Investigating the Role of Maternal Age in the Occurrence of Non-Chromosomal Congenital Anomalies

Ph.D. Thesis

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"Research is to see what everybody else has seen, and to think what nobody else has thought"

Albert Szent-Györgyi

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1 LIST OF ABBREVATIONS

AMA	advanced maternal age
CA	chromosomal anomaly
CHD	congenital heart defect
CI	confidence interval
EUROCAT	European Concerted Action on Congenital Anomalies and Twins (a network of population-based congenital anomaly registries across Europe)
GDM	gestational diabetes mellitus
GRADE	Grading of Recommendations Assessment, Development and Evaluation (a tool for grading the quality of evidence)
HCCSCA	Hungarian Case-Control Surveillance of Congenital Abnormalities
HCAR	Hungarian Congenital Abnormality Registry
ICD	International Classification of Diseases
IVF	*in vitro* fertilization
KSH	*Központi Statisztikai Hivatal* (Hungarian Central Statistical Office)
MEDLINE	Medical Literature Analysis and Retrieval System Online (the bibliographic database of the National Library of Medicine)
NCA	non-chromosomal anomaly
NTD	neural tube defects
OR	odds ratio
PECO	population, exposure, comparator, outcome (a framework for formulating scientific questions)
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International prospective register of systematic reviews
QUIPS	Quality in Prognostic Studies (a tool to assess the study quality and risk of bias)
ROBVIS	a risk of bias visualization tool for systematic reviews
RR	risk ratio
SD	standard deviation

STROBE strengthening the reporting of observational studies in epidemiology (a checklist of items that should be included in observational research articles)

WHO World Health Organization

2 STUDENT PROFILE

2.1 Vision and mission statement, specific goals

My vision is a world where women receive state-of-the-art prenatal care, ensuring the best possible outcomes for the next generation. My mission is to promote the adoption of innovative screening and monitoring techniques in prenatal care. My specific goal is to elevate screening methods for non-chromosomal birth defects to the highest possible standard, enhancing early detection and intervention worldwide.

2.2 Scientometrics

Number of all publications:	5
Cumulative IF:	21.391
Av IF/publication:	4.278
Ranking (SCImago):	D1:2, Q1:2, Q3:1
Number of publications related to the subject of the thesis:	2
Cumulative IF:	13.4
Av IF/publication:	6.7
Ranking (Sci Mago):	D1:2, Q1:, Q2: -
Number of citations on Google Scholar:	31
Number of citations on MTMT (independent):	11
H-index:	3

The detailed bibliography of the student can be found on pages 96-97.

2.3 Future plans

I plan to expand my research in prenatal care by utilizing my extensive knowledge in this area. A thorough understanding of healthcare necessitates combining practical experience with academic knowledge. I am committed to actively engaging in prenatal patient care to improve my skills and expand my perspective. Through daily contact with pregnant women, my aim is to gain a deep knowledge of their distinct demands, challenges, and worries.

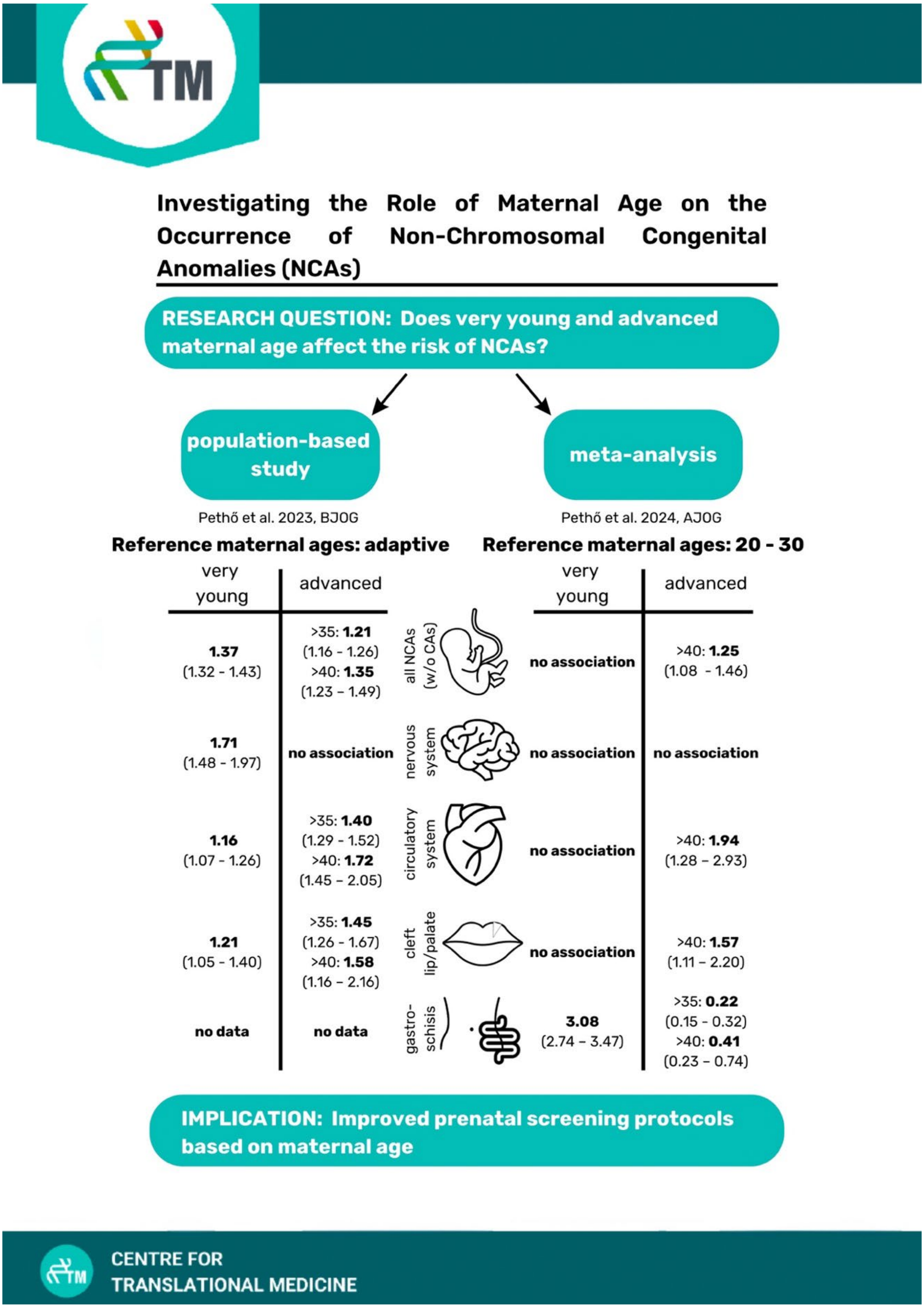
With the cohesion of my research and clinical experiences in the field of prenatal medicine, my goal is to build a professional path that raises prenatal care to the highest possible level, thereby improving the well-being of mothers and their babies.

3 SUMMARY OF THE THESIS

There is a well known association between maternal age at birth and non-chromosomal congenital anomalies (NCAs), but the exact details are still under active research. Our aim was to identify maternal age groups with elevated risk of NCAs and to analyze the age-dependent risk variation of different anomalies. To improve our comprehension and practical use, we conducted a thorough investigation utilizing a database that encompasses the entire population of Hungary over almost three decades, as well as a meta-analysis of existing population-based studies worldwide. We found strong evidence that the risk of occurrence of NCAs – excluding cases with concomitant chromosomal anomalies (CAs) – is higher for mothers over 40: RR = 1.25 (CI: 1.08–1.46) in the meta-analysis and 1.35 (CI: 1.23–1.49) in the population based study. The elevated risk in case of very young mothers was also evidenced in the population based study, however, the risk increase for the same age group in the meta-analysis did not turn out to be significant. The year-by-year data available in the population based data enabled a more precise delineation of the lowest risk maternal age range: mothers between 23 and 32 years age had the lowest chance for NCAs. When investigating specific NCA categories, concordance between the two studies was strongest for the circulatory system and cleft lip and palate, with both showing elevated risk in the 40+ age group.

The findings underscore the importance of revising current prenatal screening protocols to ensure they also account for maternal age. The results suggest that it may be beneficial to use maternal age as a screening criterion for both fetal echocardiography and neurosonography. In addition, public health policy should incorporate educational campaigns targeting high-risk age groups to emphasize the significance of prenatal care and screening. Customized counseling taking into account risks specific to different age groups can improve the effectiveness of prenatal care and assist pregnant women in making well-informed decisions.

4 GRAPHICAL ABSTRACT



5 INTRODUCTION

5.1 Overview of the topic

5.1.1 What is the topic?

The focus of my research is to investigate the impact of maternal age on the occurrence of non-chromosomal congenital anomalies (NCAs) in order to identify specific age-related risk categories and improve prenatal screening protocols.

5.1.2 What is the problem to solve?

The issue lies in the limited evidence regarding the exact relationship between maternal age and the occurrence of NCAs. This lack of clarity makes it difficult to develop accurate prenatal screening protocols and public health strategies.

5.1.3 What is the importance of the topic?

The importance of this topic cannot be overstated, as congenital anomalies are frequent with 3-5% worldwide(1, 2) and play a significant role in infant mortality (6% of infant death worldwide)(3) and morbidity rates (approximately 20%)(4, 5) as well as result in substantial healthcare expenses(6). By understanding the influence of maternal age on NCAs, we can improve prenatal screening protocols and public health strategies, consequently reducing the occurrence and impact of these anomalies on families and the healthcare system.

5.1.4 What would be the impact of our research results?

The outcomes of our research will have significant impact by enhancing prenatal screening protocols and public health strategies. Healthcare providers can enhance the effectiveness of prenatal care by identifying maternal age groups that are at a higher risk for NCAs. Public health campaigns can be customized to provide education and assistance to age groups that are at a higher risk, ultimately decreasing the occurrence and effect of NCAs. Furthermore, our findings will provide direction for future investigations and policy choices focused on improving maternal and child health outcomes.

5.2 Maternal age – a critical factor in pregnancy outcome

Over the previous few decades, women's typical delivery age has increased in developed countries.(7) Postponing childbearing is a complex phenomenon caused by social and cultural changes.(8, 9) A growing number of couples are conceiving their first child while the mother is between the ages of 30 and 35.(10) According to the literature, advanced maternal age (AMA) begins at age 35 (≥ 35 years old) but this is by far not a universal definition, and a distinct age limit could be established for each adverse perinatal outcome. The proportion of births to mothers over the age of 35 has doubled since 1990, accounting for approximately 20% of births in 2021; birth rates among mothers in their forties have also steadily increased during this time.(11) In addition to social trends, innovations in assisted reproduction techniques are increasingly allowing women to have children after the age of 35 or even 40.(12)

Many studies have linked postponement of childbearing to a variety of pregnancy and fetal complications (13-15) as well as recommendations for managing these high-risk pregnancies.(16) AMA has been linked to an increased risk of gestational diabetes mellitus(17-19), hypertensive disorders of pregnancy(20, 21), preterm delivery(22, 23), fetal growth restriction(24, 25), stillbirth(26, 27), and cesarean delivery(28, 29), among other complications. The results of the large epidemiological studies were also confirmed by studies with animal models, which make it possible to explore the mechanisms behind poor pregnancy outcomes and to develop therapeutic methods.(30) AMA, even in older pregnant women without additional health conditions like gestational hypertension or diabetes, is still associated with poorer obstetric and perinatal outcomes. This suggests that advanced maternal age alone is a significant and independent risk factor.(31) In addition to high-risk pregnancies and perinatal outcomes, AMA plays an important role in congenital anomalies. This association is strong and well known in relation to chromosomal anomalies, however, in case of NCAs, it is less coherently reported in the literature.

Very young maternal age (< 20 year old) is also a major risk factor for adverse pregnancy outcomes (higher rates of eclampsia, low birth weight and preterm delivery to mention the most important).(32, 33) The global adolescent birth rate has decreased by more than 30 percent between 2000 and 2022, going from 65 to 42 births per 1,000 adolescent girls aged 15-19.(34) This trend is the result of education, better access to contraception and social changes.(35) Very young maternal age does not seem to be an independent risk factor for most outcomes. Rather, the increased risk appears to be an consequence of the circumstances associated with becoming pregnant without planning as an adolescent.(36) Substance abuse, higher rates of sexually transmitted infections, poorer nutritional conditions and low socioeconomic status may explain poorer pregnancy outcomes.(37-39)

5.3 Congenital anomalies – the leading cause of neonatal mortality and morbidity

Congenital anomalies are structural or functional abnormalities that develop during intrauterine life and can be detected intrauterinely, at birth, or, occasionally, during infancy.(40) Congenital anomalies affect three to five percent of all births worldwide (1, 2), which is a main cause of infant mortality(41) and morbidity, responsible for the loss of 25.3–38.8 million disability-adjusted life years globally.(42) According to the EUROCAT survey, the average relative frequency of birth defects in Europe was 23.9 per thousand births in 2010.(43) The 2010 Global Burden of Disease study estimates that congenital anomalies account for 6% of infant deaths worldwide(3), while other studies show that approximately 20% of neonatal and infant mortality is associated with congenital anomalies.(4, 44)

The overall occurrence of significant birth defects has remained consistent over time. However, both increasing (e.g. atrioventricular septal defect, tetralogy of Fallot, omphalocele) and decreasing (e.g. anencephaly, common truncus, transposition of the great arteries, and cleft lip with and without cleft palate) trends were observed for certain conditions.(5)

Congenital anomalies impose a significant burden on society as a whole, particularly on affected families and the health and social care systems. Furthermore, congnital anomaly-related hospitalizations are extraordinarily costly, accounting for 4.1% of all hospitalizations and 7.7% of entire hospital expenses (among patients under 65 years), and with an estimated annual expense of $22.2 billion in the United States in 2019.(6) These facts emphasize the global significance of congenital anomalies in research, prevention and screening. It is essential to prioritize appropriate intervention as a matter of public health. .Several known maternal lifestyle risk factors and chronic illnesses are clearly associated with the occurrence of congenital anomalies. For example, a meta-analysis found that maternal tobacco use during pregnancy increases the risk of congenital anomalies (OR = 1.18; CI: 1.03–1.34).(45) The risk-increasing effect of maternal diabetes is also considered in genetic screening. A comprehensive study found that pre-gestational diabetes has a significant effect (RR = 2.66; CI: 2.04–3.47).(46)

5.4 Potential association between NCAs and maternal age

Among congenital anomalies, chromosomal anomalies (CAs) are clearly associated with advanced maternal age (47-50), a long-standing fact that has resulted in the current professional screening protocols.(51, 52) However, there is no consensus with regard to the degree of association between NCAs and maternal age.

While the role of maternal age in the development of NCAs is generally accepted, the literature is inconsistent regarding the risk of NCAs in specific age groups. This is a major issue not only because of the trend towards delayed childbearing but also because of the risks of adolescent pregnancies. Some studies show a risk-increasing effect only for the very young(53) (generally defined as under 20 years old) or only for the advanced-aged(generally defined 35 years old or older) population(54), while others for both age categories.(55, 56)

When examining the effect of maternal age on NCAs, a comprehensive analysis is justified not only by the inconsistent data. Studies are very heterogeneous in terms of age categories and the classification of NCAs: On the one hand, there is no universally accepted reference age category, on the other hand, anomalies are classified in various ways that may or may not correspond to International Classification of Diseases (ICD) categories.

The underlying maternal age releted factors contributing to the increased risk of NCAs are known, even though the precise biological links remain undetermined. The susceptibility of the very young age group can be largely attributed to the teratogenic effects resulting from the lifestyle and living conditions of mothers who became pregnant at a very young age, as well as their limited adoption of primary prevention measures. In detail, these factors may encompass smoking, drug and alcohol abuse (the combined prvelalence of substance is 41.0%), low socioeconomic status, low level of education, and a lack of sufficient folic acid supplementation which is common in case of intentional childbearing.(57) Insufficient consumption of folic acid is unequivocally linked to an elevated susceptibility to neural tube defects.(58) To what extent AMA is responsible directly and indirectly (i.e. via age-releated chronic diseases) for the increased risk of NCAs is not yet established. The necessary basic research – e.g. that would clarify the role of age-related decline of oocyte quality and deteriorated repair processes in increased risk of NCAs – is still missing.

6 OBJECTIVES

6.1 Study I. – Investigating the Impact of Maternal Age on the Development of Non-Chromosomal Congenital Anomalies in the Hungarian Population between 1980 and 2009

The aim of this study was to use our distinct database to determine the specific 10-year period of maternal age in Hungary that has the lowest risk for NCAs. Additionally, we also wanted to compare other maternal ages to this specific period in order to offer an original perspective on the relationship between maternal age and NCAs. The reason for this approach was to enhance our comprehension of age-related vulnerabilities and provide insights for modifying prenatal screening protocols according to the maternal age.

