–1.34)	NA	NA	12
Zheng et al. <sup>12</sup>	2019	Parental smoking	Congenital malformations	1.92 (1.61–2.30)	< 0.001	88.9%	33
		Parental smoking	CHD	2.10 (1.32–3.35)	0.001	92.5%	6
		Parental smoking	Oral clefts	1.87 (1.47–2.39)	0.001	86.4%	18
		Parental smoking	Digestive system	1.17 (1.05–1.32)	0.727	0.0%	2
Nicoletti et al. <sup>11</sup>	2014	Parental smoking	CHD	1.11 (1.03–1.19)	0.001	58.7%	29
		Parental smoking	Nervous system	1.74 (1.61–2.30)	0.020	63.0%	6
		Parental smoking	Musculoskeletal system	1.27 (1.16–1.39)	< 0.001	78.5%	48
		Parental smoking	Eye, ear, face and neck	1.28 (1.19–1.37)	< 0.001	53.7%	53
Yu et al. <sup>26</sup>	2019	Parental smoking	Cryptorchidism	1.18 (1.12–1.24)	0.100	30.0%	20
Zhang et al. <sup>24</sup>	2017	Parental smoking	CHD	1.11 (1.04–1.18)	< 0.001	69.0%	43
Zhao et al. <sup>25</sup>	2019	Parental smoking	CHD	1.17 (1.09–1.25)	< 0.001	63%	59
Luteijn et al. <sup>15</sup>	2014	Maternal infectious disease	CHD	1.56 (1.13–2.14)	0.083	41.2%	10
		Maternal infectious disease	Neural tube defects	3.33 (2.05–5.40)	0.031	49.6%	11
		Maternal infectious disease	Orofacial clefts	1.96 (1.33–2.91)	0.104	36.9%	11
		Maternal infectious disease	Digestive system	1.72 (1.09–2.68)	NA	0.0%	4
		Maternal infectious disease	Limb reduction defects	2.03 (1.27–3.27)	NA	0.0%	3
Ye et al. <sup>30</sup>	2019	Maternal infectious disease	CHD	3.54 (1.75–7.15)	0.080	46.0%	7
Shi et al. <sup>31</sup>	2014	Maternal fever	CHD	1.53 (1.36–2.73)	0.158	35.4%	7
Balsells et al. <sup>32</sup>	2012	GDM	Congenital anomalies	2.66 (2.04–3.47)	0.001	69.0%	9
		PGDM	Congenital anomalies	1.16 (1.07–1.25)	0.690	0.0%	15
Zhao et al. <sup>33</sup>	2015	GDM	Congenital malformation	1.18 (1.11–1.26)	0.342	9.9%	17
		PGDM	Congenital malformation	2.44 (1.92–3.10)	< 0.001	78.3%	13
Simeone et al. <sup>34</sup>	2015	PGDM	CHD	3.8 (3.0–4.9)	NA	NA	12
Hoang et al. <sup>35</sup>	2017	GDM	CHD	3.59 (3.03–4.50)	NA	NA	NA
Cai et al. <sup>66</sup>	2014	Maternal overweight	CHD	1.08 (1.02–1.15)	0.020	52%	11
		Maternal moderate obesity	CHD	1.15 (1.11–1.20)	0.190	35%	5
		Maternal severe obesity	CHD	1.39 (1.31–1.47)	0.990	0.0%	5
Zhu et al. <sup>37</sup>	2018	Maternal overweight	CHD	1.06 (1.02–1.10)	0.001	60.1%	17
		Maternal obesity	CHD	1.17 (1.14–1.20)	0.161	25.5%	17
Tanoshima et al. <sup>13</sup>	2015	Maternal drug intake	Congenital malformation	2.44 (2.13–2.80)	< 0.001	0.0%	28
Yu et al. <sup>39</sup>	2016	Maternal drug intake	CHD	2.08 (1.55–2.79)	< 0.001	0.0%	20
Feng et al. <sup>40</sup>	2014	Maternal drug intake	Cleft lip	3.37(2.09–5.42)	< 0.001	8.5%	11
Tanoshima et al. <sup>13</sup>	2015	Maternal drug intake	Neural tube defects	7.41 (4.61–11.90)	< 0.001	0.0%	14
		Maternal drug intake	Urogenital anomalies	3.10 (2.28–4.22)	< 0.001	0.0%	20
		Maternal drug intake	Musculoskeletal anomalies	3.32 (2.26–4.88)	< 0.001	0.0%	18
Gao et al. <sup>14</sup>	2017	Maternal drug intake	Congenital malformation	1.18 (1.08–1.29)	0.605	0.0%	12
		Maternal drug intake	CHD	1.36 (1.17–1.59)	0.214	23.4%	12
Yakoob et al. <sup>41</sup>	2013	Maternal drug intake	CHD	2.01 (1.18–3.42)	0.100	52%	4
		Maternal drug intake	Cleft lip	3.11 (1.79–5.43)	0.690	0.0%	4
		Maternal drug intake	Neural tube defects	3.56 (1.19–10.67)	0.520	0.0%	3
Grigoriadis et al. <sup>42</sup>	2014	Maternal drug intake	CHD	2.50 (1.32–4.73)	0.081	51.9%	5
Feng et al. <sup>44</sup>	2015	Maternal supplementation	CHD	0.72 (0.63–0.82)	< 0.001	79.4%	17
Xu et al. <sup>45</sup>	2016	Maternal supplementation	CHD	0.60 (0.49–0.71)	< 0.001	88.8%	20
Wen et al. <sup>16</sup>	2012	ART	CHD	1.64 (1.30–2.07)	< 0.001	91.0%	21
		ART	Nervous system	2.01 (1.27–3.20)	< 0.001	89.3%	15
		ART	Genitourinary system	1.69 (1.33–2.15)	< 0.001	86.4%	17
		ART	Digestive system	1.66 (1.28–2.16)	< 0.001	72.5%	19
		ART	Musculoskeletal system	1.48 (1.03–2.02)	< 0.001	90.8%	18
		ART	Ear, face, and neck	1.43 (1.01–2.05)	< 0.001	84.5%	15
Zheng et al. <sup>17</sup>	2018	ART	CHD	1.22 (1.01–1.47)	0.070	35.2%	18
		ART	Musculoskeletal, urogenital and digestive system malformations	1.16 (1.05–1.27)	0.352	6.6%	17
		ART	Nervous system malformations	1.28 (1.07–1.53)	0.868	0.0%	16
Massaro et al. <sup>47</sup>	2015	ART	Congenital malformation	1.27 (1.02–1.59)	0.780	0.0%	9
Qin et al. <sup>48</sup>	2015	ART	Congenital malformation	1.33 (1.24–1.43)	< 0.001	66%	57

