„CAROL DAVILA" UNIVERSITY
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PARTICULAR PHENOTYPICAL TRAITS AND CONGENITAL ANOMALIES IN THE NEONATAL PERIOD - RISK FACTORS

PHD THESIS SUMMARY

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INTRODUCTION

The idea that we are the result of the continuous interaction between gene, psyche, eating habits/lifestyle and environment represented the point "zero"/starting point of this PhD thesis, titled: "*Particular Phenotypic Traits and Congenital Anomalies in the Neonatal Period - Risk Factors*". *Gene/*genetic inheritance, physical traits, phenotype, genotype, ethnicity, race; *Psyche*/soul, personality, character, stress; *Eating and living habits*/ food, drinking water, tobacco, alcohol, drugs, medication, lifestyle, living conditions; *Environment*/ecosystem, microclimate, pollution, meteorological phenomena and other natural phenomena that can have influences on the human body.

During the past years an increase in congenital anomalies in the neonates born worldwide, but also nationally, was observed. Working in a neonatology ward, I was able to directly observe this: - in a state maternity hospital with the number of births amounting in average 1900/year, and with a significant number of neonates with congenital anomalies and particular phenotypic traits. Questions such as: "Why?"; "What led to the increase in the incidence of congenital anomalies?"; „Could the ecosystem in which the mother of the neonate lived be important? And if yes, what is the contribution of the environment? What is more important: the genetic factor, the risk factors that we can directly intervene upon (for example: smoking, alcohol, teratogenic drug treatments), the risk factors related to various pathologies of the mother or the environment factor?" emerged. These were just some of the questions I asked myself and which led me to choose ***congenital anomalies and risk factors*** as the main subject of my PhD thesis.

The importance of the research theme is given by the studies in the literature that appreciate the fact that the incidence of congenital anomalies in the neonate is increasing.

The following are known, among others, as risk factors that have led to an increase in the frequency of congenital anomalies in neonates in recent years:

-1) increased age at which a woman decides to have a child;

-2) the development of assisted human reproduction techniques → in vitro fertilization (IVF) is associated with a 30-40% increase in the risk of major congenital anomalies, compared to natural conception;

-3) the increase in the incidence of twin pregnancies (either as a result of IVF, or due to the increase in the age at which a woman gives birth to her first child) → monozygotic versus dizygotic twins—statistically it has been observed that the rate of malformations per fetus in monozygotic twin pregnancies is 2-3 times higher;

-4) the increase in global pollution, as well as in our country (the level of pollution in Romania exceeds the EU limits).

This PhD thesis has as main objective the determination of risk factors, other than genetic ones, that contribute to the appearance of a particular phenotypes/congenital anomalies in neonates. The results obtained from the study carried out in this PhD thesis can help us, where applicable, with a *primary prophylaxis* regarding the occurrence of congenital anomalies in pregnancy.

The secondary objectives of the PhD thesis are:

- to predict the predominant profile of the pregnant woman predisposed to having a neonate with a congenital anomaly/particular phenotypic trait;
- to define the predominant profile of the neonate with congenital anomaly/particular phenotypic trait;
- the need for the existence in every maternity hospital in the country of a register of neonatal malformations (a register that could help us have a better knowledge of the incidence and type of congenital anomaly, as well as possible associated risk factors); the final goal being the creation of a *UNIQUE NATIONAL REGISTRY OF NEONATAL MALFORMATIONS*;
- the collaboration of specialists from different fields/the formation of multidisciplinary teams with the aim of establishing the diagnosis and providing the earliest possible treatment for these patients with congenital anomalies/rare diseases (some of them being with special needs);
- making informative notes and recommendations for public use, as well as NGOs for helping patients with phenotypes and/or congenital anomalies. Providing psychological and financial support for the affected families;
- increasing the promotion by doctors of a healthy lifestyle (especially family physicians, obstetricians, neonatologists, pediatricians, geneticists etc.)