6.2 Study II. – Investigating the Impact of Maternal Age on the Development of Non-Chromosomal Congenital Anomalies Worldwide

The objective of this study was to perform a comprehensive meta-analysis investigating the occurrence of NCAs based on maternal age. Despite thorough investigation on this subject, the full scope and characteristics of the association between maternal age and NCAs are still uncertain. The existing literature lacks a unanimous agreement on the specific particulars of this relationship. The objective of this study was to elucidate these factors and offer valuable perspectives for formulating age-specific guidelines for prenatal screening and public health strategies.

7 METHODS

7.1 Study I.

7.1.1 Study design

We conducted a population-based study in Hungary over a span of nearly 30 years to examine the occurrence of NCAs in relation to the age of the mothers. This study collected cases from the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA), and the total number of live births during the study period from the Hungarian Central Statistical Office (KSH). Instead of comparing arbitrary age categories, we used the restricted cubic spline model to identify high- and low-risk maternal age groups.(59) We present our population-based study in accordance with the guidelines outlined in the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guideline.(60)

7.1.2 Setting

Our study examines the HCCSCA, which was established in 1980 and ended in 2009.(61) The data collection process underwent modifications in 1997, specifically impacting the collection of matched controls that were not utilized in the present study. Consequently, this led to slight adjustments in the structure of the HCCSCA. The data collected from 1980 to 2009 through the HCCSCA was consolidated into a single, validated database.(62) In 2002, the legal basis of data privacy was called into question and data collection was halted until 2005 following the concerns raised by a mother.

Physicians in Hungary have been required to report patients as cases with congenital anomalies to the Hungarian Congenital Abnormality Registry (HCAR) since 1962. This reporting obligation applies from birth until the end of the first postnatal year. The HCAR, established in 1962, was the inaugural international registry of congenital anomalies with a national focus.(63) Starting from 1984, the prenatal diagnostic centers were required to inform the HCAR about any prenatally diagnosed fetuses with or without elective termination of pregnancy, if they were found to have malformations. The HCCSCA has registered cases from the HCAR since 1980.

7.1.3 Ethics and patient consent

The data analysis was conducted with the approval of the Scientific and Research Ethics Committee of the Medical Research Council, Hungary (BMEÜ/920-3/2022/EKU). Our study did not report any registry data that could be identified. There is no legal requirement for obtaining informed consent in order to register a baby with a congenital anomaly.

7.1.4 Participants

Cases with CAs in the HCAR were enrolled to the HCCSCA if they met all the following selection criteria: (1) reported to the HCAR within 3 months after birth or elective termination of pregnancy, (2) none of the three mild congenital anomalies (hip dislocation, congenital inguinal hernia, and large haemangioma) were present alone, and (3) did not have congenital anomaly-syndromes caused by gene mutations or chromosomal anomalies with preconceptional origin. In our analysis, we excluded cases with incomplete data or the co-presence of chromosomal anomalies **(Figure 1)**. The main task of the HCCSCA has been the detection of teratogenic/fetotoxic agents and other environmental effects during pregnancy resulting in the development of birth defects. The case group contains live births, stillbirths, and elective terminations of pregnancies following prenatal malformation diagnosis. For the number of controls, the total number of live births by maternal age in Hungary during the study period was obtained from the KSH.

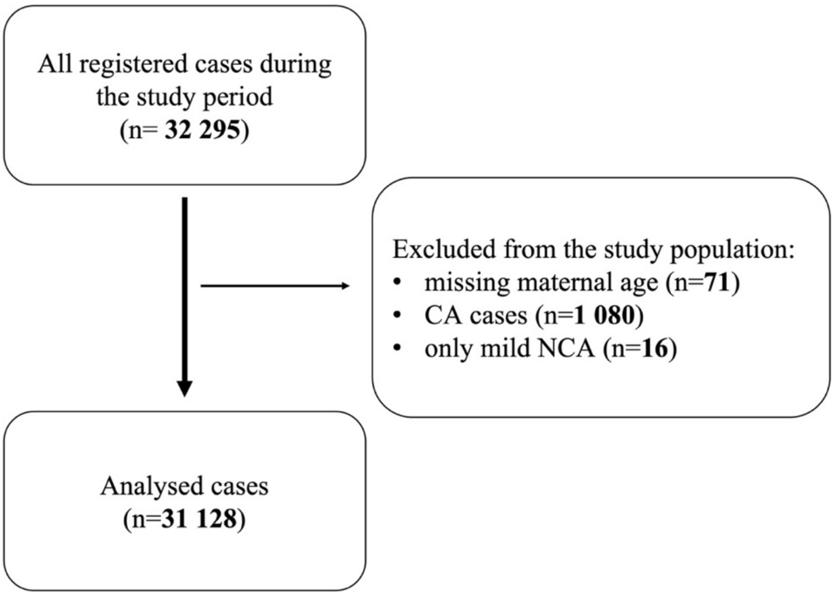


Figure 1. *Study plan(64)*

7.1.5 Variables and data sources

The data collection process recorded the following information for each patient: NCA(s), gender, maternal age, paternal age, birth date, birth weight, gestational age, place of mother's residence, birth order, mother's and father's level of education, employment status and type of employment, mother's marital status, outcome of previous pregnancies, maternal diseases during pregnancy (specified by month), medication during pregnancy (specified by month), and the mother's smoking habits and alcohol consumption patterns.(62)

The maternal age was documented at the moment of childbirth or termination of pregnancy as a result of fetal anomaly. Data regarding maternal diseases, lifestyle factors, and medication usage during pregnancy were gathered through various methods. Initially, mothers submitted their comprehensive medical records pertaining to their current pregnancy, which were then meticulously documented by professionals (prospective, medically recorded data). Subsequently, a questionnaire was sent via mail to the mothers, which included inquiries regarding maternal illnesses, drug treatments during pregnancy, and pregnancy supplements. The information collected was retrospective and based on self-reports from the mothers. Finally, nurses from different regions visited all the mothers. They assisted mothers in gathering and presenting their medical records and completing the supplementary data collection questionnaire.

7.1.6 Bias and evidence synthesis

The maternal ages were documented using birth certificates, guaranteeing a high degree of precision in the data. The distinct characteristics of data collection and verification additionally bolster the dependability of the data. Nevertheless, the categorization of results was not uniform throughout the extensive duration of the study. When converting various ICD categories, the groupings used may not always align perfectly.

We employed the GRADEpro tool to evaluate the degree of evidence underlying our findings.(65) The GRADE is a standardized methodology that allows for clear and consistent evaluation of evidence quality, and thus enables the judgement of the reliability of study results.

7.1.7 Statistical methods

Primary data extraction and organization were carried out in Microsoft Excel. Statistical analysis was carried out in R (version 4.1.3).(66)

The aim of our analysis was to determine high risk maternal age for each NCA category. We used a two-way approach.

First, we identified the best ten-year period of maternal age corresponding to the anomaly's lowest relative frequency. Risk was calculated as: number of cases among live births + stillbirths + elective terminations of pregnancies following prenatal diagnosis of malformation / total number of live births in the population. Risk ratios (RR) for each year were determined by taking the best ten-year period as a "reference risk". (Despite the case-control approach, RR could be used because data collection included the whole population.) Cases with maternal age less than 13 (1 case) and greater than 45 years (9 cases) were excluded because the very low number of cases in these maternal age ranges would have made the regression unreliable. The confidence interval (CI) of relative frequency was estimated according to Agresti and Coull.(67)

Second, we fitted a non-linear, non-parametric logistic regression model on the original data (namely, a restricted cubic splines model using 5 knots at the .05 .275 .5 .725 and .95 quantiles, as recommended in literature; explanatory variable: maternal age; response variable: presence or abscence of NCAs) using the "rms" R package (version 6.2.0).(68) The resulting relative frequency estimates of the regression were transformed to the RR scale in order to enable graphical representation in the figure showing the year-by-year risk estimates calculated above.

A grouping of NCA categories based on high risk maternal age was done by considering the confidence bands in addition to assessing the shape of the curves: a curve may appear U shaped at first glimpse but the risk increase is not necessarily statistically substantiated in both directions, i.e. the CI-band may contain the RR = 1 line corresponding to zero effect.

All CIs were calculated at a confidence level (1-α) of 95%.

7.2 Study II.

We documented our systematic review and meta-analysis based on the guidance of the PRISMA 2020 guideline (69), and we adhered to the Cochrane Handbook for Systematic Reviews of Interventions.(70) The protocol of the study was prospectively registered on PROSPERO (International Prospective Register of Systematic Reviews) (71) (registration number CRD42021283593), and we adhered to it, with some deviations: Title modification for the purpose of enhancing clarity and conciseness; Subgroup analyses were performed without prior specification; The searches involved examining reference lists of eligible articles for screening purposes. Only population-based studies that provided precise NCA counts were included in order to facilitate risk assessment. For the sake of simplicity in understanding, Risk Ratios were utilized instead of Odds Ratios. Publication bias was assessed only visually. However, these modifications primarily pertain to technical aspects and do not change the underlying conceptual framework of the study.

7.2.1 Literature search and eligibility criteria

Information sources

The search was systematically carried out in three extensive medical databases: MEDLINE (via PubMed), the Cochrane Library (CENTRAL), and Embase on October 19, 2021.

Search strategy

We conducted a systematic search using the following search term: ("maternal age" OR "maternal ages" OR "mother age" OR "mother ages") AND (((congenital OR birth) AND (anomaly OR anomalies OR disorder OR disorders OR malformation OR malformations OR defect OR defects)) OR congenital abnormalities. The search was conducted without any language restrictions or filters. In addition, we examined the bibliography of the eligible articles.

Eligibility criteria

The research question was formulated utilizing the PECO framework. We included population-based studies reporting on pregnant women (P). We did not have pre-defined exclusion criteria (e.g., age range, country, comorbidities) for our population. Eligible studies compared different maternal age groups (E and C) regarding NCAs. We examined every pre-defined age group reported by the eligible studies. Our primary outcome (O) was the rate of all NACs combined, while the secondary outcomes were the various specific structural defects. We did not have pre-defined diagnostic criteria for the NCAs. Studies not reporting the total number of patients and the number of NCAs by age group were not eligible. The following exclusion criteria were pre-defined: CAs as target outcomes; case-control or cohort studies; case series; and case reports.

7.2.2 Study selection and data extraction

Study selection

After removing duplicates, the selection was performed independently by three review authors, first by title, then by abstract, and finally based on full text according to the aforementioned criteria. Endnote v20 (Clarivate Analytics, Philadelphia, PA, USA) reference manager software was used for the selection. We calculated Cohen's kappa coefficient after each selection process to measure interrater reliability.(72) Disagreements were resolved through consensus. In cases where consensus could not be reached, a final decision was made with the participation of a fourth independent review author. The study selection process is shown using the PRISMA 2020 flowchart (**Figure 5**).

Data extraction

The author and two additional researchers independently gathered data from the eligible articles. In instances of disagreement, the decision was made by reaching a consensus. If a consensus could not be reached, a final decision was made by including a fourth researcher. The following data were extracted with a standardized collection method to an MS Excel sheet (Office 365, Microsoft, Redmond, WA, USA): first author, the year of publication, study population, study period, study site (region), study design, demographic data of the patients, total number of patients in the age groups, number of NCAs in the age groups, and further information necessary for assessing the risk of bias in the studies.

To investigate which maternal age increases the probability of particular NCAs, we utilized the age categories from the included studies or defined new ones by combining two or more age groups. The age group of 20- to 30-year-old mothers was used as a reference group. In defining the age groups, the ideal 10-year period was based on other studies, including our own work.(64) We aimed to look at very young mothers (under 20 years), advanced maternal age (35 years or older, as commonly defined); and mothers over 40. In addition, we created additional groupings for the 30–35 and 35–40 maternal ages so that the potential association between maternal age and risk change of a given NCA may be more accurately determined. A study was included in the data analysis, if data was available for the reference age category and at least one additional age category for at least one NCA. To ensure consistency, we classified the endpoints according to ICD-10.

7.2.3 Quality assessment

The author and an additional researcher performed the risk of bias assessment independently with the help of the Quality in Prognostic Studies (QUIPS) tool.(73) Disagreements were resolved by a third researcher. A web-based Risk of Bias VISualization (ROBVIS) tool for systematic reviews was used to visualization of the results.(74)

7.2.4 Data synthesis and analysis

As a general rule, we carried out a mathematical synthesis if there were at least three matching articles regarding the age groups and NCAs. In a very few cases, when for non of the age groups were at least 3 studies available for the given anomaly, we carried out the meta-analysis of even only two studies to get at least a limited information.

All statistical analyses were made with R (66) using the 5.5.0 version of meta (75), and the 0.0.9000 version of the dmetar (76) packages.

We anticipated considerable between-study heterogeneity in the study population; therefore, a random-effects model was used to pool effect sizes. RRs with 95% CI was calculated as a random effects estimate with the metabin function of the meta R package. The Mantel-Haenszel method(77-79) was used to pool RRs. Since the exact Mantel-Haenszel method was used, we did not apply continuity correction to handle zero cell counts.(80)

For outcomes with at least five studies, a Hartung-Knapp adjustment was used.(81, 82) We applied the Paule-Mandel method (83) to estimate the between-study variance (tau squared).

Additionally, between-study heterogeneity was investigated by Cochrane's Q test. Significant heterogeneity was considered at $p < 0.1$. Higgins & Thompson's I^2 statistics and 95% CI (82) were reported to illustrate the total variation across studies due to between-study heterogeneity.

Following the recommendations of IntHout et al.(84), where applicable, we also reported the prediction intervals (i.e., the expected range of effects of future studies) of the pooled estimates.

A Cochrane Q test was used between subgroups to assess the age group differences. The null hypothesis was rejected at a 5% significance level. We used forest plots to summarize the results graphically.

Publication bias (a.k.a. small study effect) was assessed visually using funnel plots (forest function of the meta R package), where asymmetry suggests potential bias. Formal assessment was not carried out if less than 10 studies were available, due to the increased risk of unreliable or misleading conclusions.

8 RESULTS

8.1 Study I: Population-based registry analysis

8.1.1 Participants

Over the study period, a total of 31,128 cases of NCAs were identified in Hungary, alongside 2,808,345 live births recorded during the same timeframe. **Table 1** presents the age distribution of the study population, showing that 7.66% of all births fell into the very young (under 20 years) and 6.62% into the advanced (35 years or more) maternal age categories. Additionally, mothers over 40 accounted for 1.11% of births. This means that 14.28% of births were in the maternal age groups expected to pose an increased risk.

Mean maternal age was practically the same among cases (26.0 years; SD = 5.4) and in the total population (26.1 years; SD = 5.1).

Table 1. *Age distribution of cases and total population by age(64)*

Maternal age	Number of live births in Hungary 1980 - 2009	Number of cases of NCA in Hungary 1980 – 2009
13 – 19	214 718	3 060
20 – 24	940 062	10 474
25 – 29	981 027	10 073
30 – 34	486 657	5 182
35 – 39	154 753	1 893
40 – 45	31 128	446

8.1.2 Characteristics of the study population

Thanks to the population-wide data collection, we had individual information about the cases. In the table below, we summarized some of this information. (**Table 2**) The most notable is the sex of the fetuses, which is around 65% male.

Table 2. *Baseline characteristics table(64)*

	maternal age: all (13–45 years) [count: 31,118]		
	count	**mean**	**SD**
birth mass (g)	30,908	3,018	707
gestation period (weeks)	30,995	38.5	3.2
paternal age (years)	1,851	32.1	6.4
	count	**proportion**	
gender			
male	20,046	65.64%	
female	10,492	34.36%	
NA	*580*		
birth order			
primiparous	16,309	55.76%	
multiparous	12,939	44.24%	
NA	*1,870*		
maternal smoking			
smoker	2,776	35.51%	
nonsmoker	5,041	64.49%	
NA	*23,301*		
maternal education			
managerial	1,377	15.26%	
professional	2,450	27.14%	
skilled worker	2,376	26.32%	
semiskilled	2,327	25.78%	
unskilled	496	5.50%	
NA	*22,092*		

8.1.3 Risk of NCAs by maternal age category

The relative frequency of NCAs in the study period was 1.1% (excluding cases with only mild anomalies and cases with concomitant chromosomal anomalies, as described in the methodology earlier).