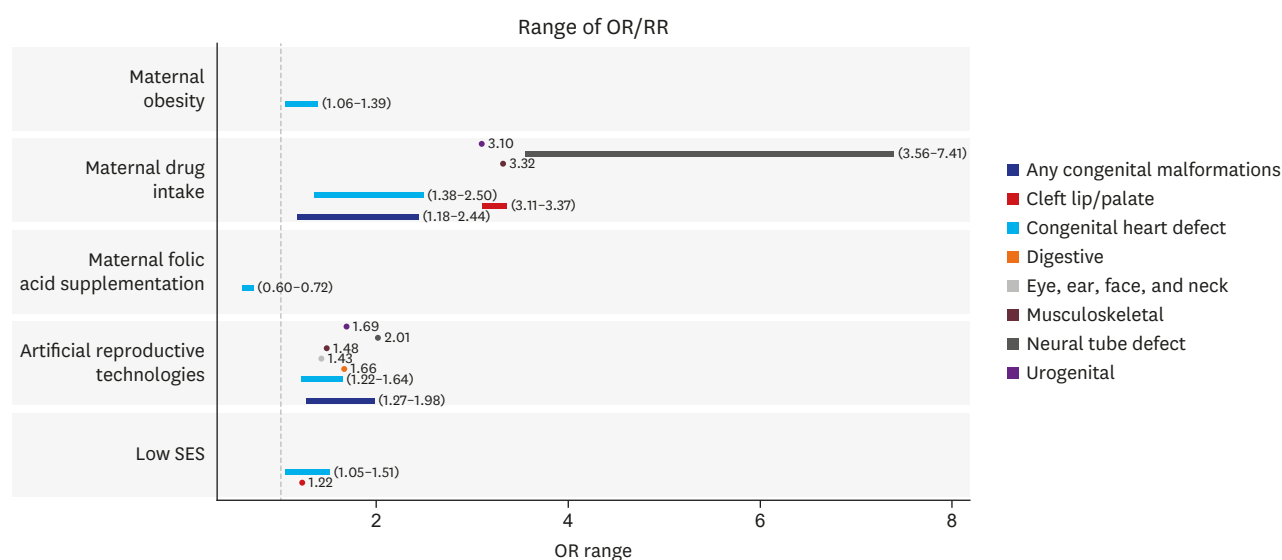
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**Table 2.** (Continued) Results of effectiveness of congenital anomalies in the significant studies of meta-analysis included for non-genetic risk factors

Study	Study year	Exposure	Outcome	Pooled OR or RR (95% CI)	$P_{\text{heterogeneity}}$	$I^2$ (%)	# of studies
Qin et al. <sup>49</sup>	2016	ART	Congenital malformation	1.37 (1.29–1.45)	0.010	41%	28
Lacamara et al. <sup>50</sup>	2017	ART	Congenital malformation	1.98 (1.86–2.11)	NA	NA	20
Yu et al. <sup>51</sup>	2014	Socioeconomic status	CHD	1.11 (1.03–1.21)	< 0.001	61.3%	6
Deguen et al. <sup>52</sup>	2016	Socioeconomic status	Cleft lip	1.22 (1.10–1.36)	0.193	30.7%	7
Daliri et al. <sup>53</sup>	2019	Baby' gender (boys)	Congenital malformation	1.25 (1.00–1.55)	0.038	52.9%	8
		Preterm delivery	Congenital malformation	2.51 (1.71–3.69)	< 0.001	82.7%	9
		Consanguineous marriage in families of children	Congenital malformation	0.39 (0.29–0.49)	< 0.001	99.9%	16
		Paternal age (45+)	Congenital malformation	1.05 (1.02–1.07)	< 0.001	67.7%	3
Oldereid et al. <sup>9</sup>	2018	Paternal smoking	Congenital malformation	1.75 (1.25–2.44)	< 0.001	61.4%	3
		Paternal age	Congenital malformation	1.05 (1.02–1.07)	< 0.001	67.7%	3
		Paternal smoking	CHD	1.75 (1.25–2.44)	< 0.001	61.4%	5
		Paternal smoking	Orofacial defect	1.51 (1.16–1.97)	0.024	61.4%	2
Peng et al. <sup>10</sup>	2019	Paternal smoking	Brain tumors	1.12 (1.03–1.22)	0.847	0.0%	14
		Paternal age increase	CHD	1.02 (1.00–1.04)	0.040	NA	4
		Paternal smoking increase	CHD	1.42 (1.17–1.74)	< 0.001	NA	10
		Paternal drinking	CHD	1.47 (1.05–2.07)	< 0.001	NA	7
Zwink et al. <sup>8</sup>	2011	Exposure to toxicant	CHD	2.15 (1.53–3.02)	< 0.001	NA	7
		Maternal overweight	Anorectal malformations	1.26 (1.06–1.50)	0.320	11.3%	3
		Maternal obesity	Anorectal malformations	1.64 (1.35–2.00)	0.440	0.0%	3
		Maternal history of diabetes during pregnancy	Anorectal malformations	1.81 (1.23–2.65)	0.220	30.0%	5
Ortiz-Neira et al. <sup>7</sup>	2012	Maternal pre-gestational diabetes	Anorectal malformations	4.51 (2.55–7.96)	0.250	27.4%	4
		Breech delivery	DDH	3.75 (2.25–6.24)	< 0.001	NA	15
		Baby's gender (girl)	DDH	2.54 (2.11–3.05)	< 0.001	NA	24
		Left side of hip	DDH	1.54 (1.25–1.90)	< 0.001	NA	10
de Hundt et al. <sup>6</sup>	2012	Family history of DDH	DDH	1.39 (1.23–1.57)	0.541	NA	4
		First born	DDH	1.44 (1.12–1.86)	< 0.001	NA	5
		Breech delivery	DDH	5.74 (4.44–7.42)	< 0.001	89%	22
de Hundt et al. <sup>6</sup>	2012	Baby's gender (girl)	DDH	3.75 (3.03–4.64)	< 0.001	62%	14
		Family history of DDH	DDH	4.77 (2.79–8.15)	< 0.001	88%	16

OR = odds ratio, RR = risk ratio, CI = confidence interval, ART = artificial reproductive technologies, CHD = congenital heart defect, DDH = developmental dysplasia of the hip, NA = not applicable, GDM = gestational diabetes mellitus, PGDM = pre-gestational diabetes mellitus.