- the involvement of physicians (of any specialty) and the general public in improving the environment, under the premise: “A clean environment, a better life!”

I. General Part

The general part consists in three chapters, each belonging to a different field of study:

Chapter 1, entitled "**Human-Environment Interaction**" brings vast information from the ***field of environmental ecology***. The *concept of monitoring* is presented and explained, the national and international organizations with the role of protecting the environment and health are listed, the term environmental pollution and its causes of production are defined, the types of pollution are exemplified. The chapter has sub-sub-chapters dedicated to air, water and soil pollution, as well as the sub-sub-chapter named "*Effects of pollution on the human body*".

Chapter 2, entitled "**The Gene and Congenital Anomalies**", belongs to the ***medical field***, specifically medical genetics, clinical genetics, cardiology and neonatology. It consists of four sub-chapters: **Glossary of genetics** (terms from genetics are defined), **Classification of Congenital Diseases** (for a better knowledge and recognition of congenital diseases), **Suspicion in Neonatology of a Congenital Disease** (working tool for the neonatologist) and **Congenital Heart Defects in Neonates - Synthesis**.

I decided to dedicate a special space to congenital heart defects in my PhD thesis, since these are the most common congenital anomalies encountered, but nevertheless, they can sometimes go undiagnosed (including severe forms!). The sub-chapter is divided into several sub-sub-chapters, as follows:

2.4.1. *Epidemiological generalities in CHD* → epidemiological data (incidence, prevalence), etiology, risk factors.

2.4.2. *The neonate with CHD* → the complete clinical examination of the neonate with cardiac problems (including the dysmorphic neonate), short list of the most common genetic syndromes that also associate CHD, the FOCUS method for rapid detection of organic or functional cardiac changes (malformation/structural changes/ functional changes).

2.4.3. *CHD Summary* → cardiac malformations with critical neonatal manifestations, brief review of CHD in neonates (according to the alphabetical index).

Chapter 3 of the general part, entitled "**Psyche, Food and Lifestyle**", belongs mostly to the ***field of psychology***. *Personality typologies* are classified. This chapter also deals with notions regarding healthy eating and lifestyle (*Physical activity, Sleep, Social activity*,

Intellectual stimulation, Mental balance, Dangerous lifestyle, Consumption/Abuse of toxic substances).

II. Personal Contributions

This PhD thesis is based on a retrospective observational study, in which no intervention is made in any way, but only the risk factors with possible contribution to the appearance of a particular phenotypes and congenital anomalies in neonates are analyzed.

From the beginning, it was desired for the steps necessary to carry out the study and the final goal of the current work to be only *prophylactic*, nothing being invasive. Starting from the title of the paper, "*Particular Phenotypic Traits and Congenital Anomalies in the Neonatal Period - Risk Factors*", we deduce which is the *population included* in the study, as well as the *main objective* of the PhD thesis. These are *extremely vulnerable subjects, namely neonates*; no analyzes (of any kind) were taken for research purposes. The ***main objective*** is to determine risk factors, other than genetic ones, that contribute to the occurrence of congenital anomalies and particular phenotypic traits in the neonatal population; in fact, *primary prophylaxis* is desired in terms of their prevention.

Starting from the data known from the specialized literature regarding the complexity of the factors that can trigger or aggravate a disease, the present work aims to analyze mainly the risk factors of congenital anomalies and particular phenotypic traits in the neonatal period. Consequently, I chose to address two studies in the special part:

- The **first study** named "*Study 1 - Non-genetic Risk Factors Associated with Congenital Anomalies/ Particular Phenotypic Traits in the Neonatal Period*" wanted, as the title implies, to identify risk factors existing before conception and/or during pregnancy, with a role in triggering congenital anomalies and particular phenotypic traits in the neonate. The *study* is retrospective, observational, case-control type; it puts emphasis on the *mother-fetus binomial*, on the strong connection that exists in the assembly of these two elements, seeking to identify the possible influences of the maternal traits, the existing conditions of the mother, the behavioral type, the maternal psyche, and lifestyle, on the existence of neonatal congenital anomalies. Of course, there is a risk that should not be overlooked, in terms of the possible erroneous attribution of a relationship of simple correlation as being a relationship of causation.