All NCAs (ICD-10 Q00-Q89):

In the first step, all NCAs were analyzed together **(Figure 2)**. We found a risk-increasing effect for both the advanced and the very young maternal age. The lowest risk ten-year period turned out to fall between 23 and 32 years (light gray shading); both lower (RR = 1.2; CI: 1.17–1.23) and higher (RR = 1.15; CI: 1.11–1.19) maternal age pose an almost identically increased risk of anomalies. The year-by-year RRs (circle markers) imply an increasing trend in both directions. The fitted regression line (black, with a dark gray confidence band) stresses that both very young and advanced maternal age increase risk even more. Even though the confidence range becomes wider in the very young and old maternal age groups due to the low number of cases, the trend is still clear.

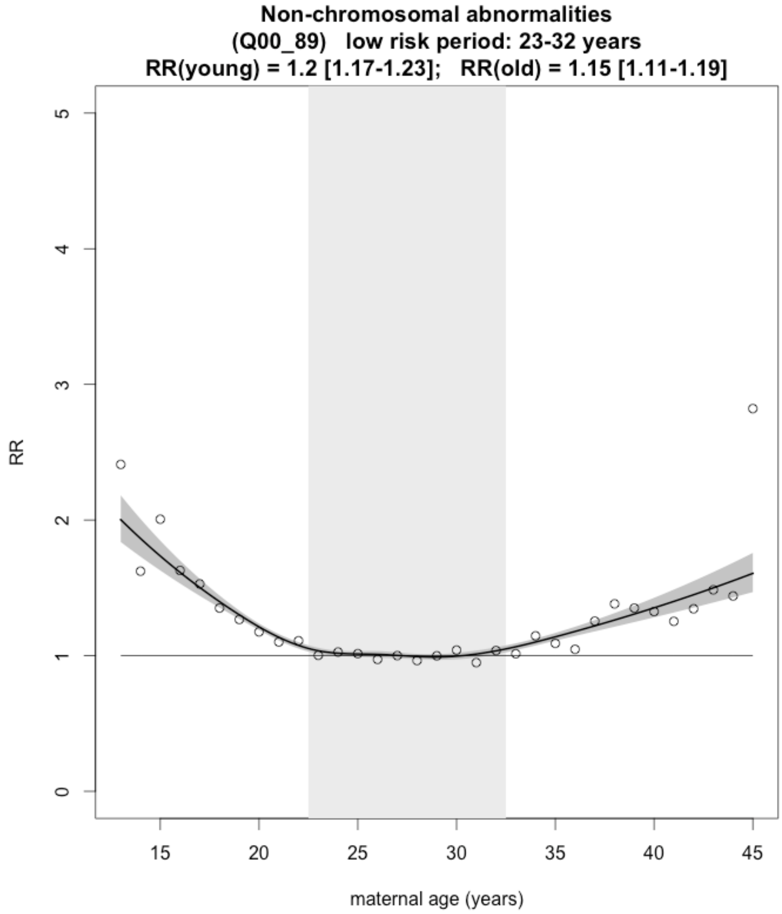


Figure 2. *Analysis of all NCAs by maternal age(64)* The figure shows the estimated risk ratios of NCAs as a function of maternal age with the best ten-year-period as "reference risk" (circle markers). The best ten-year-period is highlighted with light gray. The black curve shows the result of the restricted cubic splines regression, the dark gray area is its confidence range.

In the next step, NCAs were analyzed one-by-one by ICD categories (**Figure 3** and **Table 6**).

Congenital malformations of the nervous system (ICD-10: Q00-Q07)

The lowest risk ten-year-period in this category was detected between 26-35 years of maternal age. This is also the only NCA category where only the young maternal age is significantly associated with increased risk. Looking at the entire low-age group (< 26 years), the risk increase is 25%. For the very young age group (< 20), there is an even higher risk: RR = 1.71 (CI: 1.48–1.97).

Congenital malformations of eye, ear, face and neck (ICD-10: Q10-Q18)

The best-ten-year period for this type of anomaly was between 30–39 years. The advanced maternal age above this period shows a risk-increasing effect while young age (< 30 years) does not. Looking at the figure, the results appear to be somewhat inconsistent, because the risk increase already becomes significant above 35 years, which is still in the best-ten-year period. The risk increase is especially high above 40 years: RR = 2.09 (CI: 1.25–3.49).

Congenital malformations of the circulatory system (ICD-10: Q20-Q28)

The lowest risk ten-year-preriod falls between 23–32 years. Outside this age range, there is an increase in risk at both very young (< 23 years; RR = 1.07; CI: 1.01–1.13) and advanced maternal ages (> 32 years; RR = 1.33; CI: 1.24–1.42), but it is more pronounced in vase of the advanced age group. From a clinical point of view, it is noteworthy that within the advanced maternal age group, the risk was particularly elevated in mothers over 40 years: RR = 1.72 (CI: 1.45–2.05).

Congenital malformations of the respiratory system (ICD-10: Q30-Q34)

According to our analysis, respiratory system anomalies could not be proven to be associated with maternal age. Though a lowest risk ten-year-preiod was determined here as well, this is unlikely to reflect reality due to the scarcity of cases and the associated increased role of random data variation.

Cleft lip and cleft palate (ICD-10: Q35-Q37)

The lowest risk ten-year-period was found to be between 25–34 years of maternal age for this group of NCAs. There is an increase in risk both below (RR = 1.07; CI: 1.01–1.13) and above (RR = 1.33; CI: 1.24–1.42) this maternal age range, but it is more pronounced at advanced ages. In this case, too, mothers aged 40 and above faced the highest risk: RR = 1.58 (CI: 1.16–2.16).

Congenital malformations of the digestive system (ICD-10: Q38-Q45)

The lowest risk was for maternal age between 24 and 33 years, with both lower (RR = 1.23; CI: 1.14–1.31) and older (RR = 1.15; CI: 1.02–1.29) maternal age as a significant risk-increasing factor. The most pronounced increase in risk was observed for mothers under the age of 20: RR = 1.46 (CI: 1.31; 1.64).

Congenital malformations of genital organs (ICD-10: Q50-Q56)

The lowest risk ten-year-period was found between 25–34 years. Both the younger (RR = 1.15; CI: 1.08–1.22) and the more advanced (RR = 1.16; CI: 1.04–1.29) maternal age increases the risk – to a similar extent – The risk is expected to increase by around 30% for mothers both under 20 and over 40 years.

Congenital malformations of the urinary system (ICD-10: Q60-Q64)

The lowest risk ten-year-period was detected between 15–24 years. Higher maternal age increases the risk (RR = 1.34; CI: 1.19–1.50), with an even higher risk above 40: RR = 2.27 (CI: 1.53–3.38). Thought the below 20 age category overlaps with the lowest risk age range, a risk increase could still be detected: RR = 1.29 (CI: 1.04–1.60).

Congenital malformations and deformations of the musculoskeletal system (ICD-10: Q65-Q79)

The optimal age range is between 27–36 years. Both the younger (RR = 1.17; CI: 1.12–1.23) and the older (RR = 1.29; CI: 1.14–1.44) maternal age increases the risk. The probability of these anomalies increases most in people under 20 years of age: RR = 1.57 (CI: 1.46–1.70).

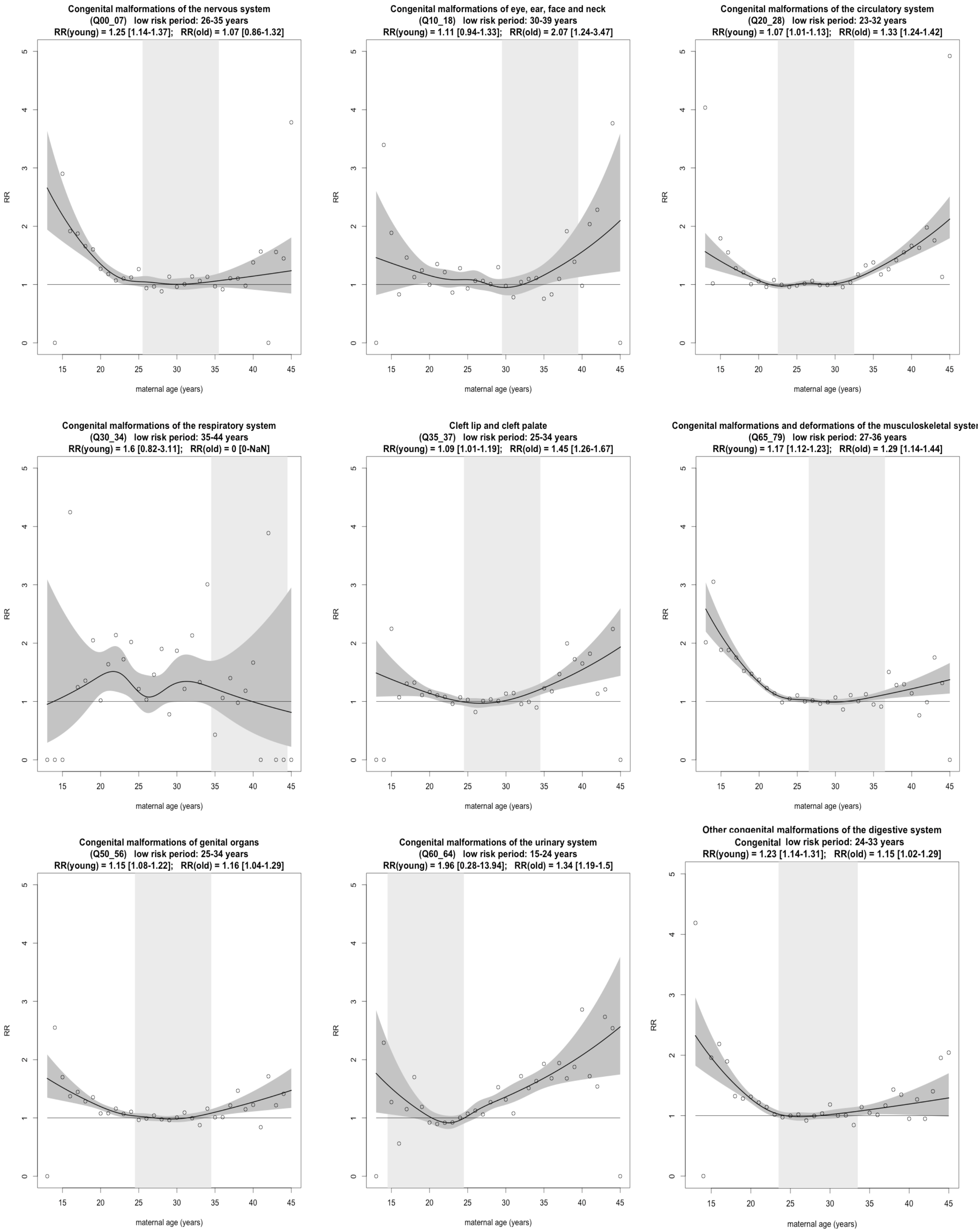


Figure 3. *Summary results for the NCA categories(64)*

8.1.4 Level of evidence

The level of evidence was only assessed in case of the overall outcome “all NCAs combined”. Here, we found a “moderate” level (i.e. level 3 on a 4-level scale with levels “very low”, “low”, “moderate”, and “high”) of evidence certainty for both the very young (< 23 years) and the advanced (> 32 years) age groups. The main reason for this is the observational study design and the lack of inclusion of confounders in the analysis.

Table 3. *Grading of the primary outcomes(64)*

Certainty assessment							№ of patients(1)		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	maternal ages	maternal ages	Relative (95% CI)	Absolute (95% CI)		
all non-chromosomal congenital anomalies together - very young maternal age												
1	observational studies	not serious	not serious	not serious	not serious	dose response gradient			RR 1.20 (1.17 to 1.23)	1 fewer per 100 (from 1 fewer to 1 fewer)[a]	⊕⊕⊕◯ Moderate	CRITICAL
all non-chromosomal congenital anomalies together - advanced maternal age												
1	observational studies	not serious	not serious	not serious	not serious	dose response gradient			RR 1.15 (1.11 to 1.19)	-	⊕⊕⊕◯ Moderate	CRITICAL

8.2 Study II: Meta-analysis

8.2.1 Study selection

After duplicate removal, 15,547 studies were identified by our search in the three screened databases, from which 72 full-text articles were included in our synthesis following the selection process shown in **Figure 4** below.

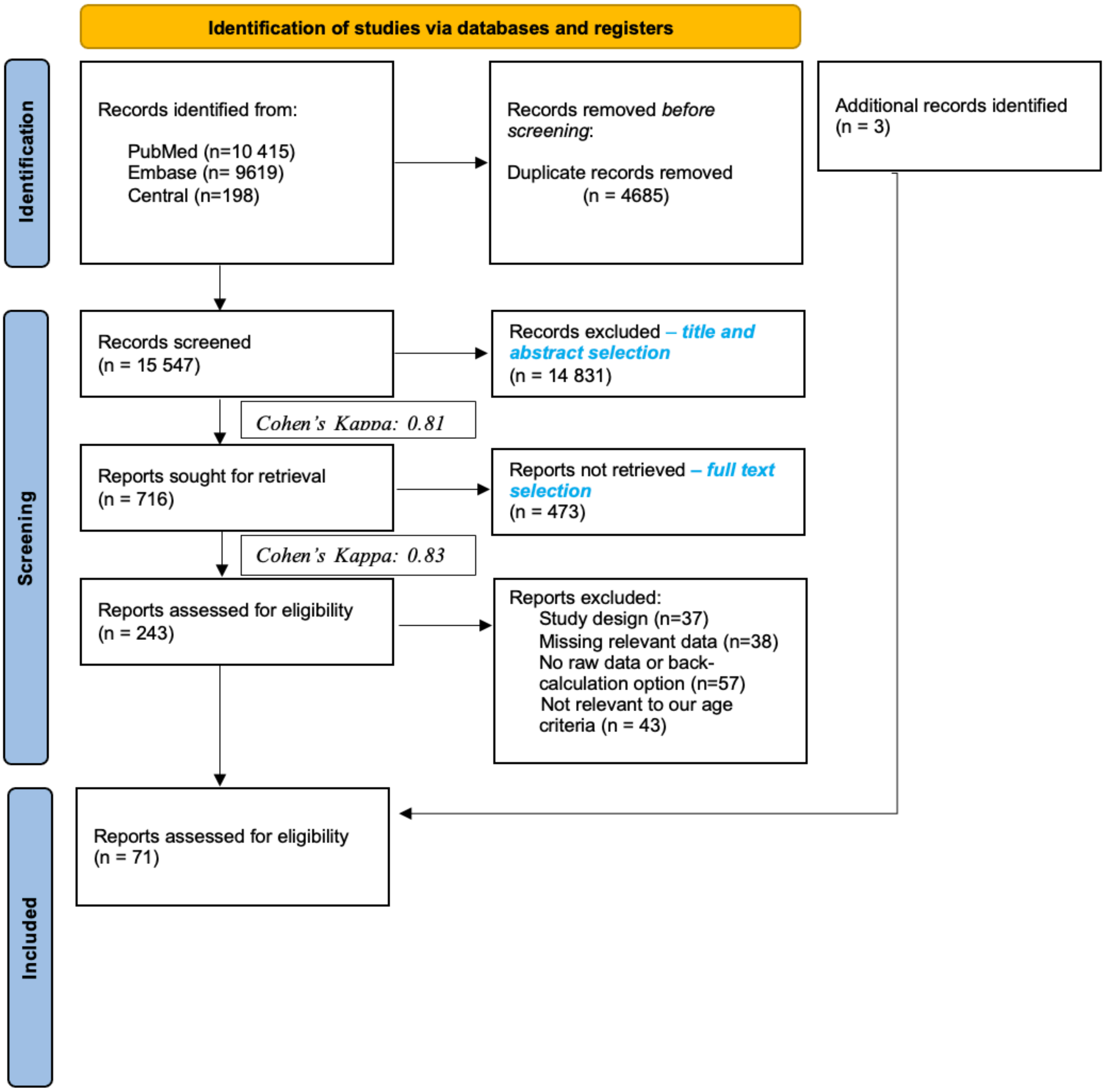


Figure 4. *PRISMA 2020 flowchart representing the study selection process(85)*

8.2.2 Study characteristics

The baseline characteristics of the included studies are detailed in **Table 4.** Our meta-analysis includes population-based studies from all over the world: 37 studies come from the Americas, 17 from Europe;, 14 from Asia, 3 from Australia, and 1 from Africa; the precise geographic location is indicated in the baseline table. In terms of the data collection period, the included studies encompass an overall timeframe between 1940 and 2018. All studies are population-based, with 36 studies carried out at the national level, 34 at the subnational level, and two at the multinational level, mostly based on the corresponding registries.

8.2.3 Risk of bias assessment

The results of the risk of bias assessment are presented in **Figure 5**. The overall risk of bias (possible levels are low, medium and high) is 88% low, 12% moderate, and 0% high. The two component bias aspects with the highest risk were the bias due to confounding (38% low, 62% moderate, 0% high) and bias due to participation (51% low, 49% moderate, 0% high). The main source of risk of bias in both cases is the limited reporting of population characteristics.