**Fig. 2.** The range of ORs or RRs in the association between non-genetic risk factor and congenital anomalies (significantly association only). OR = odds ratio, RR = risk ratio, SES = socioeconomic status.

and congenital anomalies. The study showed a positive association of toxic chemicals, such as solvents, pesticides, and heavy metals, with congenital anomalies, based on 5 case-control studies and 1 prospective cohort study (pooled OR, 1.31; 95% CI, 1.06–1.63).<sup>21</sup>

### 3) Parental substance use

#### ① Parental smoking

We identified 7 systematic reviews and meta-analyses reporting an association between parental smoking exposure and congenital anomalies. Results from 2 studies suggested a positive association between parental smoking and the risk of any type of congenital anomalies. Salmasi et al.<sup>22</sup> found that the overall estimate of the RR for congenital anomalies by fetal exposure to maternal smoking during pregnancy was 1.18, based on 12 studies (95% CI, 1.04–1.34). Zheng et al.<sup>12</sup> found that fetal exposure to secondhand smoking was associated with an increased risk of congenital malformations in a meta-analysis of 33 case-control studies (pooled OR, 1.92; 95% CI, 1.61–2.30). The results of 4 of the remaining 5 meta-analyses included in our umbrella review suggested a positive association between parental smoking and the risk of CHD.<sup>11,12,23–25</sup> Zheng et al.<sup>12</sup> showed a significant association between fetal exposure to secondhand smoking and various types of congenital anomalies, such as oral clefts, based on 18 case-control studies (pooled OR, 1.87; 95% CI, 1.47–2.39), and digestive system defects (pooled OR, 1.17; 95% CI, 1.05–1.32) in 2 case-control studies. Similarly, Nicoletti et al.<sup>12</sup> showed that prenatal exposure to secondhand smoke resulted in a significantly increased risk of congenital anomalies, such as nervous system defects (pooled OR, 1.74; 95% CI, 1.61–2.30) in 6 case-control studies, musculoskeletal system defects (pooled OR, 1.27; 95% CI, 1.16–1.39) in 48 case-control studies, and eye, ear, face, and neck defects (pooled OR, 1.28; 95% CI, 1.19–1.37) in 53 case-control studies. Yu et al.<sup>26</sup> reported that maternal smoking was positively associated with cryptorchidism, based on 5 cohort studies and 15 case-control studies (pooled OR, 1.18; 95% CI, 1.12–1.24).

#### ② Maternal alcohol consumption

We identified 3 meta-analyses reporting associations between maternal alcohol consumption and congenital anomalies. Sun et al.<sup>27</sup> conducted meta-analyses of CHD and maternal alcohol consumption before and during pregnancy, using 23 case-control or cohort studies, and found no significant association between maternal alcohol consumption and congenital anomalies. Likewise, according to Wen et al.,<sup>28</sup> who included 23 case-control or cohort studies in their meta-analysis, no statistically significant association was found between alcohol consumption and CHD. Bell et al.<sup>29</sup> performed a meta-analysis (33 case-control or cohort studies) to evaluate the risk of orofacial clefts associated with maternal alcohol consumption, and found no statistically significant association.

### *Maternal history*

#### 1) Maternal infectious disease

We identified 3 meta-analyses analyzing the association between maternal infectious diseases and congenital anomalies, such as CHD, cleft lip/palate, neural tube defect, digestive tract defects, and hydrocephaly. Luteijn et al.<sup>15</sup> reported an association between maternal influenza and congenital heart diseases from a meta-analysis of 4 case-control and 6 cohort studies (pooled OR, 1.56; 95% CI, 1.13–2.14). A study by Ye et al.<sup>30</sup> supported this conclusion by showing the association between maternal viral infection, such as rubella virus and CHD, based on 7 case-control studies (pooled OR, 3.54; 95% CI, 1.75–7.15). The association between maternal infectious diseases and other congenital diseases was further reported; neural tube defects (pooled OR, 3.33; 95% CI, 2.05–5.40), orofacial clefts (pooled OR, 1.96; 95% CI, 1.33, 2.91), digestive system defects (pooled OR, 1.72; 95% CI, 1.09–2.68), and limb



reduction defects (pooled OR, 2.03; 95% CI, 1.27–3.27).<sup>15</sup> Shi et al.<sup>31</sup> showed an association between maternal fever in the first trimester and CHD in a meta-analysis of 7 case-control studies (pooled OR, 1.53; 95% CI, 1.36–2.73).

### 2) Pre-gestational and gestational diabetes mellitus

We identified 4 systematic reviews and meta-analyses on pre-gestational and gestational diabetes mellitus (PGDM and GDM) as risk factors for congenital anomalies. Balsells et al.<sup>32</sup> and Zhao et al.<sup>33</sup> showed associations between maternal diabetes mellitus and congenital anomalies. They divided maternal diabetes into PGDM and GDM and found that GDM, based on 9 cohort studies (pooled RR, 2.66; 95% CI, 2.04–3.47), and PGDM, based on 15 cohort studies (pooled RR, 1.16; 95% CI, 1.07–1.25), were significantly associated with congenital anomalies. Zhao et al.<sup>33</sup> also found significantly positive associations between GDM and congenital malformation, based on 17 cohort studies (pooled RR, 1.18; 95% CI, 1.11–1.26), and PGDM and congenital malformation, based on 13 cohort studies (pooled RR, 2.44; 95% CI, 1.92–3.10). Simeone et al.<sup>34</sup> found a significantly positive association of PGDM and GDM with CHD (pooled OR, 3.8; 95% CI, 3.0–4.9). In addition, Hoang et al.<sup>35</sup> reported an association between maternal diabetes and any type of CHD (pooled RR, 3.59; 95% CI, 3.03–4.50).

### 3) Maternal obesity

We identified 2 meta-analyses reporting the association between maternal obesity and CHD. Cai et al.<sup>36</sup> found that maternal body mass index (BMI) was associated with CHD. A dose–response effect of maternal overweight (pooled OR, 1.08; 95% CI, 1.02–1.15), moderate obesity (pooled OR, 1.15; 95% CI, 1.11–1.20), and severe obesity (pooled OR, 1.39; 95% CI, 1.31–1.47) on the occurrence of CHD was found. Similarly, Zhu et al.<sup>37</sup> demonstrated that CHD was associated with maternal BMI based on a meta-analysis of 17 case-control and cohort studies. Maternal overweight status (pooled OR, 1.06; 95% CI, 1.02–1.10) and obesity (pooled OR, 1.17; 95% CI, 1.14–1.20) significantly elevated the risk of CHD based on 13 case-control studies and 4 cohort studies. On the other hand, underweight maternal status was not associated with CHD.

### 4) Other maternal history

We identified 3 meta-analyses investigating the effect of other maternal histories and congenital anomalies. Davenport et al.<sup>38</sup> showed that prenatal exercise did not increase the OR for congenital anomalies, based on 10 randomized controlled trial studies (pooled OR, 1.23; 95% CI, 0.77–1.95). Yu et al.<sup>39</sup> found no significant association between maternal polycystic ovary syndrome and congenital anomalies, based on 6 cohort studies (pooled RR, 0.94; 95% CI, 0.36–2.42). Feng et al.<sup>40</sup> showed that there was no evidence of association between maternal parity and the risk of CHD based on 14 case-control studies and 2 cohort studies (pooled OR, 1.01; 95% CI, 0.97–1.06).