- **The second study** entitled "*Study 2 - Environmental Pollution/ Influences on the Fetus*" analyzed environmental pollution at the level of different regions of the country, during the study. To carry out Study 2 I used important sources of data: the Ministry of the Environment, County Reports on the state of the environment, the National Agency for Environmental Protection. Only the regions of origin of the mothers hospitalized in the maternity ward that accounted for a significant number of subjects in the conducted study were analyzed. Study 2 looks for possible correlations between the pollution level of the region (the residence of the mother) and cases of neonatal anomalies (incidence, severity).

In carrying out the scientific research I used the following as *working hypotheses*:

1. Detailed anamnesis of maternal medical history and of the current pregnancy (which may reveal possible risk factors);

2. The general clinical examination of the neonate (which may reveal a particular phenotype or congenital anomalies);

3. Complete ultrasound examination (POCUS/ Point-of-Care Ultrasound; FOCUS/Focused cardiovascular ultrasound);

4. Data from official sources regarding environmental pollution in various areas of the country.

I Observation the fact that the research was facilitated by the equipment included in and the possibility of permanent access to the multidisciplinary team, through good collaboration with different surgical and medical departments from other pediatric/neonatology hospitals. The Bucur Maternity Hospital, namely the Neonatology Department, has benefited in recent years, with the affiliation to the support hospitals for patients with SARS-CoV-2, from new equipment (medical equipment, renovation of the wards, new installations), including two ultrasound machines. The access to the full ultrasound examination (POCUS) of the neonates from the study was due to the author's previous direct ultrasound experience (competence in general ultrasound and echocardiography).

STUDY 1 - NON-GENETIC RISK FACTORS ASSOCIATED WITH CONGENITAL ANOMALIES/ PARTICULAR PHENOTYPIC TRAITS IN THE NEONATAL PERIOD

Study 1 has as starting point the *mother-fetus binomial*, this powerful interdependence and mutual interaction that exists in the "Uterine Universe of the Mother". Only the mother action → fetus relationship is addressed, not the fetus action → mother relationship. *It is a retrospective, observational, case-control study*.

Target Group/ Case Group/ Group with Malformations/ Group with Control Patients

The inclusion criteria of the patients were:

-***Location***: Emergency Clinical Hospital "St. Ioan" Bucharest, Bucur Maternity.

-***Period***: during the study of the PhD thesis (October 2017- August 2021).

N.B.: *With the Observation that 5 neonates from 2016 are also included in the study (2 with malformations, 3 in the control group!*)

-***Subjects***: neonates (full-term, premature or post-mature) who present par*ticular phenotypic traits* and/or *congenital anomalies* and are admitted to the Neonatology Department of the maternity (born in Bucur Maternity or not within the hospital but then admitted to the maternity ward, or from other maternity hospitals and transferred to the ward).

Control group

The inclusion criteria of the patients were:

-***Location***: Emergency Clinical Hospital "St. Ioan" Bucharest, Bucur Maternity.

-***Period***: during the study of the PhD thesis (October 2017- August 2021).

N.B.: With the Observation that 5 neonates from 2016 are also included in the study (2 with malformations, 3 in the control group!)

-***Subjects***: neonates (full-term, premature or postmature) who do NOT present particular phenotypic traits and/or congenital anomalies. The selection of the members of the control group will be made, randomly, among the neonates admitted to the Bucur Maternity Hospital, but with the exclusion of the subjects included in the target group.