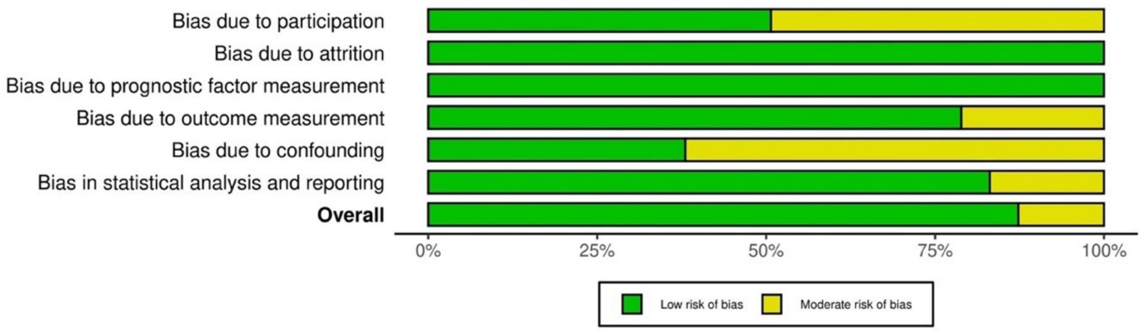


Figure 5. *Risk of bias assessment using the ROBVIS tool(85)*

8.2.4 Heterogeneity and publication bias

Most of our analyses showed a significant and high level (i.e., $I^2 > 75\%$) of heterogeneity. This is attributable to the diversity of geographical regions, population sizes, date and duration of the study periods represented by the included studies.

Upon visual inspection of the funnel plots no significant plot asymmetry was found that would suggest publication bias.

Table 4. *Baseline charecteriscics of the included articles(85)*

Author (year)	Ref	Country	Study period	Total	Cases	Age category	Congenital anomalies
Agopian 2009	(86)	Texas (USA)	1999 - 2004	2208758	325	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Omhalocele
Baer 2014	(87)	California (USA)	2005 - 2010	3070957	1279	<19, 20-24, 25-29, 30-34, ≥35	Gastroschisis
Beckman 1976	(88)	Sweden	1950 - 1973	61061	280	<24, 25-29, 30-34, ≥35	Cleft palate, Cleft lip with or without cleft palate, Polydayctyly, Syndactyly, Clubfoot
Bergman 2015	(89)	Europe	2001 - 2010	5871855	10929	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Hypospadiasis
Baird 1994	(90)	Canada	1966 - 1981	576815	702	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Isolated Cleft palate, Cleft lip and cleft palate
Bodnár 1970	(91)	Hungary	1958 - 1967	115215	2100	<19, 20-24, 25-29, 30-39, ≥40	all NCAs, Nervous system, Circulatory system, Urogenital anomalies, Musculoskeletal

							system, Digestive system
Borman 1986	(92)	New Zeland	1978	52143	104	<20, 20-24, 25-29, ≥30	Anencephlaus, Spina bifida
Borque 2021	(93)	Canada	2012 - 2018	1001080	231	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Gastroschisis
Bugge 2017	(94)	Greenland (Denmark)	1989 - 2015	26666	33	<20, 20-24, 25-29, 30-34, 35-39, 40-44, ≥45	Gastroschisis, Omphalocele
Byron 1998	(95)	Australia	1980 - 1990	358679	59; 104	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Gastroschisis, Omphalocele
Canfield 2009	(96)	Texas (USA)	1999 - 2003	1827317	514; 643	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Anencephlaus, Spina bifida
Canon 2012	(97)	Arkansas (USA)	1998 - 2007	196050	1455	<20, 20-24, 25-29, 30-34, ≥35	Hypospadiasis
Croen 1995	(98)	California (USA)	1983 - 1988	1028255	29848	<20, 20-24, 25-29, 30-34, 35-39, ≥40	all NCAs
DeRoo 2003	(99)	Washington (USA)	1987 - 1990	298138	608	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Cleft lip and cleft palate
Dott 2003	(100)	Metropolitan Atlanta (USA)	1968 - 1999	1029143	249	<20, 20-24, 25-34, ≥35	Diaphragmatic hernia

Dudin 1997	(101)	Palestina	1986 - 1993	26934	148	15-19, 20-24, 25-29, 30-39, ≥40	Neural tube defects
Fedrick 1976	(102)	Scotland (UK)	1961 - 1972	1162939	3246	<20, 20-24, 25-29, 30-34, 35-39, 40-44, ≥45	Anencephlaus
Feldman 1982	(103)	New York, Brooklyn (USA)	1968 - 1976	173670	179	<20, 20-24, 25-29, 30-34, ≥35	Neural tube defects
Forrester 2004	(104)	Hawaii (USA)	1986 - 2000	281866	544	<19, 20-24, 25-29, 30-34, 35-39, ≥40	Cleft lip and cleft palate
Forrester 1999	(105)	Hawaii (USA)	1986 - 1997	229584	150	19≥, 20-24, 25-29, 30-34, 35-39, ≥40	Omphalocele, Gastoschisis
Forrester 2000	(106)	Hawaii (USA)	1986 - 1997	246231	245	19≥, 20-24, 25-29, 30-34, 35-39, ≥40	Anencephaly, Spina bifida, Encephalocele
Friedman 2016	(107)	USA	2005 - 2013	24836777	5985	<20, 20-24, 25-29, 30-34, ≥35	Gastroschisis
Gupta 1967	(108)	Nigeria	1964	4220	15	15-19 20-24, 25-29, 30-34, 35-39, 40-44	CHD
Hansen 2021	(109)	Australia	1990 - 2016	765419	8173	<20, 20-24, 25-29, 30-34, 35-39, ≥40	CHD
Hay 1972	(110)	USA	1961 - 1966	8475600	1063	<20, 20-24, 25-29, 30-34, 35-39, ≥40	Anencephlay, Spina bifida, Hydrocephalus,

							Congenital heart defects, Cleft lip without cleft palate, Cleft lip and palate, Cleft palate without cleft lift, Tracheoesophageal fistula and other esophageal defects, Omhalocele, Imperforate anus and other anorectal defects, Hypospadiasi, Position foot defects, Polydactyly, Syndactyly, Reduction deformities
Hollier 2000	(111)	Dallas (Texas, USA)	1988 - 1994	102728	3466	<20, 20-24, 25-29, 30-34, 35-39, ≥40	all NCAs

Jaikrishan 2012	(112)	India	1995 - 2011	141540	1370	15-19, 20-29, ≥30	Clubfood, CHD, Cleft palate/lip, NTD, Hypospadiasis
Janerich 1972	(113)	New York State (USA)	1945 - 1970	4555614	4450	15-19; 20-24; 25-29; 30-34; 35-39; 40-44	Spina bifida
Janerich 1972	(114)	New York State (USA)	1945 - 1967	4074079	3090	15-19; 20-24; 25-29; 30-34; 35-39; 40-44	Anencephaly
Jaruratanasirikul 2016	(115)	Southern Thailand	2009 - 2013	186393	269	<20; 20–<25; 25–<30;30–<35; ≥35	Oral clefts
Jones 2016	(116)	USA	1995 - 2012	21040437	8866	<20; 20-24; 25-29; 30-34; 35<	Gastroschisis
Kazaura 2004	(117)	Norway	1967 - 1998	1869388	699	<20; 20–24; 25–29; 30–34; 35–39; ≥40	Gastroschisis, Omphalocele
Kirby 2013	(118)	USA	1995 - 2005	13233235	4713	<20; 20-24; 25-29; 30-34; 35<	Gastroschisis
Liu 2013	(119)	Canada	2002 - 2010	2283223	26488	<19; 20-24; 25-29; 30-34; 35-39; 40<	CHD
Liu 2019	(120)	Canada	2004 - 2015	3327762	1517	<19; 20-24; 25-29; 30-34; 35-39; 40<	Spina bifida, Anencephaly/encephalocele

Li 2019	(121)	Zhejiang Province (China, People's Republic of)	2010 - 2016	1748023	2790	<20; 20-25; 30-35; ≥ 35	Kidney and urinari tract defects
Loc-Uyen 2015	(122)	Texas (USA)	1999 - 2011	4970525	2549	<19; 20-24; 25-29; 30<	Gastroschisis
Luo 2019	(123)	Shenzhen (China, People's Republic of)	2003 - 2017	591024	777	<25; 25−;30−; 35<	Cleft lip and palate
Martinez-Frias 1984	(124)	Spain	1976	264502	52	<19; 20-24; 25-29; 30-34; 35-39; 40<	Gastroschisis, Omphalocele
Materna-Kiryluk 2009	(125)	Poland	1998 - 2002	716089	8683	<19; 20-24; 25-29; 30-34; 35-39; 40<	all NCAs (Excluded muskuloskeletal defects),Diaphragmatic hernia,Gastroschisis, Omphalocele, Neural tube defects, Microcephalus, Hydrocephalus, Congenital heart

							defects, Hypospadiasis, Renal agenesis or hypoplasia, Cystic kidney disease, Hydronephrosis, cleft palate, cleft lip with or without cleft palate, Oesophageal atresia, Small intestinal/large intestinal atresia or stenosis, Anal atresia or stenosis
McGivern 2015	(126)	Europe	1980 - 2009	11478586	3373	<20; 20-24; 25-29; 30-34; 35<	Diaphragmatic hernia
Miller 2011	(127)	Atlanta (Georgia, USA)	1968 - 2005	1301340	5289	<35; 35<	CHD
Mucat 2019	(128)	Malta	2000 - 2014	55943	2225	20-24; 25-29; 30-34; 35-39; 40<	all NCAs, Nervous system, Eye,ear, face, neck, Circulatory

							system, Respiratory system, Digestive system, Genital organs, Urinary system, Muskoloskeletal system
Nazer 2007	(129)	Chile	1996 - 2005	21083	1767	<15; 15-19; 20-24; 25-29; 30-34; 35-39; 40-44; 45<	all NCAs
Nazer 2013	(130)	Chile	2002 - 2011	15636	1174	<15; 15-19; 20-24; 25-29; 30-34; 35-39; 40-44; 45<	all NCAs
Parkes 2020	(131)	England, Scotland (UK)	2003 - 2010	219486	5154	<19; 20-29; 30-39; 40<	all NCAs
Pasnicki 2013	(132)	Poland	1988 - 2007	192438	2769	<18; 20-24; 25-29; 30-34; 35-39; 40<	all NCAs, Nervous system, Circulatory system, Cleft lip and cleft palate, Digestive system, Genital

							organs, Urinary system, Muskoloskeletal system, Other
Persson 2019	(133)	Sweden	1992 - 2012	2050491	28628	>24; 25-29; 30-34; 35<	CHD
Petrova 2009	(134)	Norway and Arkhangelskaja Oblast (Russia)	1995 - 2004	434567	615	<19; 20-24; 25-29; 30-34; 35<	Neural tube defects: Anencephalus, Spina bifida
Pradat 1992	(135)	Sweden	1981 - 1986	573422	1605	<20; 20-24; 25-29; 30-34; 35-39; 40-44; >44	CHD
Purkey 2019	(136)	California (USA)	2008 - 2012	2054516	6325	<19; 20-24; 25-29; 30-34; 35<	CHD
Rankin 1999	(137)	Northern England (UK)	1986 - 1996	426694	296	11–19; 20–24; 25–29; 30–34; 35–39; >40	Gastroschisis, Omphalocele, Omphalocele
Rankin 2000	(138)	Northern England (UK)	1984 - 1996	507405	934	11–19; 20–24; 25–29; 30–34; 35–39; >40	Neural tube defects
Rider 2013	(139)	Utah (USA)	1999 - 2008	480125	8510	<24; 25-29; 30-34; 35-39; 40-60	all NCAs

Roeper 1987	(140)	California (USA)	1968 - 1977	3297071	166	<19; 20-24; 25-29; 30-34; 35-39; 40<	Gastroschisis, Omphalocele
Salihu 2003	(141)	New York State (USA)	1992 - 1999	2153955; 2149340	595	<19; 20-24; 25-29; 30-34; 35-39; 40<	Omphalocele, Gastroschisis
Salim 2019	(142)	Brazil	1996 - 2014	4270114	5062	<19; 20-29; 30-34; 35-39; 40<	Circulatory system
Sarkar 2013	(143)	India	2011 - 2012	12896	286	<20; 20-30; 30<	all NCAs
Sever 1982	(144)	Los Angeles County (California, USA)	1966 - 1972	2945555	962	<14; 15-19; 20-24; 25-29; 30-34; 35-39; 40-44; 45<	Anencephalus, Spina bifida, Encephalocele, Neural tube defects, SUM
Shields 1981	(145)	Denmark	1940 - 1971	2406654	548	<19; 20-24; 25-29; 30-34; 35-39; 40-44; 45<	Cleft palate
Short 2019	(146)	USA	2006 - 2015	17686317	3489	<19; 20-24; 25-29; 30<	Gastroschisis
StLouis 2017	(147)	USA	1999 - 2007	13105878	138999	<19; 20-24; 25-29; 30-34; 35<	all NCAs, Anencephalus, Spina bifida,Encephalocele, Anotia/microtia, Common truncus CHD,Transposition of

							the great arteries ,Tetralogy of Fallot,Atrioventricular septal defect without Down syndrome, Hypoplastic left heart syndrome, Coarctation of the aorta, Aortic valve stenosis, Cleft palate without cleft lip, Cleft lip with and without cleft palate, Esophageal atresia/tracheoesophageal fistula, Pyloric stenosis, Rectal and large intestinal atresia/stenosis, Hypospadiasb, Upper limb deficiency,

							Lower limb deficiency, Any limb deficiency,Diaphragmatic hernia, Gastroschisis, Omphalocele
Tan 1996	(148)	England, Wales (UK)	1987 - 1993	4873547	1043	<20; 20-24; 25-29; 30-34; 35-39; >40	Gastroschisis, Omphalocele
Tan 2005	(149)	Singapore	1994 - 2000	328077	7870	<20; 20-24; 25-29; 30-34; 35-39; >40	all NCAs
Tan 2008	(150)	Singapore	1993 - 2002	460532	121	<20; 20-24; 25-29; 30-34; 35-39; >40	Gastroschisis, Omphalocele
Williams 2005	(151)	Atlanta (USA)	1968 - 2000	877604	211	<20; 20–24; 24<	Gastroschisis
Xie 2016	(152)	Hunan Province (China, People's Republic of)	2005 - 2014	925413	17753	<20; 20-24; 25-29; 30-34; 35<	all NCAs
Xie 2018	(153)	China (People's Republic of)	2012 - 2016	673060	6289	<20, 20-24, 25-29, 30-34, ≥35	Congenital heart defects
Xu 2011	(154)	China (People's Republic of)	1996 - 2007	6308594	1601	<19; 20-24; 25-29; 30-34; 35<	Gastroschisis

Zhang 2012	(155)	China (People's Republic of)	2012	62526	976	<25; 25-30; 35<	all NCAs
Yang 2006	(156)	California (USA)	1989 - 1997	2506188	550	<20; 20-24; 25–29; 30–34; 35–39; 40–55	Diaphragmatic hernia
Zhou 2020	(157)	Southern Jiangsu (China, People's Republic of)	2014 - 2018	238712	1707	<19; 20-24; 25-29; 30-34; 35<	all NCAs

8.2.5 Risk of NCAs by maternal age category

The role of maternal age in the occurrence of NCAs: **Table 5** summarizes our results. By default forest plots and summary statistics were prepared including all eligible studies regadless of concommittant CAs.

<u>All NCAs</u> (**Figure 6, Figure 7**)

Regarding our primary outcome, i.e. analyzing all NCAs combined, we found that age > 35 (RR = 1.31, CI: 1.07–1.61) and especially age > 40 (RR = 1.44; CI: 1.25–1.66) increase the risk of NCAs For this outcome we conducted two subgroup analyses to investigate the question more deeply. First, we examined the age risk of all NCAs excluding cases with co-occurrent chromosomal anomalies, we found significant results for the > 40 age category (RR = 1.25; CI: 1.08–1.46). Next, we carried out the analysis for studies where the presence of chromosomal anomalies was allowed: the risk of NCAs was found to increase with maternal age > 35 (RR = 1.26; CI: 1.12–1.42) and > 40 (RR = 1.63; CI: 1.26–2.09).

<u>Congenital malformations of the nervous system (Q00–Q07)</u>

Despite the analysis of up to 10 studies for each age group, we found no singificant association between maternal age and congenital nervous system malformations (see **Supplementary Figure 3** of the article).