## *Maternal drug, supplement, and food consumption*

### 1) Maternal drug intake

We identified 5 meta-analyses reporting maternal drug intake associated with congenital anomalies. Results from 2 studies suggested a positive association between maternal drug intake and the risk of congenital anomalies. Based on 28 cohort studies, Tanoshima et al.<sup>13</sup> found an association between valproic acid intake and congenital malformation (pooled RR, 2.44; 95% CI, 2.13–2.80), CHD (pooled RR, 2.08; 95% CI, 1.55–2.79), cleft lip (pooled RR, 3.37; 95% CI, 2.09–5.42), neural tube defects (pooled RR, 7.41; 95% CI, 4.61–11.90), urogenital anomalies (pooled RR, 3.10; 95% CI, 2.28–4.22), and musculoskeletal

anomalies (pooled RR, 3.32; 95% CI, 2.26–4.88).<sup>13</sup> A positive association between exposure to fluoxetine and congenital malformation was shown in the study by Gao et al.,<sup>14</sup> based on 12 cohort studies (pooled RR, 1.18; 95% CI, 1.08–1.29). In addition, fluoxetine was positively associated with CHD (pooled RR, 1.36; 95% CI, 1.17–1.59). However, urogenital anomalies, musculoskeletal anomalies, and nervous system defects were not significantly associated with fluoxetine.<sup>14</sup> Yakoob et al.<sup>41</sup> found no significant association between maternal intake of  $\beta$ -blockers and congenital anomalies overall. However, maternal  $\beta$ -blockers intake was significantly associated with subtypes of congenital anomalies, such as CHD (pooled OR, 2.01; 95% CI, 1.18–3.42), cleft lip (pooled OR, 3.11; 95% CI, 1.79–5.43), and neural tube defects (pooled OR, 3.56; 95% CI, 1.19–10.67).<sup>41</sup> Grigoriadis et al.<sup>42</sup> showed that CHD was associated with maternal antidepressant use (pooled OR, 2.50; 95% CI, 1.32–4.73). However, Wang et al.<sup>43</sup> showed no significant association between selective serotonin reuptake inhibitors (SSRIs) and CHD.

### 2) Maternal supplementation

We identified 2 meta-analyses reporting the association between maternal supplementation and congenital anomalies. Feng et al.<sup>44</sup> reported a significantly negative association between folic acid supplementation and CHD, based on a meta-analysis based on 1 randomized controlled trial, 1 cohort study, and 16 case-control studies (pooled RR, 0.72; 95% CI, 0.63–0.82). Xu et al.<sup>45</sup> also reported a meta-analysis showing a negative association of maternal folic acid supplementation and CHD, which was based on 20 case-control studies (pooled OR, 0.60; 95% CI, 0.49–0.71).

### 3) Maternal diet

We identified 1 meta-analysis reporting the association between maternal diet and congenital anomalies. Li et al.<sup>46</sup> showed that no significant association was found between maternal coffee consumption and neural tube defects.

### *Artificial reproductive technologies*

We identified 6 meta-analyses reporting the association between artificial reproductive technologies (ART) and congenital anomalies. Two of these showed a positive relationship between ART and CHD. Wen et al.<sup>16</sup> reported a pooled OR of 1.64 (95% CI, 1.30–2.07), while Zheng et al.<sup>17</sup> reported a pooled RR of 1.22 (95% CI, 1.01–1.47). In addition, Wen et al.<sup>16</sup> reported associations of ART and nervous system (pooled OR, 2.01; 95% CI, 1.27–3.20); genitourinary system (pooled OR, 1.69; 95% CI, 1.33–2.15); digestive system (pooled OR, 1.66; 95% CI, 1.28–2.16); musculoskeletal system (pooled OR, 1.48; 95% CI, 1.03–2.02); and ear, face, and neck (pooled OR, 1.43; 95% CI, 1.01–2.05) anomalies. Zheng et al.<sup>17</sup> showed an association between ART and various congenital anomalies. Massaro et al.<sup>47</sup> and Qin et al.<sup>48</sup> published 2 papers about the positive association between ART and congenital malformation (pooled RR, 1.33; 95% CI, 1.24–1.43 and pooled RR, 1.37; 95% CI, 1.29–1.45). Several studies have shown a weak relationship between in vitro fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) and several types of congenital malformations.<sup>17</sup> Zheng et al.<sup>17</sup> reported a significant association between the risk of musculoskeletal, urogenital, and digestive system malformations based on 17 cohort studies (pooled RR, 1.16; 95% CI, 1.05–1.27); risk of nervous system malformations based on 16 cohort studies (pooled RR, 1.28; 95% CI, 1.07–1.53). However, Zheng et al.<sup>17</sup> did not find evidence of association between IVF/ICSI and risk of congenital anomalies such as cleft lip and/or palate; eye, ear, face, and neck; and respiratory system malformation. Massaro et al.<sup>47</sup> showed a positive association between congenital malformation and ICSI as opposed to IVF (pooled OR, 1.27; 95% CI, 1.02–

1.59).<sup>49</sup> Lacamara et al.<sup>50</sup> also showed a positive association between ICSI and congenital malformation, based on 5 cross-sectional studies and 15 cohort studies (pooled OR, 1.98; 95% CI, 1.86–2.11).

#### *Socioeconomic status*

We identified 2 meta-analyses reporting on socioeconomic status (SES) and congenital anomalies. Yu et al.<sup>51</sup> reported that SES indices, including maternal educational attainment, family income, and maternal occupation, were negatively associated with an increased risk of CHD (pooled RR [95% CIs] ranging from 1.05 [1.01–1.09] to 1.51 [1.02–2.24]). Deguen et al.<sup>52</sup> showed a significant association between low neighborhood SES and cleft palate, compared to high SES and found a significantly higher cleft lip rate in deprived neighborhoods, based on 6 case-control studies and 1 ecological study (pooled OR, 1.22; 95% CI, 1.10–1.36). However, no significant difference was observed according to SES in terms of neural tube defects and CHD.<sup>52</sup>

#### *Multiple factors*

We identified 6 meta-analyses reporting on the role of multiple factors and congenital anomalies. Results from 2 studies suggested a strong positive association between multiple factors and the risk of any congenital anomalies. Daliri et al.<sup>53</sup> found that congenital malformation was associated with multiple risk factors, such as the baby's sex (boys), preterm delivery, consanguineous marriage in the families of the children, paternal age (> 45 years), and paternal smoking. Oldereid et al.<sup>9</sup> found that the overall estimate of the OR for paternal age (> 45 years) and birth defects was 1.05 (95% CI, 1.02–1.07). They showed an association between exposure to multiple factors, such as paternal age > 45 years (pooled OR, 1.03; 95% CI, 0.99–1.06) and paternal smoking (pooled OR, 1.75; 95% CI, 1.25–2.44), and CHD. In addition, they found positive associations between paternal smoking and 2 types of congenital anomalies, i.e., orofacial defect (pooled OR, 1.51; 95% CI, 1.16–1.97) and brain tumors (pooled OR, 1.12; 95% CI, 1.03–1.22). However, they found no significant association between paternal age (> 45 years) and 3 types of congenital anomalies, such as spinal bifida, orofacial defects, and gastroschisis, compared to a younger paternal age. Peng et al.<sup>10</sup> found a positive association between multiple factors and CHD and reported that increased paternal age, paternal smoking, paternal drinking, and exposure to toxicants were associated with the occurrence of CHD. Zwink et al.<sup>8</sup> found positive associations between parental risk factors, such as maternal overweight, maternal obesity, maternal history of diabetes during pregnancy, and anorectal malformations. Results from 2 studies suggested a strong positive association between multiple factors and the risk of musculoskeletal anomalies. De Hundt et al.<sup>6</sup> and Ortiz-Neira et al.<sup>7</sup> reported the overall estimates of developmental dysplasia of the hip (DDH) upon exposure to multiple factors, such as breech delivery, baby's sex (girl), family history of DDH, mode of delivery, and first birth.