The working methods for the evaluation of the subjects included in the study were:

1. Detailed anamnesis of maternal medical history and current pregnancy (it may reveal possible risk factors);

2. The general clinical examination of the neonate (it may reveal a particular phenotype or congenital anomalies);

3. Complete ultrasound examination (POCUS/ Point-of-Care Ultrasound; FOCUS//Focused cardiovascular ultrasound → can confirm the intrauterine diagnosis of the malformation, can show new congenital anomalies, can make the final diagnosis of neonatal congenital anomalies).

Used materials:

- A) Observation sheet/ Detailed medical history/ Questionnaire filled in by mothers.

- B) Complete and thorough **Clinical Examination** of the neonate with a particular phenotype and/or congenital anomaly:

- complete clinical examination, accompanied by the neurological examination of the neonate;
- centimeter;
- scale;
- graphs for the positioning on percentiles of the obtained values https://percentagecalculator.net/; WHO growth chart; bulk-calculator-wt-hc-l-fenton-2013;
- depending on the general condition of the neonate, other evaluation methods are sometimes necessary: thermometer, pulse oximeter, cardiac function monitor, capnograph, etc.

-**C) Performing ultrasounds** (abdominal, brain/transfontanellar and cardiac ultrasound) of all neonates in the target group and the control group.

The statistical analysis was performed using ***IBM SPSS Statistics 21*** and ***Microsoft Office Professional Plus 2019***, and the statistical tests were ***Chi-Squared Test*** and ***Mann–Whitney-U Test***.

The Study 1 database included 273 neonates, divided as follows:

-A ***group with malformed neonates***, including 144 neonates (52.7% of cases) and

-A ***control group (neonates without malformations)***, including 129 healthy neonates (47.3% of cases).

Study 1 Conclusions

Odds Ratio (OR):

-***Neonates who had a single umbilical artery*** *are 4.6 times more likely to develop a malformation compared to those who did not have a single umbilical artery.*

-*Women who have* ***cancer within their heredo-collateral antecedents*** *are 8.5 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who have* ***genetic diseases within their heredo-collateral antecedents*** *are 8.07 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who have* ***diabetes within their heredo-collateral antecedents*** *are 2.8 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who have* ***mild genetic diseases*** *are 16.12 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who have* ***mental illnesses*** *are 6.4 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who have* ***neurological diseases*** *are 2.47 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*The chances of giving birth to a child with malformations increase by 18% in the case of women who have faced* ***maternal infections (usually cervical infections and urinary infections)***.

-*Women who had* ***viral infections during pregnancy*** *are 1.83 times more likely to give birth to children with malformations compared to women who do not have such antecedents.*

-*Women who* ***consume alcohol*** *are 4.49 times more likely to give birth to children with malformations compared to women who do not consume alcohol.*

-*Women who were* ***exposed to a toxic environment at work*** *are 2.7 times more likely to give birth to a child with malformations compared to women who were not exposed to a toxic environment.*

-***Roma women*** *are 1.6 times more likely to give birth to children with malformations compared to Romanian women.* (***N.B.***: A possible cause includes the existence of *marriages between blood relatives, pregnancies not properly managed* and the higher frequency of *underage mothers* in this ethnic group etc.).

The predominant profile of the pregnant woman predisposed to having a neonate with a congenital anomaly/ particular phenotypic trait:

Regarding the ***predominant profile of the pregnant woman predisposed to having a neonate with a congenital anomaly/ particular phenotypic trait****, we can conclude as follows:*

Woman ***around the age of 28 ½ years****, more frequently from the* ***urban environment, not married****, sometimes with a* ***slightly lower education level*** *(compared to the general population), possible* ***social case*** *(precarious standard of living etc.),* ***alcohol user, exposed to a toxic environment at work****, with a* ***greater exposure time to screens (phone/computer),*** *GI PI, with* ***heredo-collateral antecedents*** *(****cancer, genetic diseases, diabetes****), with* ***personal pathology history*** *(****mild genetic diseases, mental diseases, neurological diseases****), with* ***urinary/uterine infections*** *and* ***viral infections*** *during pregnancy, who gained* ***more than 15 kg*** *during the pregnancy, with* ***blood group incompatibility*** *and* ***polyhydramnios*** *in pregnancy.*

The predominant profile of the neonate with a certain malformation:

The neonate with a ***cardiac malformation (severe form)***: Born from an ***underage*** mother, ***alcohol drinker, of Roma ethnicity, pregnancy without medical monitoring***, ***neonate with lower Apgar Score at 1 minute*** (*compared to the existing 1-minute Apgar Score in neonates with another type of malformation*).