<u>Congenital malformations of the circulatory system (Q20–Q28)</u> **(Figure 8)**

We found a risk-increasing effect of maternal age > 40 (RR = 1.94; CI: 1.28–2.93). Among the diseases of the circulatory system, we also specificly analyzed the group of congenital heart defects (CHD) **(Figure 9)**, where we also found risk-increasing effect for advanced maternal age: for the > 35 group: RR = 1.50; CI: 1.11–2.04; and for the > 40 group: RR = 1.75; CI: 1.32–2.32 was found. For the very young maternal age (< 20) group a preventive effect was observed (RR = 0.87; CI: 0.78–0.97).

Cleft lip and cleft palate (Q35–Q37) **(Figure 10)**

Maternal age > 40 elevated the risk of cleft lip and cleft palate (RR = 1.57; CI: 1.11–2.20). Regarding cleft palate separately (see **Supplementary Figure 10** of the article), we found an even higher risk with advanced maternal age, which appears as early as the 35th year (for age> 35: RR = 1.78; CI: 1.16–2.73; and for age > 40: RR = 1.77; CI: 1.48–2.11).

Congenital malformations of the digestive system (Q38–Q45) **(Figure 11)**

We found a risk-increasing effect for maternal age > 40 (RR = 2.16; CI: 1.34–3.49).

Congenital malformations of the urinary system (Q60–Q64)

We could not detect an association between maternal age and congenital malformations of the urinary system after analyzing three eligible population-based studies with homogeneous age categories (see **Supplementary Figure 13** of the article).

Congenital malformations and deformations of the musculoskeletal system (Q65–Q79)

We did not find an association with maternal age. However, this can also be explained by the low number of studies and their heterogeneity, and also by the complex nature of the group (see **Supplementary Figure 14** of the article).

Other malformation categories

Regarding the congenital malformations of the eye, ear, face, and neck (Q10–Q18), congenital malformations of the respiratory system (Q30–Q34), and congenital malformations of genital organs (Q50–Q56), we did not find enough studies with homogenous age groups and NCAs to carry out a mathematical synthesis.

On the other hand, we found a clear association between maternal age and some individual malformations. The risk of omphalocele was higher in both very young (age < 20, RR = 1.44; CI: 1.08–1.92) and advanced maternal age (age > 40, RR = 2.57; CI: 1.77–3.73) group. Based on 22 eligible articles (age < 20, RR = 3.08; CI: 2.74–3.47), gastroschisis shows a strong association with very young maternal age (**Figure 12**).

Additionally, we also re-sorted our study level outcomes by year of publication to detect any apparent trend in case of outcomes where sufficient number of articles were available to have any chance to reliably assess any effect (see **Supplementary Figures 38-47** of the article) and we could not find any convincing trend upon visual inspection. As an alternate approach, we also analyzed the subset of studies published from 2005 onward (see **Supplementary Figues 48-57** of the article): no clear and convincing trend could be identified, only weak trends in a few cases (summarized in **Supplementary Table 6**).

Table 5. Summary of our results based on ICD-10 categories (85)

Congenital anomaly	ICD-10 Category	Age < 20	N	Age 30-35	N	Age 35-40	N	Age > 35	N	Age > 40	N
All NCAs (with or without CAs)	Q00-Q89	1.08 (0.89; 1.32)	14	1.23 (0.85; 1.78)	13	1.47 (0.87; 2.49)	9	1.31 (1.06; 1.61)	13	1.44 (1.25; 1.66)	11
All NCAs (without CAs)	Q00-Q89	1.21 (0.59; 2.49)	5	1.54 (0.55; 4.32)	6	1.73 (0.45; 6.70)	5	1.37 (0.76; 2.45)	6	1.25 (1.08; 1.46)	6
All NCAs (with CAs)	Q00-Q89	1.15 (0.87; 1.52)	10	1.02 (0.99; 1.06)	7	1.20 (0.99; 1.44)	4	1.26 (1.12; 1.42)	7	1.63 (1.26; 2.09)	6
Nervous system	Q00-Q07	1.16 (0.74; 1.81)	10	1.64 (0.70; 3.81)	8	2.56 (0.64; 10.32)	5	1.53 (0.80; 2.94)	8	1.56 (0.67; 3.62)	7
Encephalocele	Q01	1.76 (0.44; 7.12)	3	1.51 (0.33; 6.83)	3	no data		1.43 (0.57; 3.60)	3	no data	
Congenital hydrocephalus	Q03	1.19 (1.02; 1.38)	2	no data		no data		no data		no data	
Spina bifida	Q05	1.30 (0.93; 1.82)	9	1.15 (0.65; 2.06)	8	1.79 (0.61; 5.31)	5	1.39 (0.75; 2.59)	8	1.96 (0.72; 5.31)	5
Anencephaly	Q00.0	1.40 (0.98; 1.99)	9	1.15 (0.72; 1.84)	8	1.20 (0.53; 2.72)	6	1.02 (0.60; 1.72)	8	1.30 (0.71; 2.38)	6
Circulatory System	Q20-Q28	0.87 (0.68; 1.11)	3	1.09 (1.00; 1.20)	3	1.18 (0.94; 1.49)	3	1.33 (0.97; 1.82)	3	1.94 (1.28; 2.93)	4
Congenital Heart Defects	Q20-Q26	0.87 (0.78; 0.97)	10	1.45 (0.83; 2.52)	10	1.91 (0.65; 5.62)	6	1.50 (1.11; 2.04)	10	1.75 (1.32; 2.32)	6
Cleft lip and palate	Q35-Q37	0.93 (0.76; 1.14)	6	1.58 (0.77; 3.22)	6	1.85 (0.59; 5.75)	4	1.47 (0.95; 2.28)	6	1.57 (1.11; 2.20)	4
Cleft palate	Q35	0.99 (0.56; 1.73)	6	1.42 (0.66; 3.06)	8	2.08 (0.54; 7.99)	5	1.78 (1.16; 2.73)	8	1.77 (1.48; 2.11)	5
Digestive System	Q38-Q45	0.98 (0.71; 1.37)	2	no data		no data		no data		2.16 (1.34; 3.49)	2
Urinary System	Q60-Q64	no data		0.97 (0.75; 1.26)	3	no data		0.86 (0.57; 1.29)	3	no data	
Hypospadiasis	Q54	0.99 (0.91; 1.07)	5	1.06 (0.96; 1.17)	4	no data		1.11 (0.88; 1.39)	4	no data	
Musculoskeletal System	Q65-Q79	0.88 (0.72; 1.08)	2	no data		0.93 (0.71; 1.22)	2	0.94 (0.65; 1.37)	2	0.90 (0.55; 1.46)	3
Congenital Diaphragmatic Hernia	Q79.0	0.96 (0.88; 1.06)	5	1.74 (0.52; 5.80)	4	no data		1.52 (0.79; 2.91)	5	no data	
Omphalocele	Q79.2	1.44 (1.08; 1.92)	14	1.13 (0.85; 1.50)	14	1.35 (0.98; 1.87)	13	1.47 (1.20; 1.79)	14	2.57 (1.77; 3.73)	13
Gastroschisis	Q79.3	3.08 (2.74; 3.47)	22	0.32 (0.23; 0.44)	17	0.27 (0.16; 0.47)	12	0.22 (0.15; 0.32)	17	0.41 (0.23; 0.74)	11

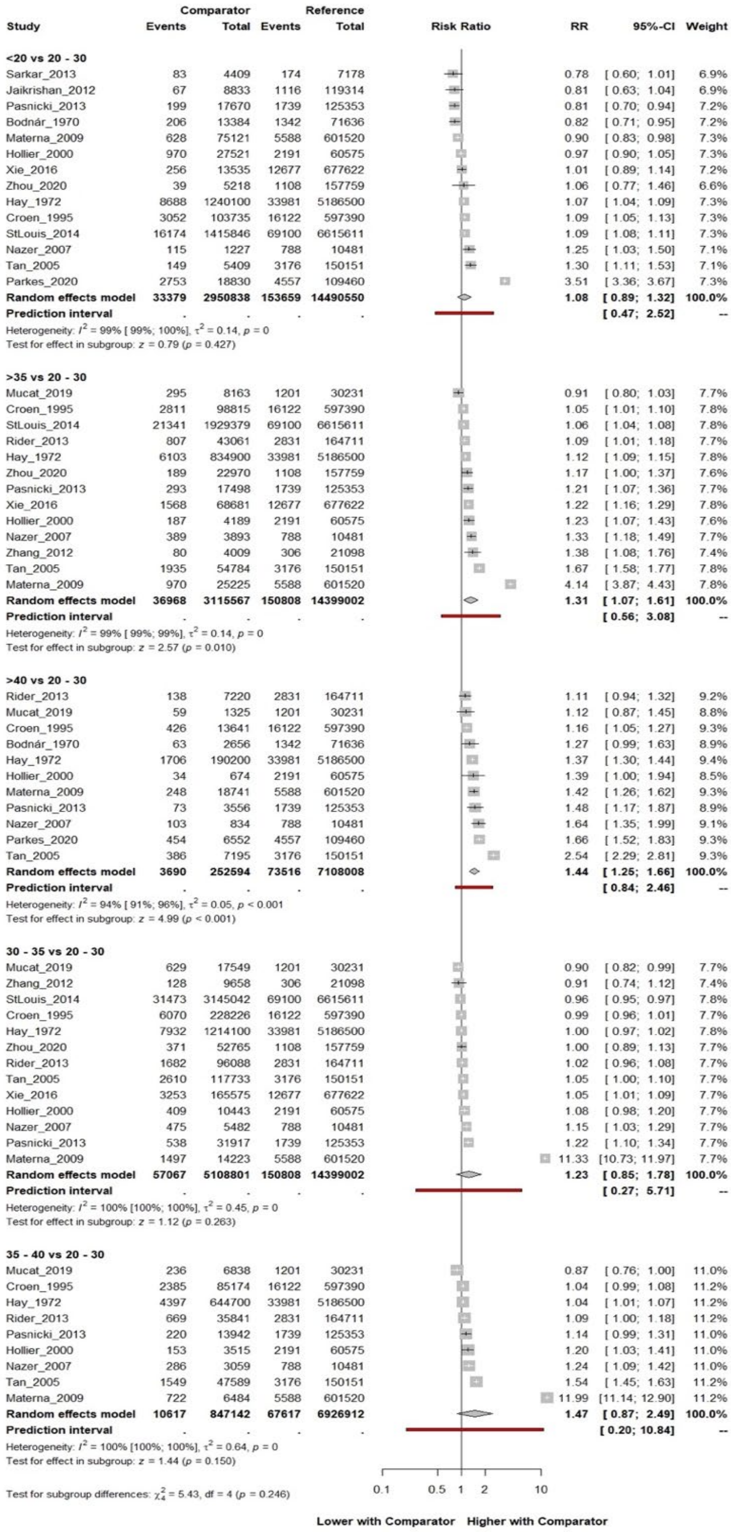


Figure 6. *Forest plot representing the RR with 95% CI of all non-chromosomal anomalies (ICD-10: Q00-Q89) in different age groups compared to the 20-30 age group(85)*

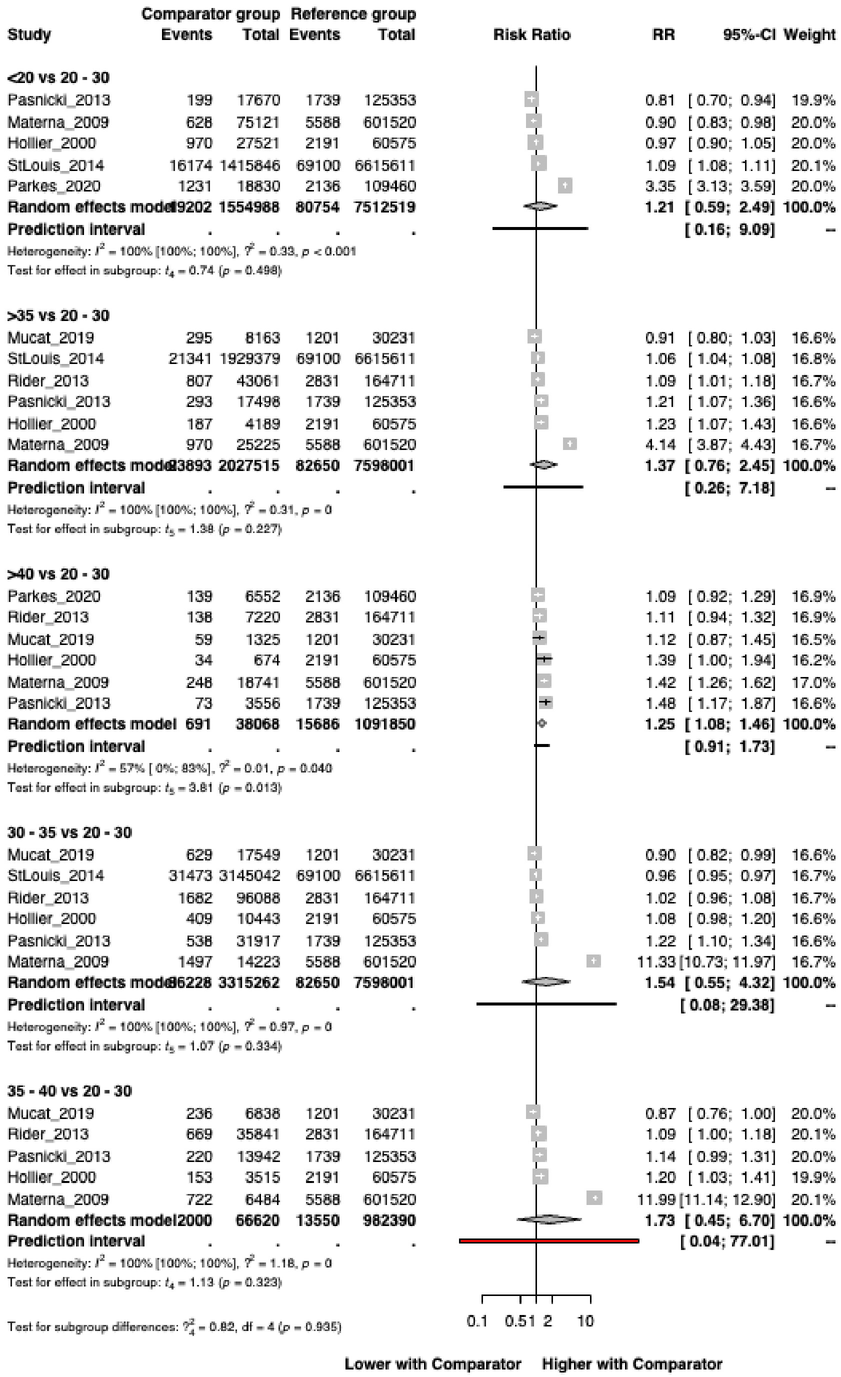


Figure 7. *Forest plot representing the RR with 95% CI of all NCAs combined (excluding studies where co-incidence of CAs was allowed) ICD-10 Q00-Q89 in different age groups compared to the 20-30 age group (85)*