### **Genetic risk factors with evidence of association**

**Table 3** provides a summary of the association between genetic factors and congenital anomalies. We summarized the evidence for these associations in the included meta-analyses in **Table 4** and **Fig. 3**. More detailed results about the association between each factor and congenital anomaly are described below.

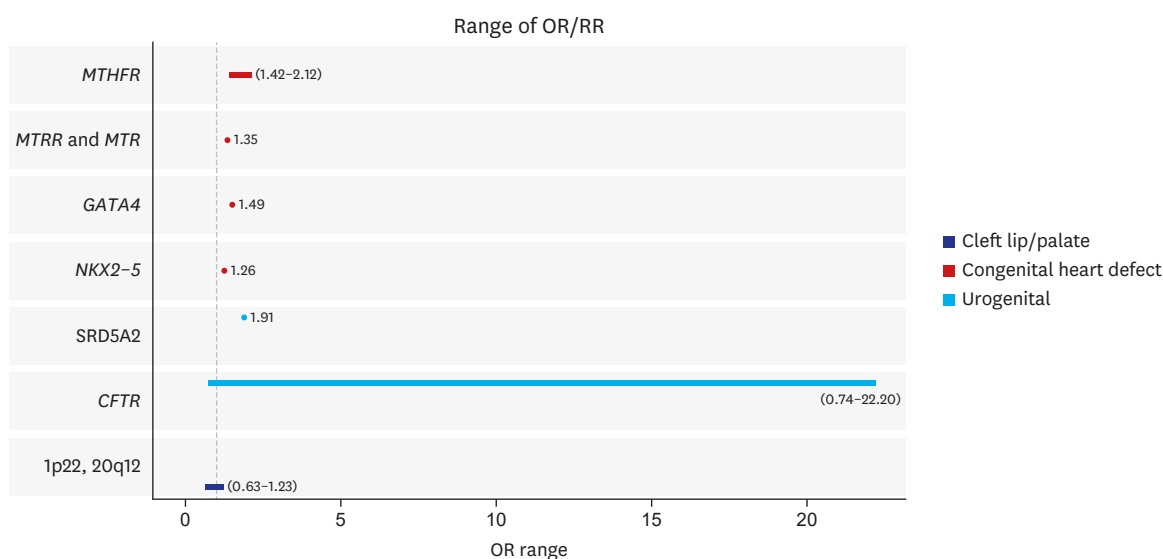
#### *MTHFR*

In *MTHFR*, which encodes methylenetetrahydrofolate reductase, 2 polymorphisms, C677T and A1298C, have been investigated. Eleven meta-analyses have investigated the association

**Table 3.** The summary of evidence on significance with association between genetic risk factors for congenital diseases

Risk factors	#	Any congenital malformations	Congenital heart defect	Cleft lip/palate	Neural tube defect	Digestive tract defect	Urogenital tract defect	Respiratory tract defect	Musculoskeletal defect	Eye, ear, fact and neck defect
<i>MTHFR</i>	12		C677T ↑ (7/11) <sup>a</sup> A1298C ↑ (5/5)	C677T → (1/1) A1298C → (1/1)						
<i>MTRR</i> and <i>MTR</i>	2		A66G ↑ (2/2) A2756G → (1/1)							
<i>GATA4</i>	1		354A ↑ (1/1)							
<i>NKX2-5</i>	1		63A ↑ (1/1)							
<i>SRD5A2</i>	1						CG allele ↑ (1/1)			
<i>CFTR</i>	1						5T ↑ (1/1) M470V ↓ (1/1) ΔF508 ↑ (1/1)			
1p22, 20q12	1			rs560426 A/G ↑ (1/1) C allele ↓ (1/1)						

*MTHFR* = methylene tetrahydrofolate reductase, *MTRR* = methionine synthase reductase gene, *MTR* = methionine synthase, *GATA4* = GATA binding protein 4, *NKX2-5* = NK2 homeobox 5, *SRD5A2* = steroid 5 alpha-reductase type 2 gene, *CFTR* = cystic fibrosis transmembrane conductance regulator, ↑ = positive pooled estimate association, → = no effect, ↓ = negative pooled estimate association, # = the numbers of meta-analysis studies.  
<sup>a</sup>(numbers of significant pooled estimate/total numbers of meta-analysis).



**Fig. 3.** The range of ORs or RRs in the association between non-environmental risk factor and congenital anomalies (significantly association only). OR = odds ratio, RR = risk ratio, *MTHFR* = methylene tetrahydrofolate reductase, *MTRR* = methionine synthase reductase gene, *MTR* = methionine synthase, *GATA4* = GATA binding protein 4, *NKX2-5* = NK2 homeobox 5, *SRD5A2* = steroid 5 alpha-reductase type 2 gene, *CFTR* = cystic fibrosis transmembrane conductance regulator.

of congenital diseases with C677T,<sup>54-64</sup> All meta-analyses analyzed the risk of CHD and only 1 study examined both congenital heart disease and orofacial cleft. Three studies showed no significant association between C677T and congenital heart disease, while 7 studies described a positive association with these conditions. In particular, the largest odds ratio was found in the study by Yuan et al.,<sup>56</sup> which reported a significant positive effect in a homozygote model based on 21 case-control studies. However, Chen et al.<sup>54</sup> reported 2 negative ORs, for the recessive model and for the homozygote model, each based on 6 case-control studies. Yang et al.<sup>57</sup> conducted a meta-analysis using 42 studies; both *MTHFR* C677T and A1298C polymorphisms were associated with the risk of CHD.<sup>56</sup> A TT model (pooled RR, 1.30; 95% CI, 1.14–1.48) and a TT+CT model (pooled RR, 1.09; 95% CI, 1.04–1.14) showed a significant positive relationship with congenital heart disease. Only 1 study found a statistically non-significant association between orofacial cleft and C677T in mothers,

**Table 4.** Results of effectiveness of congenital anomalies in the significant studies of meta-analysis included for genetic risk factors