The neonate with ***genetic malformations (severe forms)***: Comes from a ***mother consuming alcohol***, living in an ***urban environment*** (usually).

The neonate with ***kidney malformations (mild forms)***: Comes from a mother from an ***urban residence*** *(*in *71.43%*), who has ***diabetes or cancer in their heredo-collateral antecedents***, neonate (M/F= 1:1).

The neonate with ***kidney malformations (severe forms)***: Comes from a mother from an ***urban residence*** (in *88.24*% of cases), with ***allergies*** and ***renal diseases*** *in* ***their heredo-collateral antecedents***, ***alcohol consumer***, is more often a ***neonate born at full-term, male***.

The neonate with ***cheilognathopalatoschisis***: Comes *significantly more often* from ***Rh negative mother***, the presence during pregnancy of ***oligohydramnios*** or ***polyhydramnios***, and is more frequently neonate born ***at full-term***.

Neonates with ***Genetic Syndromes***: more frequently come from a ***smoking mother*** (***actively/passively***), with ***rural residence*** in *57.14*% of cases, presence during pregnancy of ***polyhydramnios*** or/and ***green amniotic fluid at birth***, are more frequently neonates ***at full-term***. (62.5%), SGA, M/F= 1: 1.

STUDY 2 - ENVIRONMENTAL POLLUTION/ INFLUENCES ON THE FETUS

If Study 1 is focused on the mother-fetus binomial (relationship), Study 2, entitled "Environmental pollution/influences on the fetus", is attributed to the *ecosystem* in which we live, being directed to the human-environment binomial. By definition, * ECOSYSTEM = an ensemble consisting of biotope and biocenosis, within which close relationships are established both between the organisms and between them and abiotic factors. Natural unit that includes all living organisms (biocenosis) and the environment (biotope) in which they live. The relationships between living organisms and environmental factors are realized through the mutual exchange between matter and energy. [*dexonline.ro definition*]

The study aims at analyzing *environmental pollution* throughout the study at the level of the predominantly encountered counties (in the database, as the maternal domicile).

To carry out Study 2 I used sources from the *official public data* of the Ministry of the Environment, the County Reports on the state of the environment and the National Agency for Environmental Protection.

The study aims to look for possible *correlations between the pollution level within the region (mother's domicile) and cases of neonatal anomalies (incidence, severity)*.

I would like to Observation that, at national level, we do not have clear evidence of severe neonatal malformations, nor studies carried out within the country, which would refer to the action of the environment and non-genetic risk factors on the fetus.

Characteristics of Study 2:

- ***Location***: Emergency Clinical Hospital "St. Ioan" Bucharest, Bucur Maternity.

-***Period***: during the study of the PhD thesis (October 2017- August 2021).

N.B.: *With the Observation that 5 neonates from 2016 are also included in the study (2 with malformations, 3 in the control group!*)

-***Subjects***: common database with Study 1

-Material and method:

-***Material: Study 1 database*** and ***official public data*** *of the Ministry of the Environment, of the County Reports on the state of the environment and of the National Agency for Environmental Protection.*

-***Method***:

The database included 273 neonates hospitalized in the Bucur Maternity Hospital between June 2016 and August 2021, divided into 2 groups as follows: a group with malformations, including 144 neonates (52.7% of cases) and a group control, including 129 healthy neonates (47.3% of cases).

In conducting Study 2, I used the information from the database regarding the mother's residence (county/urban/rural environment) and the year of the neonate's birth for both groups. The subjects were distributed by year and county of origin and the results obtained between the two groups were compared.