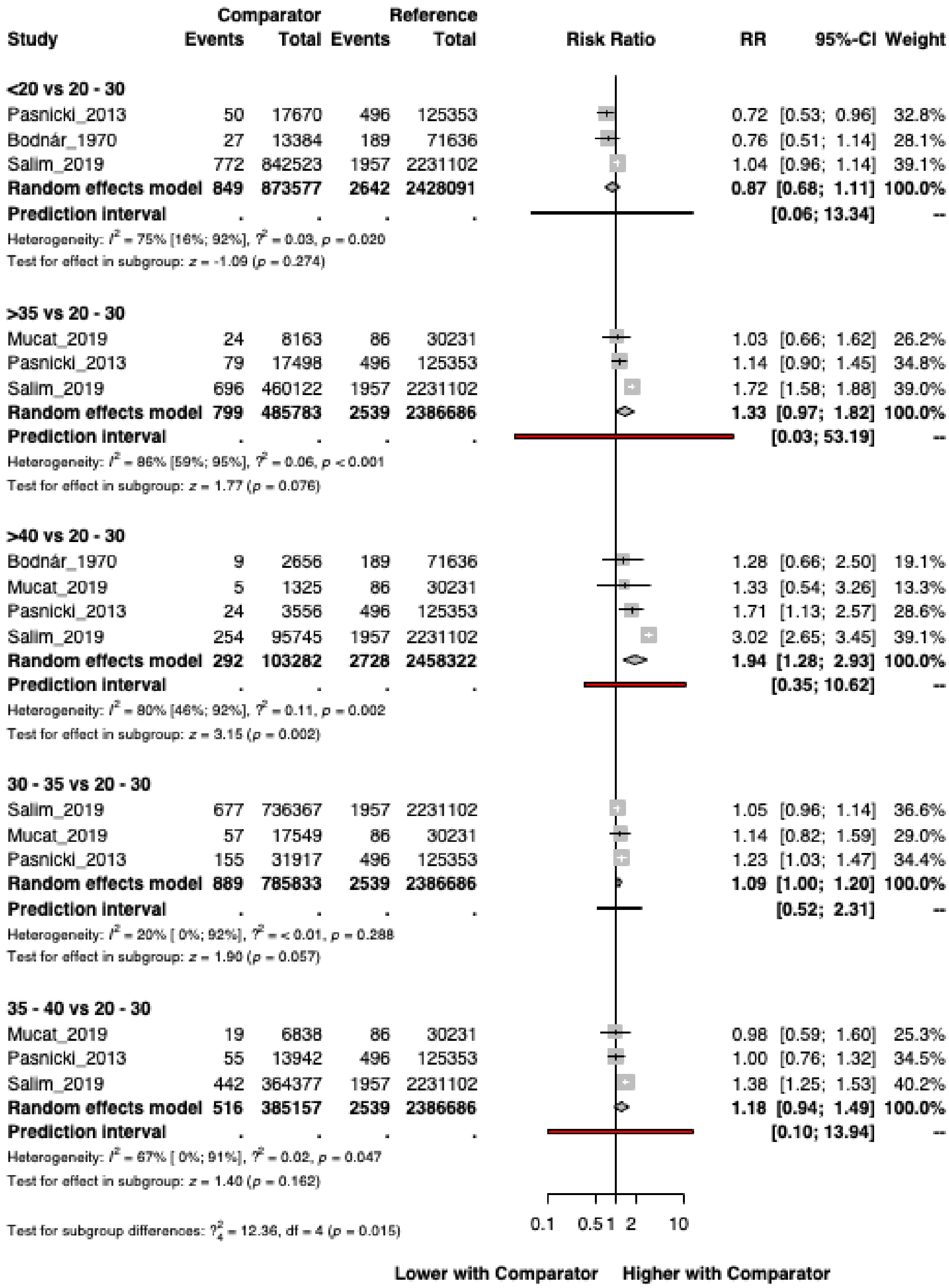


Figure 8. *Forest plot representing the RR with 95% CI of congenital anomalies of the circulatory system (ICD-10: Q20-Q28) in different age groups compared to the 20-30 age group. (85)*

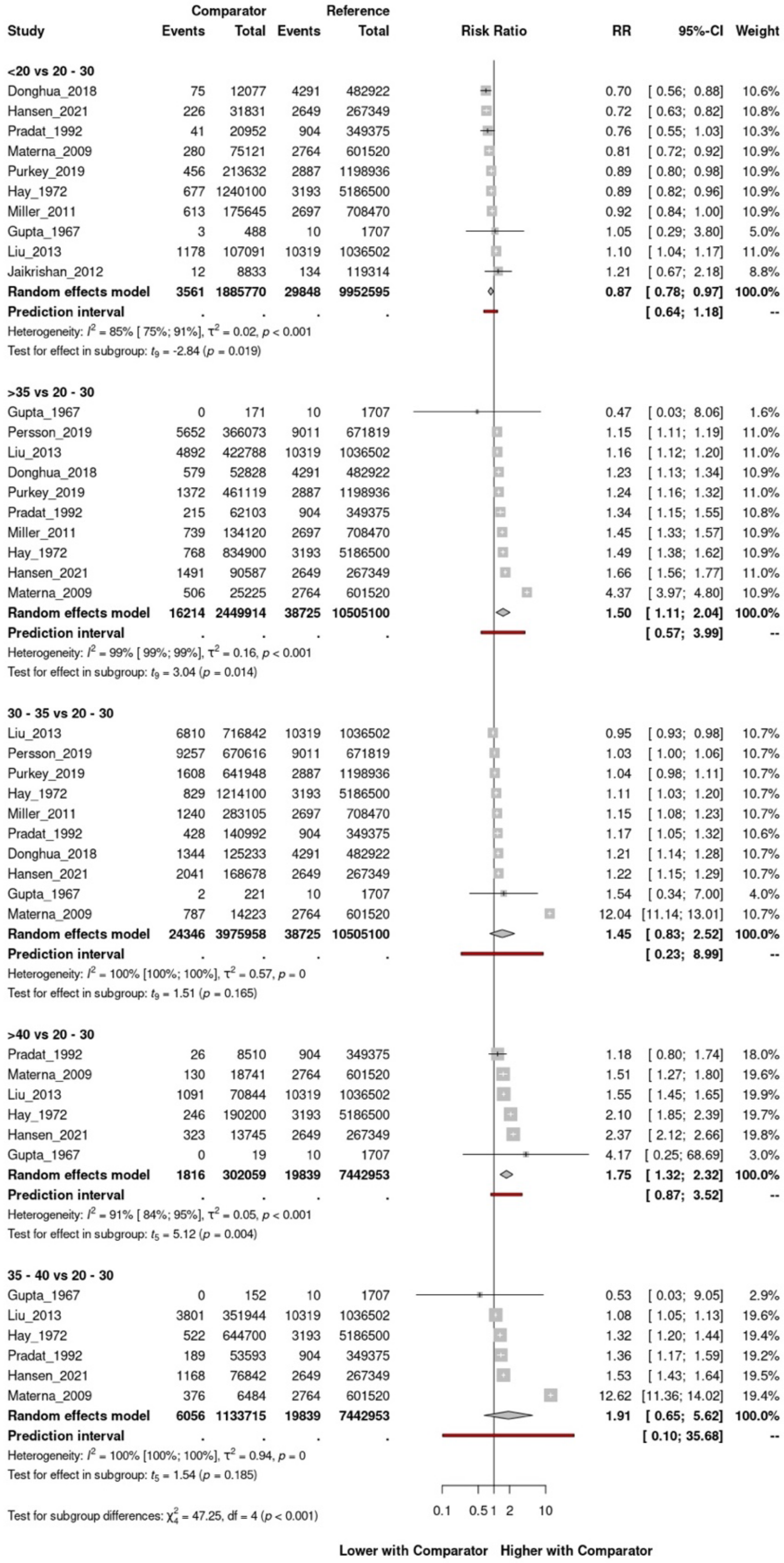


Figure 9 *Forest plot representing the RR with 95% CI of congenital heart defects (ICD-10: Q20-Q26) in different age groups compared to the 20-30 age group(85)*

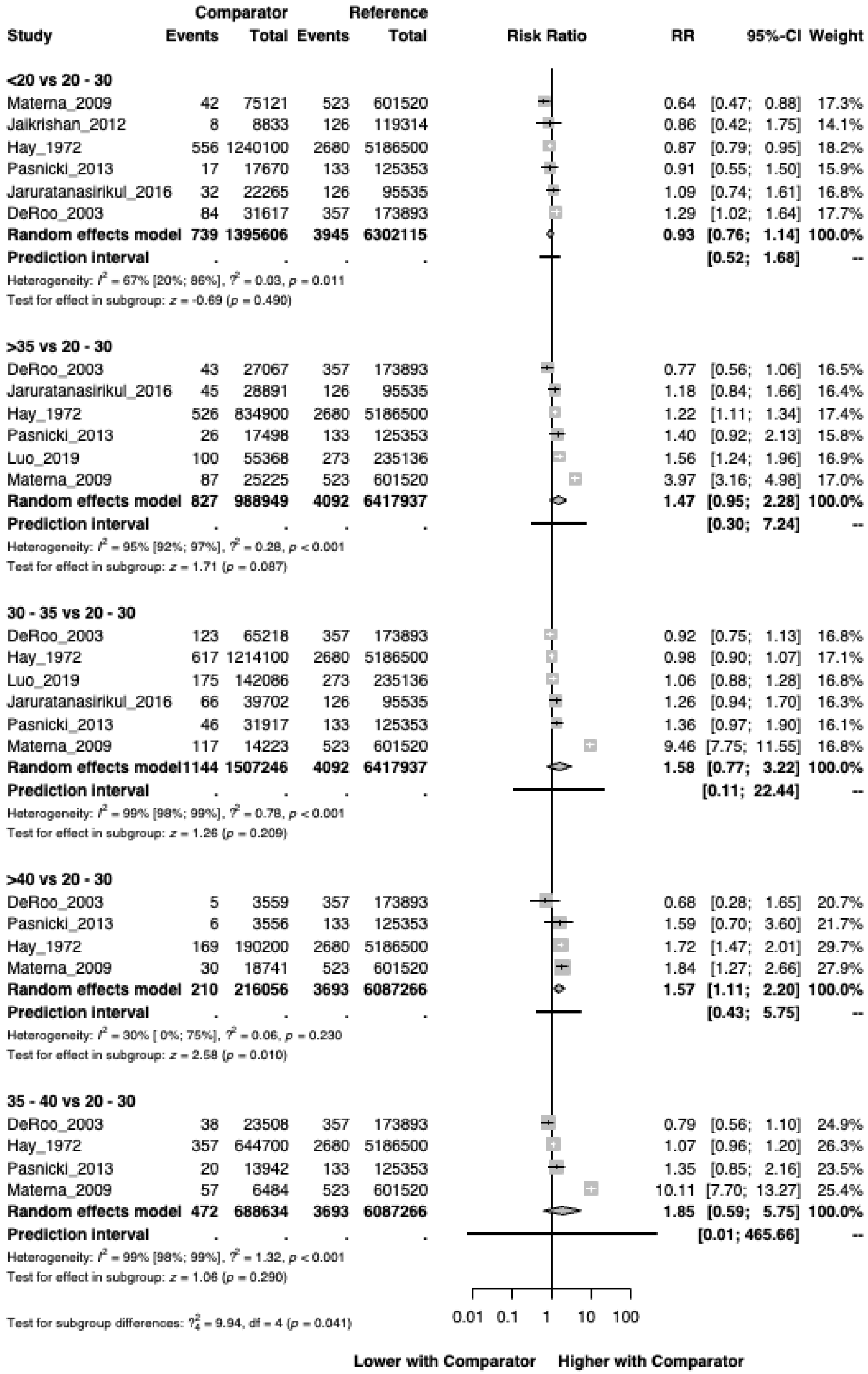


Figure 10. *Forest plot representing the RR with 95% CI of cleft lip and cleft palate (ICD-10: Q35-Q37) in different age groups compared to the 20-30 age group.(85)*

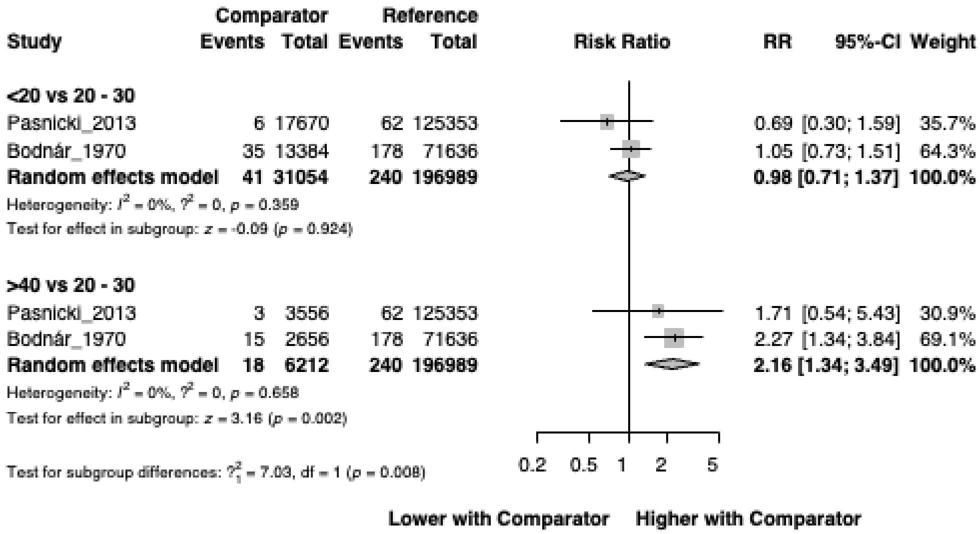


Figure 11. *Forest plot representing the RR with 95% CI of congenital anomalies of the digestive system (ICD-10: Q38-Q45) in different age groups compared to the 20-30 age group.(85)*