Study	Study year	Exposure	Models	Outcome	Pooled OR or RR (95% CI)	$P_{\text{heterogeneity}}$	$I^2$ (%)	Based on # of studies
Zhang et al. <sup>55</sup>	2018	<i>MTHFR</i> (C677T)	-	CHD	1.35 (1.11–1.64)	< 0.001	77.0%	32
			<i>MTHFR</i> (A1298C)	-	CHD	1.42 (1.10–1.84)	0.070	43.0%
Yuan et al. <sup>56</sup>	2017	<i>MTHFR</i> (C677T)	TT+CT vs. CC	CHD	1.44 (1.11–1.87)	< 0.001	78.0%	21
			T vs. C	CHD	1.30 (1.06–1.59)	0.010	85.0%	21
			TT vs. TC	CHD	1.63 (1.30–2.05)	< 0.001	61.0%	21
			TT vs. CC	CHD	1.44 (1.11–1.87)	0.006	78.0%	21
			TT vs. TC+CC	CHD	1.78 (1.36–2.33)	< 0.001	75.0%	21
Yang et al. <sup>57</sup>	2018	<i>MTHFR</i> (C677T)	TT	CHD	1.30 (1.17–1.44)	< 0.001	51.6%	58
			TT+CT	CHD	1.07 (1.04–1.11)	< 0.001	58.5%	58
		<i>MTHFR</i> (A1298C)	CC	CHD	1.44 (1.07–1.95)	< 0.001	65.9%	21
			CC+AC	CHD	1.16 (1.02–1.32)	< 0.001	81.4%	21
Xuan et al. <sup>58</sup>	2014	<i>MTHFR</i> (C677T)	T vs. C	CHD	1.25 (1.09–1.43)	< 0.001	82.3%	35
			TT vs. CC	CHD	1.49 (1.14–1.94)	< 0.001	78.9%	35
			TT vs. CT	CHD	1.31 (1.10–1.57)	0.001	53.4%	35
		<i>MTHFR</i> (A1298C)	Dominant model	CHD	1.24 (1.05–1.46)	< 0.001	74.4%	35
			Recessive model	CHD	1.40 (1.14–1.72)	< 0.001	72.2%	35
			CC vs. AC	CHD	1.35 (1.02–1.79)	0.804	0.0%	35
Wang et al. <sup>59</sup>	2013	<i>MTHFR</i> (C677T)	T vs. C	CHD	1.27 (1.18–1.37)	< 0.001	NA	19
			TT vs. CC	CHD	1.61 (1.37–1.89)	< 0.001	NA	19
			TT+CT vs. CC	CHD	1.26 (1.12–1.41)	0.057	NA	19
			TT vs. TC+CC	CHD	1.57 (1.37–1.79)	< 0.001	NA	19
Yin et al. <sup>60</sup>	2012	<i>MTHFR</i> (C677T)	CT vs. CC	CHD	1.16 (1.00–1.34)	NA	NA	13
			TT vs. CC	CHD	1.55 (1.25–1.93)	NA	NA	13
			TT+CT vs. CC	CHD	1.23 (1.06–1.41)	0.030	46.0%	13
Li et al. <sup>63</sup>	2015	<i>MTHFR</i> (C677T)	CC+TC vs. TT	CHD	1.26 (1.06–1.51)	NA	NA	16
			TT vs. CC	CHD	1.54 (1.04–2.26)	< 0.001	63.0%	16
Chen et al. <sup>54</sup>	2013	<i>MTHFR</i> (C677T)	CC vs. TT	CHD	0.50 (0.36–0.71)	0.010	67.1%	6
			Dominant model	CHD	1.45 (1.09–1.91)	0.629	0.0%	6
			Recessive model	CHD	0.46 (0.35–0.61)	0.296	18.1%	6
Yu et al. <sup>67</sup>	2014b	<i>MTRR</i> (A66G)	G vs. A	CHD	1.16 (1.02–1.33)	0.004	66.1%	8
			GG vs. AA	CHD	1.33 (1.02–1.74)	0.035	53.7%	8
			GG vs. AA+AG	CHD	1.22 (1.00–1.48)	0.001	70.2%	8
Zhang et al. <sup>68</sup>	2017	<i>GATA4</i>	A>C mutation	CHD	1.49 (1.15–1.93)	0.003	38.0%	11
Wang et al. <sup>69</sup>	2013	<i>NKX2-5</i>	63A>G mutation	CHD	1.26 (1.02–1.56)	0.009	65.1%	7
Zhang et al. <sup>70</sup>	2017	<i>SRD5A2</i>	C vs. G	Hypospadias	1.91 (1.13–3.23)	< 0.001	93.0%	6
Xu et al. <sup>71</sup>	2014	<i>CFTR</i>	5T	CBAVD	8.35 (6.68–10.43)	0.021	44.0%	19
			M470V	CBAVD	0.74 (0.60–0.91)	0.036	61.0%	5
			ΔF508	CBAVD	22.20 (7.49–65.79)	0.735	0.0%	5
Haung et al. <sup>72</sup>	2012	1p22 & 20q12	A/G variant at 1p22	Cleft lip with or without cleft palate	1.23 (1.04–1.47)	0.040	NA	4

OR = odds ratio, RR = risk ratio, CI = confidence interval, *MTHFR* = methylene tetrahydrofolate reductase, *MTRR* = methionine synthase reductase gene, *MTR* = methionine synthase, *GATA4* = GATA binding protein 4, *NKX2-5* = NK2 homeobox 5, *SRD5A2* = steroid 5 alpha-reductase type 2 gene, *CFTR* = cystic fibrosis transmembrane conductance regulator, CHD = congenital heart defect, CBAVD = congenital bilateral absence of vas deferens.

based on 8 case-control studies.<sup>64</sup> Five studies have identified associations with the A1298C polymorphism.<sup>55-57,64,65</sup> All 5 studies considered congenital heart disease, while only Verkleij-Hagoort et al.<sup>64</sup> further analyzed orofacial cleft. Moreover, all 5 studies showed an increasing effect of A1298C on CHD. The largest OR was reported by Zhang et al.<sup>55</sup> based on 11 case-control studies (pooled OR, 1.42; 95% CI, 1.10–1.84). On the other hand, A1298C in mothers and children did not have a significant effect on orofacial cleft.<sup>64</sup> Additionally, Yang et al.<sup>57</sup> also reported A1298C effects on congenital heart disease using RR. They found that the CC (pooled RR, 1.44; 95% CI, 1.07–1.95) and CC+AC genotype groups (pooled RR, 1.16; 95% CI, 1.02–1.31) had a higher risk of CHD than did the other genotype group, based on 21 case-control studies.<sup>57</sup>

#### *MTRR and MTR*

A meta-analysis of 8 case-control studies examined the association between the methionine synthase reductase gene (*MTRR*) and the risk of CHD and showed that the G allele of the A66G polymorphism had a pooled OR of 1.35 (95% CI, 1.14–1.59) for CHD.<sup>66</sup> However, there was no risk of CHD associated with the A2756G in this gene, based on 4 case-control studies. Similarly, a meta-analysis by Yu et al.,<sup>67</sup> which included 8 case-control studies, also showed that the *MTRR* A66G polymorphism was associated with a higher risk of CHD, based on 8 case-control studies (G>A: pooled OR, 1.16; 95% CI, 1.01–1.33).

#### *GATA4*

Zhang et al.<sup>68</sup> reported a meta-analysis of 11 case-control studies, and found that *GATA* 354A>C mutation was associated with an increased risk of CHD (pooled OR, 1.49; 95% CI, 1.15–1.93). However, neither *GATA4* 99G>T nor *GATA* 487C>T mutations were related to the incidence of CHD.