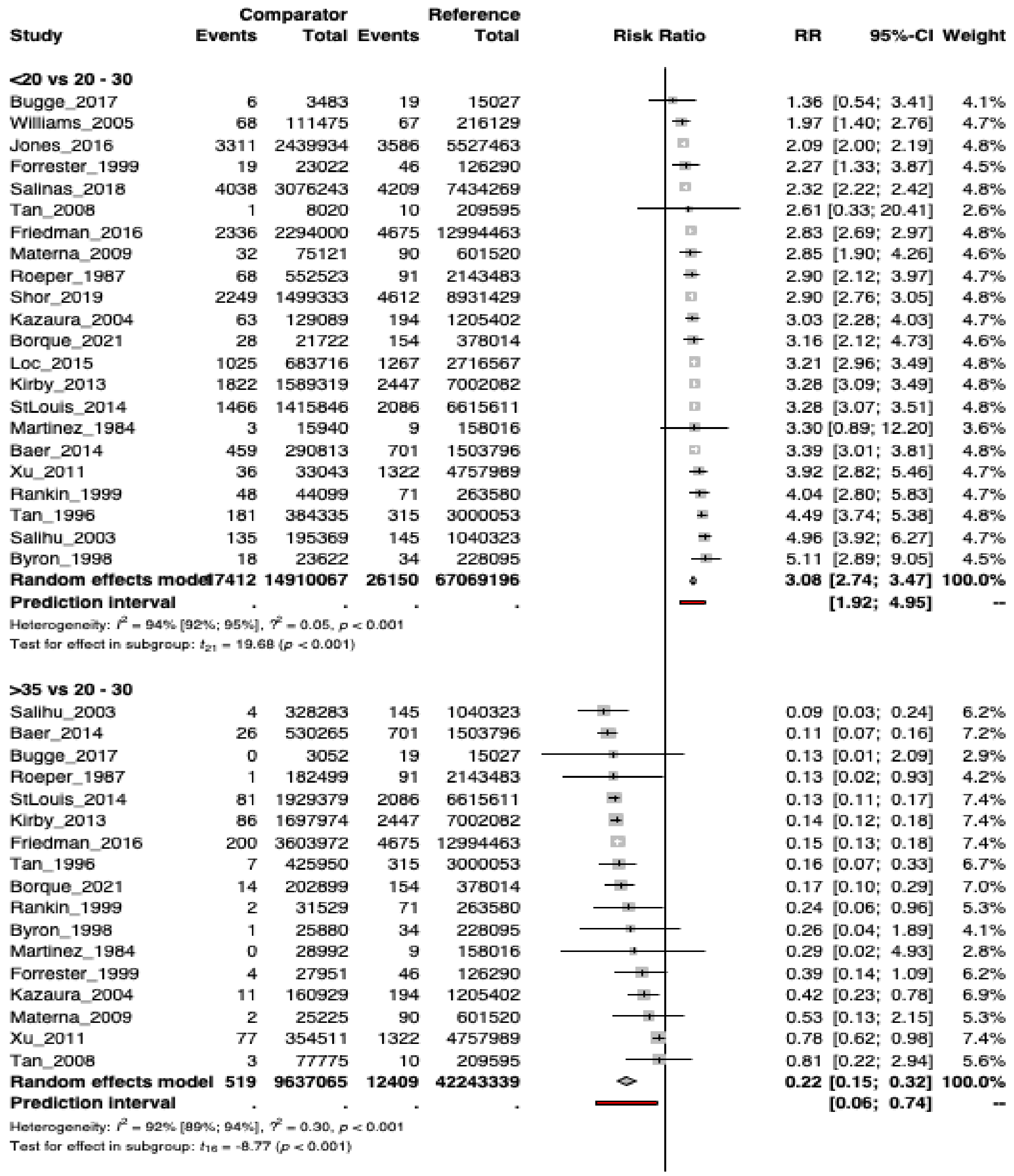


Figure 12. *(continued below)*

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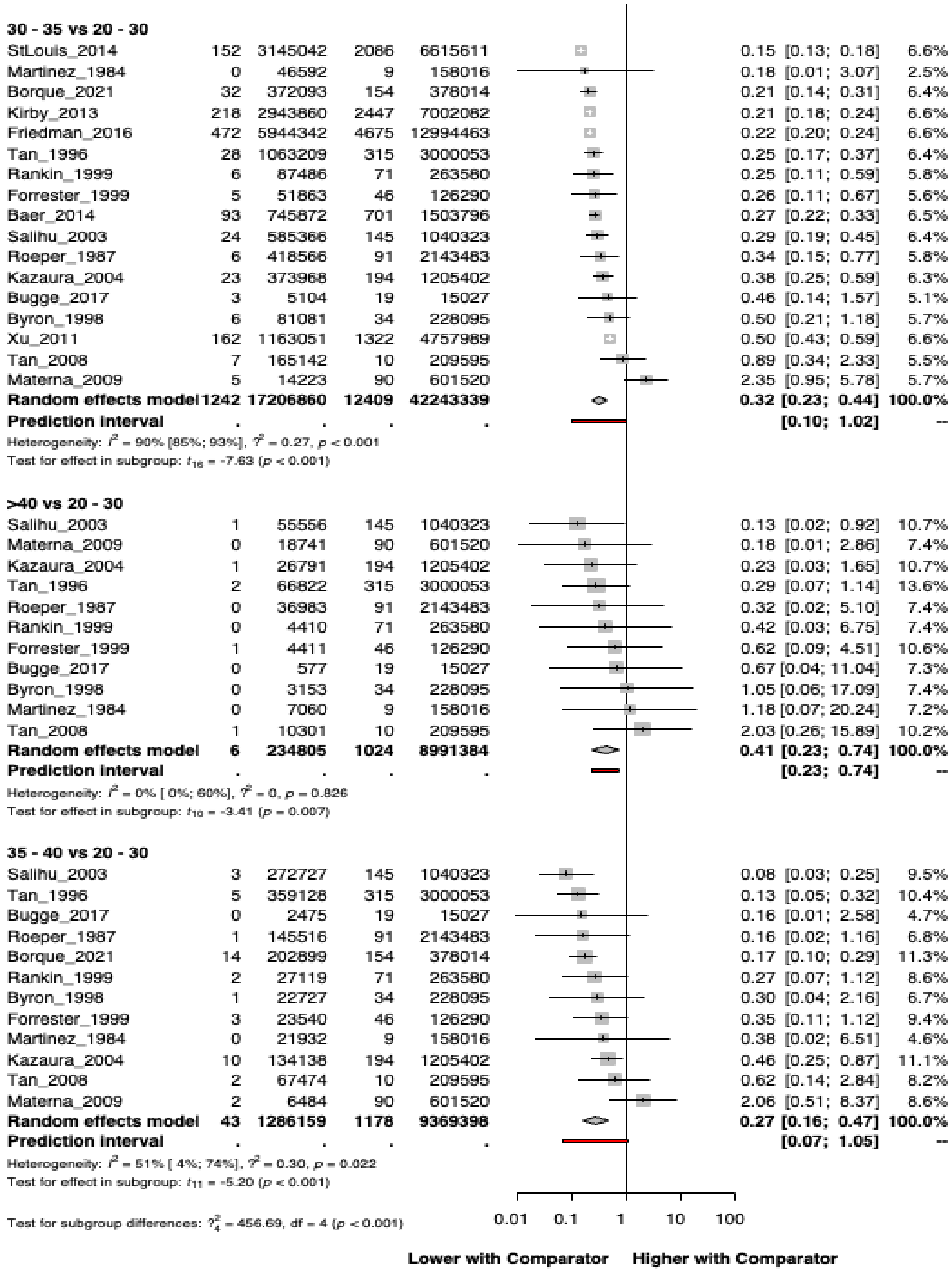

Figure 12. *Forest plot representing the RR with 95% CI of gastroschisis (ICD-10: Q79.3) in different age groups compared to the 20-30 age group.(85)*

Table 6. *Comparing the findings of both of our studies*

Congenital anomaly	ICD-10 Category	meta-analysis reference age range: 20-35			population-based analysis reference age range: individual for each		
		age < 20	age > 35	age > 40	age < 20	age > 35	age > 40
All NCAs (with or without CAs)	Q00-Q89	1.08 (0.89; 1.32)	1.31 (1.06; 1.61)	**1.44 (1.25; 1.66)**	no data	no data	no data
All NCAs (without CAs)	Q00-Q89	1.21 (0.59; 2.49)	1.37 (0.76; 2.45)	1.25 (1.08; 1.46)	**1.37 (1.32; 1.43)**	1.21 (1.16; 1.26)	1.35 (1.23; 1.49)
All NCAs (with CAs)	Q00-Q89	1.15 (0.87; 1.52)	1.26 (1.12; 1.42)	**1.63 (1.26; 2.09)**	no data	no data	no data
Nervous system	Q00-Q07	1.16 (0.74; 1.81)	1.53 (0.80; 2.94)	1.56 (0.67; 3.62)	**1.71 (1.48; 1.97)**	1.05 (0.87; 1.26)	1.25 (0.84; 1.86)
Encephalocele	Q01	1.76 (0.44; 7.12)	1.43 (0.57; 3.60)	no data	no data	no data	no data
Congenital hydrocephalus	Q03	1.19 (1.02; 1.38)	no data	no data	no data	no data	no data
Spina bifida	Q05	1.30 (0.93; 1.82)	1.39 (0.75; 2.59)	1.96 (0.72; 5.31)	no data	no data	no data
Anencephaly	Q00.0	1.40 (0.98; 1.99)	1.02 (0.60; 1.72)	1.30 (0.71; 2.38)	no data	no data	no data
Eye, ear, face, and neck	Q10-Q18	no data	no data	no data	1.25 (0.94; 1.66)	1.24 (0.92; 1.69)	**2.09 (1.25; 3.49)**
Circulatory system	Q20-Q28	0.87 (0.68; 1.11)	1.33 (0.97; 1.82)	**1.94 (1.28; 2.93)**	1.16 (1.07; 1.26)	**1.40 (1.29; 1.52)**	**1.72 (1.45; 2.05)**
Congenital heart defects	Q20-Q26	0.87 (0.78; 0.97)	1.50 (1.11; 2.04)	**1.75 (1.32; 2.32)**	no data	no data	no data
Respiratory system	Q30-Q34	no data	no data	no data	1.82 (0.83; 4.03)	1.00 (0.40; 2.51)	1.32 (0.29; 6.13)
Cleft lip and palate	Q35-Q37	0.93 (0.76; 1.14)	1.47 (0.95; 2.28)	1.57 (1.11; 2.20)	1.21 (1.05; 1.40)	**1.45 (1.26; 1.67)**	1.58 (1.16; 2.16)
Cleft palate	Q35	0.99 (0.56; 1.73)	1.78 (1.16; 2.73)	**1.77 (1.48; 2.11)**	no data	no data	no data
Digestive system	Q38-Q45	0.98 (0.71; 1.37)	no data	**2.16 (1.34; 3.49)**	**1.46 (1.31; 1.64)**	1.16 (1.02; 1.32)	1.15 (0.85; 1.57)
Genital organs	Q50-Q56	no data	no data	no data	1.36 (1.24; 1.50)	1.15 (1.03; 1.29)	1.30 (1.02; 1.66)
Urinary system	Q60-Q64	no data	0.86 (0.57; 1.29)	no data	1.29 (1.04; 1.60)	**1.90 (1.56; 2.32)**	**2.27 (1.53; 3.38)**
Hypospadiasis	Q54	**0.99 (0.91; 1.07)**	1.11 (0.88; 1.39)	no data	no data	no data	no data
Musculoskeletal System	Q65-Q79	0.88 (0.72; 1.08)	0.94 (0.65; 1.37)	0.90 (0.55; 1.46)	**1.57 (1.46; 1.70)**	1.12 (1.02; 1.23)	1.07 (0.86; 1.34)
Congenital Diaphragmatic Hernia	Q79.0	**0.96 (0.88; 1.06)**	1.52 (0.79; 2.91)	no data	no data	no data	no data
Omphalocele	Q79.2	1.44 (1.08; 1.92)	1.47 (1.20; 1.79)	**2.57 (1.77; 3.73)**	no data	no data	no data
Gastroschisis	Q79.3	**3.08 (2.74; 3.47)**	**0.22 (0.15; 0.32)**	**0.41 (0.23; 0.74)**	no data	no data	no data
Other	Q80-Q89	no data	no data	no data	**1.45 (1.25; 1.68)**	1.35 (1.15; 1.59)	1.70 (1.23; 2.36)

9 DISCUSSION

9.1 Summary of findings, international comparisons (including all studies)

The main findings of our studies support our hypothesis. The relative frequency of all NCAs combined is strongly related to maternal age. The importance of our findings lies not only in their clinical relevance, but in their quality. The population based study encompasses a long time period for a whole country with almost 3 million births, maternal age data is available by year, all level 2 malformation categories of ICD-10 were assessed, aims for a high level of transparency, and a complex statistical analysis approach was used – this is particularly apparent if we compare it with other similar studies (e.g. those included in the meta-analysis). The meta-analysis provides a higher level of evidence for a worldwide audience than our registry analysis. It is the first of its kind (i.e. analyzing all NCAs combined and also separately by categories), and we made our best to avoid typical design flaws (c.f. Ahn et al 2022, (158)): we only pooled population-based studies with matching age groups and NCA categories.

<u>All NCAs combined (ICD-10: Q00-Q89)</u>

Our meta-analysis revealed risk increase above 35 and a more relevant increase above 40. It thus confirmed the risk-increasing effect of advanced maternal age. In contrast, our population-based study found that both very young and advanced maternal age increases the risk, when all NCAs are considered collectively.

Though the meta-analysis also shows an increase in risk in very young mothers, but here statistical significance does not support a clinical association. The main reason for this is the high heterogeneity (temporal and geographical differences), which suggests that the risk-increasing effect of young maternal age may be prevalent in certain regions (6/14 of the included articles also found a risk-increasing effect of extremely young maternal age). Despite the topic being extensively researched, the age distributions of different NCAs show inconsistencies in the literature. The risk increasing effect of advanced maternal age is consistent with previous research(54, 159, 160), highlighting the importance of considering advanced maternal age as a risk factor in prenatal care and genetic counseling. The 2022 meta-analysis on the subject (158) addressed advanced maternal age as a risk factor. The increased risk observed in older mothers can be attributed to a variety of factors, including increased rate of IVF (in vitro fertilization) (161-164), increased prevalence of comorbidities particularly pregestational diabetes mellitus (165-167), and a higher likelihood of long-term exposure to environmental factors.(168, 169) In contrast to our findings, certain studies have questioned the risk-increasing effect of advanced maternal age.(53, 170) This may be explained by the fact that the increase in maternal age in Europe is especially associated with women of higher social status, which may have led to a decrease in the risk of NCA in this age group compared to previous trends.(53, 171)

Several studies indicate that advanced maternal age is linked to a decreased risk of NCAs. To explain this, researchers hypothesize that the embryonic development is more strongly influenced by the "all-or-nothing" phenomenon than the aging of the egg – this results in a higher chance surviving fetuses are anatomically normal.(172)

In line with our population-based study, Reefhuis et al.(55) demonstrated that women under 20 years and women over 35 years are at increased risk of having a fetus with an NCA. Croen et al.(173) also observed this association in their data analysis from the California Birth Defects Monitoring Program, except for the Afro-American population. Analyzing data from the EUROCAT database, Loane et al. argue (53) that greater attention should be given to the screening of adolescent mothers, as they are more prone to having multiple risk factors. Possible factors contributing to this increased risk among younger mothers encompass insufficient prenatal care, a greater prevalence of socioeconomic disadvantages, and an elevated vulnerability to nutritional deficiencies during pregnancy.(57)

The effect of advanced maternal age on the risk of chromosomal anomalies is well known. In addition to chromosomal anomalies, the prevalence of NCAs is also higher, so as a significant confounder, we excluded the co-occurrence of chromosomal anomalies from our population-based study and in the case of meta-analysis, we also performed an analysis that tests the hypothesis without the co-occurrence of chromosomal anomalies. In this case, we found a 25% increase in risk for mothers over 40. This further supports the idea that a mother's age can be an independent risk factor, since having chromosomal anomalies at the same time is one of the most significant variables that can influence the occurrence.

Congenital malformations of the nervous system (ICD-10: Q00-Q07)

In the case of neural tube defects (NTD), there is already a well-known and high level of evidence that folic acid supplementation is effective in preventing these disorders.(174) In addition to this well known preventive option, there are further possibilities for secondary prevention of this group of anomalies through neurosonography or fetal MR scans. Hence, it is crucial in clinical practice to identify risk factors in order to improve the criteria for diagnostic approaches.

Our population-based analysis reveals a significant and large increase in risk in very young mothers, but the meta-analysis shows no significant effect on the risk. The latter could be explained by the high level of heterogeneity caused by population differences. As a result, we cannot draw broad conclusions, but we do see an increase in risk locally both in advanced and very young age categories. The studies included in the meta-analysis demonstrate either no significant effect or a significant risk increase.

The literature is not consistent on the age effect in this case either. Most studies have found a 'U-shaped' relationship between maternal age and the relative frequency of NTDs.(103, 175) Other researchers suggest that a higher risk of NTD is probably associated with increased maternal age.(176) The heterogeneous results could be attributed to an inappropriate NTD definition, as grouping was not applied uniformly across studies. Some anomalies were explicitly associated with young maternal age (e.g. anencephaly)(102, 177), while other isolated anomalies were more common in older mothers(e.g. spina bifida, encephalocele).(177)

Encephalocele (ICD-10: Q01): No significant effect was found for any age category in our meta-analysis. Wen et al. discovered that younger maternal ages are specifically associated with encephaloceles. This association was not explained by maternal education level or the timing of prenatal care initiation in their study.(178) A 2024 meta-analysis found that the age of the mother was a factor in the occurrence of encephaloceles. Two publications showed a link between encephaloceles and very young maternal age, while another publication documented a connection with advanced maternal age.(179)

Congenital hydrocephalus (ICD-10: Q03): In our meta-analysis, we were only able to examine the effect of very young maternal age, and even in this category, only 2 studies could be mathematically synthesised. As a result, an increase in risk is observed in the very young age group, which, despite combining the findings of only two papers, is a mathematically significant result. Reefhuis and Honein also discovered that teenage mothers had a significantly higher risk of having hydrocephalus offsprings than mothers aged 25–29 years (OR = 1.56; CI: 1.23–1.96). The increased risk could be attributed to confounding lifestyle factors like insufficient prenatal care and exposure to harmful substances.(55) A 2023 case-control analysis also confirms this link.(180) Another study also identified a risk increasing effect of maternal age, but for very young and advanced age (U-shaped distribution).(181) In contrast, in another study maternal age was not associated with any subtype of hydrocephalus.(182) In this case, a variety of causal factors may explain the inconsistency of the literature.

Spina bifida (ICD-10: Q05): Most of the studies included in the meta-analysis found an increase in risk among mothers in the examined age groups. However, due to the large confidence intervals, the pooled values cannot statistically prove or disprove the risk-increasing effect. Consistent with our findings, the literature reviews on this subject do not acknowledge the potential for maternal age to increase the risk of spina bifida.(183, 184)

Anancephaly (ICD-10: Q00.0): Most of the articles included in the meta-analysis do not show a significant effect and the pooled value does not show evidence for the presence or absence of a risk factor. The literature does not mention maternal age as a relevant risk factor for anencephaly either.(185, 186)

<u>Congenital malformations of eye, ear, face and neck (ICD-10: Q10-Q18)</u>

There was insufficient data for mathematical synthesis in the meta-analysis. Our population-based study showed a clinically and statistically significant increase in risk over the age of 40 years. Congenital anomalies of the face and neck are one of the most difficult to diagnose prenatally (187), and there is no clear reference in the literature to the risk factor we have studied. A 2024 study in the same setting as ours (i.e. using ICD-10 categories) found no association with maternal age.(188) Given the paucity of data on this topic, further studies are needed to assess the link.

Congenital Malformations of the Circulatory System (ICD-10: Q20-Q28)

There is a clinically and statistically significant increase in risk above the age of 40 years in both the meta-analysis and the population-based study. In the case of the meta-analysis, despite the large heterogeneity, this is strong evidence. The effect of very young maternal age is not detected in the meta-analysis, and although it is significant in the population-based analysis, the effect is minimal.

The risk-increasing effect of advanced maternal age can be found in the literature(189), but most research specifically focuses on cardiac malformations within other anomalies of the circulatory system.

Congenital Heart Defects(CHD) (ICD-10:Q20-Q26): Due to the differences in ICD classifications and for conceptual reasons, this group was not included in the population-based study (only ICD main groups were analysed). In the meta-analysis, there is a statistically and clinically significant increase in risk in advanced maternal age (both 35 and over 40). There is a slight protective effect in the very young maternal age category, but this is barely clinically relevant.