#### *NKX2-5*

Seven studies were included in a meta-analysis of the association between *NKX2-5* 63A>G and congenital heart disease in the Chinese population, which showed a pooled OR of 1.26 (95% CI, 1.02–1.56).<sup>69</sup> Four studies included in meta-analysis showed no evidence for association between *NKX2-5* 60G>C and CHD.

#### *SRD5A2*

Six case-control studies were included in a meta-analysis that showed an increased risk of hypospadias associated with the V89L polymorphism of the steroid 5-alpha-reductase type 2 gene (*SRD5A2*), for the G vs C allele (pooled OR, 1.91; 95% CI, 1.13–3.23), GC vs GG genotype (pooled OR, 2.36; 95% CI, 1.35–4.13), GC+CC vs. GG genotypes (pooled OR, 2.46; 95% CI, 1.27–4.72), and CC vs. GC+GG genotypes (pooled OR, 1.91; 95% CI, 1.00–3.66).<sup>70</sup>

#### *CFTR*

Twenty-nine case-control studies were included in the meta-analysis investigating the association between congenital bilateral absence of the vas deferens (CBAVD) and polymorphism in *CFTR*.<sup>71</sup> Cystic fibrosis patients often carry variations in *CFTR*, which encodes a glycosylated transmembrane protein that is widely expressed in epithelial cells of exocrine tissues, including sweat glands, lungs, and vas deferens. Three variations showed significant associations with CBAVD: 5T (pooled OR, 8.35; 95% CI, 6.68–10.43), M470V (pooled OR, 0.74; 95% CI, 0.60–0.91), and ΔF508 (pooled OR, 22.20; 95% CI, 7.49–65.79).

#### *1p22 and 20q12 anomalies*

The C allele of a single nucleotide polymorphism at 20q12 (rs13041247), as opposed to the T allele, was associated with a reduced risk of cleft lip, with or without cleft palate, in a meta-analysis of 4 case-control studies (pooled OR, 0.63; 95% CI, 0.53–0.59).<sup>72</sup> Moreover, the rs560426 A/G variant at 1p22 was associated with an elevated risk of cleft (pooled OR, 1.23; 95% CI, 1.04–1.47).

## DISCUSSION

Overall, we reviewed 66 systematic reviews and meta-analyses that investigated the association between non-genetic or genetic risk factors and congenital anomalies. Seven



categories of non-genetic and genetic risk factors were identified that showed association with congenital anomalies. We categorized congenital anomalies into CHD, cleft lip and/or palate, neural tube defects, defects in digestive, urogenital, and respiratory tracts, musculoskeletal anomalies, and eye, ear, face, and neck anomalies. Overall, 269 associations for non-genetic risk factors and 128 associations for genetic risk factors were found.

The risk of congenital anomalies was elevated by non-genetic risk factors, such as maternal exposure to environmental exposures (air pollution and toxic chemicals), parental smoking, maternal history (infectious diseases during pregnancy, PGDM and GDM, maternal obesity), maternal drug intake, pregnancy through ART, and socioeconomic factors (low SES). The association of maternal alcohol or coffee consumption with congenital anomalies was not significant, and maternal folic acid supplementation had a preventive effect on CHD. Genes or genetic loci associated with congenital anomalies included *MTHFR*, *MTRR* and *MTR*, *GATA4*, *NKX2-5*, *SRD5A2*, *CFTR*, and 1p22 and 20q12.

Exposure to NO<sub>2</sub> increased the risk of CHD, while other pollutants showed no significant effect on CHD; however, more studies should be conducted because of the limitations of previous meta-analyses, such as small sample sizes. Air pollution could promote oxidative stress and increase production of free radicals, which may have developmental effects on the fetus.<sup>73</sup> Oxidative stress due to air pollution has been reported to be associated with adverse birth outcomes.<sup>74,75</sup>

The association between maternal alcohol consumption during pregnancy and congenital malformation was not significant. Alcohol consumption in pregnant women is socially considered as taboo; thus, women may underreport alcohol consumption status during surveys. Although maternal alcohol consumption was not associated with congenital anomalies in this study, alcohol is known to be a teratogen.<sup>76</sup> Even a small amount of alcohol consumption at any point of pregnancy can have undesirable effects on the fetus, including small size for gestational age and preterm delivery.<sup>77</sup> Heavy alcohol consumption during pregnancy can lead to fetal alcohol spectrum disorder (FASD) (an umbrella term including fetal alcohol syndrome (FAS), partial FAS, and alcohol-related neurodevelopmental disorder), manifested with intellectual disability and behavioral and developmental problems that may not necessarily be presented as congenital anomalies. Nonetheless, FASD most often co-occurs with congenital malformations in the heart, kidneys, or bones.<sup>78</sup>

Influenza could mediate teratogenic effects via several pathways; however, confounding due to the linkage between influenza and treatments with antipyretics or antivirals could be present. However, from the perspective of vaccination, whether the teratogenic effects are due to influenza or the drugs is not important, as vaccination would prevent both events.<sup>15</sup> The pathways via which influenza leads to congenital anomalies are first, hyperthermia, and second, direct passage of virus through the placenta. Hyperthermia is reported to be associated with neural tube defects,<sup>79</sup> and influenza virus can mediate teratogenic effects by placental transmission.<sup>80</sup>

CHD is associated with both maternal PGDM and GDM, but studies have shown that the associations with PGDM are stronger than those with GDM; this could be because GDM onset typically occurs after cardiac development.<sup>35</sup> Previous studies have suggested that maternal hyperglycemia could disturb energy production during embryogenesis<sup>81</sup> as well as the expression of *PAX-3*, which is a transcription factor for cardiac neural crest cells.<sup>82,83</sup>

However, non-diabetic obese pregnant women also showed a greater risk CHD in the offspring.<sup>36</sup> An explanation for this association is that obese pregnant women could be less responsive to folic acid supplementation at the standard dose than pregnant women who have a BMI in the normal range.<sup>84</sup>

A meta-analysis of 13 case-control and cohort studies showed that the use of beta-blockers during the first trimester increased the risk of CHD, cleft lip and palate, and neural tube defects.<sup>38</sup> Beta-blockers are the most commonly prescribed antihypertensive drugs during the first trimester, and about 1 in 200 pregnant women are exposed to beta-blockers.<sup>85</sup> Caton et al.<sup>85</sup> pointed out that hypertension, which is an indication for beta-blockers, might be associated with congenital anomalies and act as a confounder in these associations. The estimated risk of beta-blockers in the study by Yakoob et al.<sup>41</sup> was greater than the risk of hypertension itself, as shown in another study.<sup>86</sup> However, none of the studies included in the meta-analysis compared the risk of malformation associated with beta-blockers in comparison to other types of antihypertensive medications.

The intake of fluoxetine increases the risk of congenital malformation and CHD. Fluoxetine is among the most commonly prescribed selective serotonin reuptake inhibitors (SSRIs) in the first trimester. While most SSRIs have a half-life of about 1 day, fluoxetine has a longer half-life (1–4 days) and its metabolite norfluoxetine has a half-life of approximately 7–15 days.<sup>87</sup> Fluoxetine is known to cross the human placenta; a high concentration of fluoxetine and its metabolite are detected in umbilical cord blood.<sup>88</sup> Although the mechanisms of fluoxetine-induced congenital anomalies are unknown, serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter for signaling during embryogenesis and cardiac morphogenesis.<sup>89</sup> A mouse study showed that 5-HT has a direct effect on myocardial cell development<sup>90</sup> and 5-HT affects cardiac morphogenesis during the formation of the endocardial cushion.<sup>91</sup> Thus, it is suggested that healthcare providers and patients carefully consider the risk and benefit of fluoxetine therapy for depression during the first trimester.<sup>14</sup>

Two meta-analyses confirmed that folic acid supplementation intake reduces the risk of CHD, and most individual studies showed consistent results. According to a meta-analysis, the protective effect of folic acid supplementation against CHD is significant in China and Europe, but not in the United States, suggesting that it may be related to genetic background and environmental factors. The exact effects of folic acid supplements on heart formation are unknown, and more evidence is needed to support these findings.

Children born from pregnancies established by IVF and/or ICSI showed an increased risk of congenital defects. Excess risk of birth defects in these children may be due to the underlying infertility of the parents rather than ART itself.<sup>92,93</sup> Specific defects after ICSI may be associated with paternal subfertility against a particular genetic background.<sup>92</sup> However, the procedure of ART itself may also increase the risk of adverse birth outcomes.<sup>94</sup> It has been suggested that the risk of congenital anomalies in children born to parents who received ART should be compared with children born to infertile parents who spontaneously gave birth without ART, to assess the effect of ART per se on congenital anomaly.<sup>95</sup> A previous study showed that parents who underwent ART generally had higher SES levels. These parents may have a stronger desire for a healthy pregnancy than those who conceived naturally, which may offset the risk of any adverse effects of the ART procedure.<sup>96</sup>

The 2 *MTHFR* polymorphisms, C677T and A1298C, are the most studied genetic factors associated with congenital anomalies, specifically CHD and orofacial cleft lips. *MTHFR* encodes the enzyme that catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a methyl donor to methionine.<sup>97</sup> Thus, it is a crucial enzyme that connects the folate pathway and homocysteine metabolism.<sup>98</sup> Mutation in *MTHFR* can cause inactivation or malfunctioning of the enzyme that breaks down the amino acid homocysteine and folate.<sup>55</sup> The OR for the C677T polymorphism was much larger than that for the A1298C polymorphism. The risk of CHD associated with homozygosity was higher than that associated with heterozygosity; for example, recessive (TT vs. TC+CC) models were reported to have a greater effect than dominant models (TT+TC vs. CC). These results are not consistent with the fact that homozygosity for C677T results in 25% higher homocysteine levels than the normal genotype, which indicates low folate status.<sup>99</sup>

*MTR* encodes methionine synthase, an enzyme that transfers a methyl group from 5-methyl-tetrahydrofolic acid (5mTHF) to homocysteine and thus produces methionine and tetrahydrofolic acid (THF) by remethylation. In the early stage of embryo development, this methylation is the only method by which homocysteine is reduced and methionine is produced, and thus a defect in this gene can lead to folic acid metabolic disorder.<sup>67</sup> *MTRR* encodes methionine synthase reductase, which plays an important role in inactivating methionine synthase. Therefore, defects in *MTRR* may disrupt the course of embryonic development by influencing the levels of homocysteine, 5mTHF, THF, and methionine.

*NKX2-5* and *GATA4* are cardiac transcription factors that regulate heart development.<sup>69</sup> *NKX2-5* has been shown to participate in heart development not only in animal models, such as zebrafish, frog, chicken, and mouse, but also in humans in previous family studies.<sup>69</sup> During the first trimester of gestation, male external genitalia are developed by regulation of androgens, such as testosterone and dihydrotestosterone. Since *SRD5A2* plays a role in androgen synthesis and metabolism, it may have an effect on the differentiation of genitalia in males.

This study had several limitations. First, it did not review individual articles that were not included in the systematic reviews and meta-analyses. Thus, certain categories of risk factors could have been missed. Second, the same article may have been included in several systematic reviews and meta-analyses. The availability of several meta-analyses on a topic does not necessarily mean that there are a greater number of individual articles published on the specific topic. Thus, caution is needed in interpreting the level of evidence based on the number of meta-analyses available. Third, the quality of the studies included in this systematic review was mostly low or very low according to the AMSTAR2 criteria. The main reason for the low quality was the lack of a reference list for the studies excluded from each meta-analysis. Although AMSTAR2 provides a valuable guideline for evaluating the quality of meta-analyses and for performing meta-analyses, it may be too strict to apply to the currently available literature because few studies satisfied the AMSTAR2 criteria for high quality meta-analyses. Fourth, it should be noted that case-control studies included in each meta-analysis could be subject to recall bias, which can be quite influential in studies of congenital anomalies, where mothers tend to have feelings of guilt.<sup>100</sup>

Despite these limitations, this study provided comprehensive information regarding both non-genetic and genetic risk factors of congenital anomalies, providing insight into which topic requires further studies. Suggestions for future studies include the following. First, it is

necessary to analyze the disease burden of each risk factor of congenital anomalies by taking effect sizes and prevalence into account. There is a lack of systematic reviews that suggest health policy priorities for preventing congenital anomalies by considering the burden of disease of each individual risk factor. Second, there are relatively few systematic reviews and meta-analyses of studies on environmental and occupational risk factors for congenital anomalies. Not only toxic chemicals, but also occupational risk factors could affect birth outcomes. For example, studies on the effects of night shift work on congenital anomalies are very rare. This study provided a very comprehensive umbrella review of risk factors of congenital anomalies. Modifiable risk factors, such as maternal behaviors, medication, supplementation, and possibly environmental hazards, should be carefully considered to achieve better birth outcomes. Allocation of social resources is warranted to support such modifications, to protect mothers and children from congenital anomalies that could permanently affect development and function in children.

In conclusion, from umbrella review of systematic review and meta-analysis studies, we identified 66 studies reporting non-genetic and genetic risk factors of congenital anomalies, such as air pollution, toxic chemicals, parental smoking, alcohol consumption, maternal infectious diseases, pregestational diabetes, maternal obesity, maternal drug intake, maternal supplementation, maternal diet, and several genetic factors polymorphisms in *MTHFR*, *MTRR* and *MTR*, *GATA4*, *NKX2-5*, *SRD5A2*, *CFTR*, 1p22, and 20q12. Further studies are warranted to identify more diverse risk factors of congenital anomalies and elucidate the mechanisms of congenital anomalies.

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## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

The AMSTAR2 checklist based on the studies involved

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### Supplementary Table 2

Characteristics of included meta-analyses

[Click here to view](#)

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