The study of this subgroup of anomalies is particularly important, both in terms of their frequency and severity, as well as due to the potential for specific screening methods. Currently, fetal echocardiography is not recommended based on the mother's age.(190, 191)

Similar to our results, several studies – including a 2024 meta-analysis on the subject – report an increase in risk in advanced maternal age.(55, 188, 192, 193) Mamasoula et al. identify both very young and advanced maternal age as a risk factor and specifically highlight the association of very severe CHDs in the very young group.(194) Our study and the scientific literature are consistent on the risk-adjusting effect of advanced maternal age, but further publications are not consistent for very young mothers. This finding necessitates additional investigation to validate and explore the influence of behavioral or genetic factors.

Congenital malformations of the respiratory system (ICD-10: Q30-Q34)

The meta-analysis lacked sufficient data for mathematical synthesis. The population-based analysis yielded estimates with a wide confidence interval due to the limited sample size, so the presence or absence of risk could not be determined in this study either. Most of the studies in the literature failed to confirm or refute the existence of a link with maternal age.(170)

Varela et al. described an association between lower social status and congenital respiratory disorders(195) , which may increase the need to examine the very young age of the mother.

Cleft lip and cleft palate (ICD-10: Q35-Q37)

In this anomaly group, there is a significant increase in risk above 40 according to both our population-based analysis and the meta-analysis. In our population-based study, we found an increased risk in both the under-20 and the over-35 age groups, but the meta-analysis could not confirm this. There is no consensus in the literature on the association with maternal age either: neither its existence nor its exact nature is agreed upon. A study carried out in California showed that women older than 39 years had twice the risk of having a child with left lip and cleft palate when compared to mothers between 25 and 29 years.(196) In contrast, a 2002 meta-analysis found no association with maternal age (197), which is also confirmed by a 2010 study.(198)

Cleft palate (Q35): When analysed independently, there is a clinically and statistically significant increase in risk for cleft lip above 35, not just above 40, but smaller confidence intervals above 40 provide stronger evidence. According to a 2012 meta-analysis mothers aged 35 to 39 years had a 20% higher risk of having a child with a cleft palate, and mothers aged 40 or more had a 28% higher risk.(199)

Congenital malformations of the digestive system (ICD-10:Q38-Q45):

Our results are very contradictory, because the meta-analysis shows that there is a significant increase in risk above 40, while the population-based study shows an increase in risk already

above 35 and below 20. A severe limitation is that only two articles were included in the meta-analysis. The available evidence concerning maternal age is contradictory. Loane et al. found that young maternal age is a risk factor(53), while a meta-analysis in 2022 could not confirm the effect of maternal age in either the very young or the advanced maternal age group.(158)

Congenital malformations of genital organs (ICD-10: Q50-Q56)

In the meta-analysis, there were insufficient data to examine the maternal age groups in question. In our population-based study, we observed an increase in risk of around 15% in both the very young and advanced age categories. There is limited data available in the literature that has examined these differences as a group. The risk-increasing effect of advanced maternal age is confirmed by Reefhuis et al for male genital defects, moreover, they also found that very young maternal age is a risk-increasing effect in case of female genital defects.(55) A meta-analysis has demonstrated a risk-increasing effect of advanced maternal age when genital organ defects were merged with urinary anomalies. In this setting, the risk increase for mothers over 35 was 46%.(158)

Hypospadiasis (ICD-10:Q54): Based on the meta-analysis, we can conclude that there is no effect in the younger population while the evidence to determine the presence or absence of risk in the elderly population is insufficient. The literature supports the risk-increasing effect of advanced maternal age. According to Fisch et al. and Porter et al., advanced maternal age is associated with a marked increase in risk.(200, 201)

Congenital malformations of the urinary system (ICD-10: Q60-Q64)

The 3 studies included in this meta-analysis did not show a significant effect of advanced maternal age (study count for the rest of the age groups was insufficient). However, in our population-based study, we found a risk-increasing effect for both the very young and the advanced maternal age, with a 2-fold increase in risk above 40. Another population-based study in Washington state confirmed the risk-increasing effect of advanced maternal age, but they found only a 20% increase in risk.(202)

Congenital malformations and deformations of the musculoskeletal system (ICD-10: Q65-Q79)

Based on our population-based study, both very young and advanced maternal age have a risk-increasing effect. Based on a meta-analysis, however, we were unable to confirm the presence or absence of risk. Considering the diseases in this group, very limited data are available in the literature.

Congenital diaphragma hernia (ICD-10: Q79.0): The meta-analysis could not prove either a risk or a protective effect in any of the examined age groups. A population-based study written in 2019 did not find an association between maternal age and congenital diaphragma hernia either.(203) In contrast, a registry analysis in 2022 found that both very young and advanced maternal age pose increased risk.(204)

Omphalocele (ICD-10: Q79.2): The meta-analysis suggests that both very young and advanced maternal age increase the risk, with this risk-increasing effect being particularly pronounced over 40. Marshall et al. came to the same conclusion (205) and an earlier review article described this link as well.(206)

Gastroschisis (ICD-10: Q79.3): In our meta-analysis, we found a 3-fold increase in risk in the young and a protective effect in the older age groups. The relevant scientific literature confirms the finding for the young age group. A review in 2000 found a clear and strong risk-increasing effect of young maternal age.(207) A 2020 metaanalysis of 29 studies looking into the possible factors underlying the risk-influencing effect of young maternal age discovered that maternal smoking (RR = 1.56; CI 1.40–1.74), illicit drug use (RR = 2.14; CI 1.48–3.07), and alcohol consumption (RR = 1.40; CI 1.13–1.70) were all associated with an increased risk of gastroschisis.(208) A 2024 study discovered that the prevalence of gastroschisis increased by 61% between 1980 and 2017 in the surveillance programmes studied. The increase was observed across all age groups, with mothers under the age of 20 having the highest incidence.(209)

9.2 Strengths (including all studies)

The strengths of our research greatly enhance the dependability and application of our findings. A meta-analysis combined with a population-based study offers a thorough and strong investigation into the influence of maternal age on NCAs.

The population-based study provided several distinct advantages to our research. The extensive number of cases and controls yielded a sizeable dataset, which is crucial for rigorous statistical analysis. We employed a distinctive database and rigorous data collection techniques to guarantee the precise recording of information. The meticulous gathering of this data minimized potential biases and improved the dependability of our results. In addition, the innovative statistical methodology we utilized enabled us to depict reality with greater precision, so circumventing the constraints linked to arbitrary grouping by age.

Throughout our meta-analysis, we followed our pre-registered protocol rigorously, guaranteeing transparency and consistency in our methods. Through the implementation of a meticulous approach, we guaranteed the incorporation of a wide range of population-based publications from different geographical areas across the globe. This method enabled us to acquire a thorough and inclusive viewpoint on NCAs. Through the analysis of data from a substantial number of cases, we have improved the applicability of our conclusions, ensuring that our findings are pertinent to a wide range of people. The inclusion of studies with an international scope enhances the generalizability and application of our conclusions, offering insights that are useful on a worldwide scale.

9.3 Limitations (including all studies)

Although our research offers valuable insights into the association between maternal age and NCAs, it is crucial to recognize the inherent limitations in our study designs.

The population-based study revealed comparable constraints. Throughout the extended duration of the study, minor modifications in the screening techniques and rates of detection may have had an impact on our findings. Furthermore, the definitions of certain individual anomalies exhibited variations over time or were completely absent in certain cases, resulting in inconsistencies. Although the documents were organized based on ICD-10 categories, there were instances where it was challenging to precisely identify anomalies, which had a negative effect on the accuracy of our data. An other limitation of this study was the lack of a multivariate model, which was due to the insufficient information available on the general population compared to the detailed data on pathological cases.

A notable constraint in the meta-analysis stems from the fact that all the studies included in it have a retrospective design. The retrospective nature of this study hinders our ability to determine causality and restricts the evaluation of certain confounding variables. Publication bias is a common concern in meta-analyses, referring to the tendency of studies with non-significant results to be less likely to be published. Though we could not detect significant publication bias in our analysis, it is important to note that failing to prove the presence of bias does not prove its absence. Another source of concern may be the presence of high level of heterogeneity. However, this should only partly be considered a limitation, because heterogeneity is often a natural characteristic of the studied variable resulting from the effect of various confounders. The potential sources of heterogeneity in our study may be the following: high variability of sample sizes (smaller studies have a higher chance of random variation); the prolonged duration of the period from which studies were collected (resulting in variation of screening methods, lifestyle factors specific for age categories, the ICD categorization), geographical variations (potential variation in the detection quality screening methods, and probably even in the probability of malformations e.g. due to nutritional or socio-economic causes), categorization (not all studies used explicit ICD categories, and different editions of ICD were in use for different studies), the definition of "total number of births" (are stillbirths as well as elective abortions – carried out either due to or not due to fetal anomalies – included).

Recognizing these constraints emphasizes the necessity for careful appreciation of our discoveries and emphasizes the significance of future investigations to tackle these concerns. In order to obtain more conclusive findings and deepen our understanding of the effects of maternal age on NCAs, it is crucial to conduct prospective studies that employ consistent definitions, improved data collection methods, and incorporate multivariate analyses.

10 CONCLUSIONS

1.) Both very young (< 20 years) and advanced maternal ages (> 35 years) are associated with an increased risk of non-chromosomal congenital anomalies (NCAs) in Hungaryan population. The evidence pertaining to the advanced age category is more robust and valid worldwide.

2.) In the Hungarian population, mothers between the ages of 23 and 32 have the lowest risk of NCAs.

3.) Very young maternal age increases the risk of neurvous system anomalies in the Hungarian population.

4.) Eye, ear, face, and neck anomalies are associated to advanced maternal age in the Hungarian population.

5.) Anomalies in the circulatory system exhibit a higher risk in advanced maternal age. This relationship remains valid even in the absence of concurrent chromosomal anomalies.

6.) Congenital heart defects demonstrate higher risk at advanced (40+) maternal age and there is a suspected mild prophylactic effect in very young mothers.

7.) In the case of cleft lip and palate, both very young and advanced maternal age pose an increased risk in the Hungarian population, with this association being evident worldwide above the age of 40.

8.) Very young and advanced maternal age increase the risk of digestive system anomalies in the Hungarian population, while this risk is also evident worldwide above the age of 40.

9.) Genital organ anomalies exhibit a heightened risk in both very young and advanced maternal age groups in the Hungarian population.

10.) For urinary system anomalies, both very young and advanced maternal age increase the risk in the Hungarian population. This effect is greater in advanced maternal age group.

11.) Anomalies of the musculoskeletal system are more likely to occur in both advanced and very young mothers in the Hungarian population, but the risk is higher in younger mothers.

12.) Gastroschisis is associated with a threefold risk in very young mothers.

11 IMPLEMENTATIONS FOR PRACTICE

Early translation of research findings into clinical practice is crucial.(210, 211) It is worth considering to treat maternal age as an independent risk factor when developing prenatal screening protocols – and not only because of co-morbidities or because of the higher risk of chromosomal anomalies. Considering this factor is crucial for optimizing prenatal care and enhancing the identification of NCAs among various age groups of mothers.

Indications for fetal echocardiography and neurosonography do not currently include maternal age-based screening.(190, 191, 212, 213) Based on our results, when developing recommendations for fetal echocardiography and neurosonography, it is advisable to include advanced maternal age as an indication for fetal echocardiography and very young maternal age as an indication for fetal neurosonography. Screening protocols that take maternal age into account can improve the child's prospects by enabling timely identification for proactive medical planning, enabling parents to make informed decisions about their pregnancy. This approach recognizes the differences that women struggle with at different stages of life and contributes to personalized, effective care.

12 IMPLEMENTATION FOR RESEARCH

Methodology issues

In the analysis of the articles used for the meta-analysis, difficulties were encountered with the uniform maternal age categorisation (at least the broadly consistent use in the literature of the advanced maternal age categories /above 35/ and very young /under 20/), the lack of a standard reference age (we mark this as 20-30 based on our two analyses) and the lack of consistency with ICD categories. For future studies on this topic - in addition to eliminating the above problems - we recommend providing complete raw data (i.e. total and diseased birth count for each maternal age) for a more precise and complete synthesis of the data.

Study design

It is advisable to prioritize the analysis of the impact of maternal age by using prospective data collection in a multivariate model. Since the potential confounders are largely known (e.g. financial status, healthcare access, lifestyle choices, genetics), future research should further analyze them. This may affect the intrinsic risk increaseing effect of maternal age on NCAs. Currently, detailed data are usually available in a case-matched control model for both cases and controls, but this is not suitable for estimating true prevalence, and for population studies we do not have more detailed information on the control population. A comprehensive pregnancy registry can generate a reliable dataset for multivariate analysis and generalisable results.

New aspects

We hope, our findings will facilitate further research of the biological background. It is essential to establish the biological model behind the statistical-clinical association we have found. Due to the nature of the topic, collaboration with co-disciplines (geneticists, pediatricians, epidemiologists) can provide additional insights and valuable new aspects and enhance the quality and complexity of research.

13 IMPLEMENTATION FOR POLICYMAKERS

We have little influence on the social and societal trends that lead to delayed childbearing, so it is primarily the task of decision-makers, but also of us as practitioners, to respond to these trends with the appropriate sensitivity and effectiveness. Although the risk-increasing effects of advanced maternal age are generally more discussed in the developed world, the increased risks associated with pregnancy in very young mothers are also important to prioritise.

Regarding NCAs, at-risk mother age groups should be given top priority at several layers of prevention. To implement *primary prevention* strategies effectively, policymakers should consider the development of accessible educational programs targeting both the general population and healthcare professionals. Women should be educated about both the risk for pregnancy at particular ages and the available diagnostic methods. As *second prevention*, prioritizing comprehensive surveillance helps to implement effective monitoring systems and encourages early detection and intervention practices in healthcare facilities. Integrating emerging evidence into policy decisions helps improve early detection, intervention strategies, and outcomes for affected fetuses. Based on our studies, one of the most game-changer changes could be the provision of maternal and human resources for maternal age-based screening protocols for fetal echocardiograpy and neurosonography. As for *tertiary prevention*, mobilising adequate attention and resources is also essential, as the substantially unchanged high prevalence of NCAs in developed countries indicates that the provision of treatment protocols, rehabilitation programmes and psychosocial support can improve the quality of life of those affected.

14 FUTURE PERSPECTIVES

Building on our previous findings, we intend to continue our research on this topic in our research group, with the goal of contributing to a higher level of perinatal screening.

We should relaunch the Hungarian database, because the uniqueness of the data collection methodology and the wide range of information collected can greatly contribute to the understanding of the topic.

We plan to reproduce the meta-analysis regularly, following the current concept, as this is an intensively researched area and a significant number of new publications are expected to be published each year. It is our expectation that our publications will lead to the development of a more uniform maternal reference age and a more standardized definition of NCAs. These changes could increase the proportion of publications that can be included and synthesised, while reducing the limitations due to the expected lower heterogeneity. We also plan to conduct a meta-analysis of publications using a multivariate model.

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16 BIBLIOGRAPHY

16.1 Publications related to the thesis

1.**Pethő B,** Mátrai Á, Agócs G, Veres DS, Harnos A, Váncsa S, Bánhidy F, Hegyi P, Ács N

Maternal age is highly associated with non-chromosomal congenital anomalies: Analysis of a population-based case-control database

BJOG: AN INTERNATIONAL JOURNAL OF OBSTETRICS AND GYNAECOLOGY

2023; 130: 10 pp. 1217-1225. DOI: doi.org/10.1111/1471-0528.17461

D1, IF: 4,7

2.**Pethő B**, Váncsa Sz, Váradi A, Agócs G,Mátrai Á, Zászkaliczky-Iker F, Balogh Z, Bánhidy F,Hegyi P, Ács N

Very Young and Advanced Maternal Age Strongly Elevates the Occurrence of Non-Chromosomal Congenital Anomalies: A Systematic Review and Meta-Analysis of Population-Based Studies

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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16.2 Publications not related to the thesis

1.Galamb Á, **Pethő B**, Fekete D, Petrányi G, Pajor A.

Uterine anomalies in women with recurrent pregnancy loss

ORVOSI HETILAP 2015 Jul;156(27):1081-1084.

DOI: 10.1556/650.2015.30136. PMID: 26122902.

Q3, IF: 0,291

2. Mátrai Á, Teutsch B, Váradi A, Hegyi P, **Pethő B**, Fujisawa A, Váncsa S, Lintner B, Melczer Z, Ács N.

First-Trimester Influenza Infection Increases the Odds of Non-Chromosomal Birth Defects: A Systematic Review and Meta-Analysis. **VIRUSES**

2022; 14(12):2708.

DOI: doi.org/10.3390/v14122708

Q2, IF: 4.7

3.Mátrai Á, Teutsch B, **Pethő B**, Kaposi A, Hegyi P, Ács N

Reducing the Risk of Birth Defects Associated with Maternal Influenza: Insights from a Hungarian Case-Control Study

JOURNAL OF CLINICAL MEDICINE

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18 PUBLICATIONS