Additionally, in the case of the ***group with malformations***, the *statistical analysis between the number of cases of malformations and the air quality indicators* in the counties of origin was followed, ***aiming to detect possible correlations***. ***I Observation the fact*** that the statistical analysis was carried out only for the counties of origin of the mothers admitted to the maternity hospital that accumulated a significant number of subjects in the conducted study, namely: Bucharest, Ilfov, Giurgiu and Călărași.

The ***air pollution indicators*** used for analysis in Study 2 were ***ozone, PM10 respirable suspended particulates*** and ***nitrogen dioxide***, as these are usually among the first parameters to change when air quality is affected.

Discussions Study 2

The database of Study 2 (shared with that of Study 1) included 273 newborns, divided as follows:

-A ***group with malformed newborns***, including 144 newborns (52.7% of cases) and

-A ***control group (newborns without malformations)***, including 129 healthy newborns (47.3% of cases).

Distribution of cases according to year of birth and county of origin:

In the control group

Most of the children in the ***control group*** *were born* in the years *2017, 2018 and 2019, the highest share representing the year 2017 with 34.65%* of the cases.

Depending on ***the mother's domicile***, *more than half* of the cases come from *Bucharest*, but *neighboring counties*, such as *Ilfov, Giurgiu or Călărași* are also observed as having important shares: 56.19% Bucharest, 12.38% Ilfov, 8.57% Giurgiu (9) and 6.67% Călărași (7).

In the group with malformations

Most of the children ***with malformations*** included in the group *were born* in *2017, 2018* and *2019*, with the *highest share* representing the year *2017* with 32.87% of the cases.

Depending on the ***mother's place of residence***, *more than half* of the cases come from *Bucharest*, but *neighboring counties*, such as *Ilfov, Giurgiu or Călărași* are also observed as having important shares: *55.04% Bucharest, 16.28% Ilfov, 8.53% Giurgiu and 4.65% Călărași*.

From the point of view of the *representation of cases by year and county*, ***the two groups*** were ***similar***: The best represented year was *2017*, and the mothers' domicile was *Bucharest* in over *50%* of the cases, the mothers coming from an *urban environment* in a percentage of 65.09% in the ***control group*** and *64.34%* in the ***group with malformations***. The *distribution per years* of the cases in the study is closely related to the *number of births per year in Bucur Maternity*: of the years included in the study, 2017 had the highest number of births, and the years 2020 and 2021 were at the opposite pole, since from March 2020, the maternity ward was declared COVID support medical unit. *The counties with the largest share* of representation in the study are, as it can be deducted, Bucharest and the neighboring counties, assigned to the maternity: Ilfov, Giurgiu and Călărași. *The year 2020* had a *slightly different distribution of cases from the point of view of the counties* compared to the rest of the years: neonates with mothers from much different areas of the country. The status of the Bucur Maternity as COVID support unit increased its addressability (mothers with SARS CoV-2/ COVID infection from all regions of the country were hospitalized).

*Only in **2020 the number of cases of malformations was higher in Ilfov county** than in Bucharest (**attention should be considered**: incomplete case report in 2020, as the maternity became a COVID support unit, and thus the number of presentations to the Emergency Room of pregnant women was much lower, including the number of births!).* Otherwise, for *2017, 2018* and *2019, the **year-by-year distribution of cases in the statistically representative counties was constant**: Bucharest, Ilfov, Giurgiu, Călărași (in descending order).*

Pollution indicators

The *results obtained* from the statist*ical analysis between the number of cases of malformations and the air quality indicators* in the counties of origin ***were not relevant***.

Possible factors that contributed to this situation:

-**1**. Probably, *the most important aspect* would be that, for Bucharest and the counties more often found in the database (Ilfov, Giurgiu and Călărași), ***the annual average values of the air quality indicators are within normal parameters*** (in several reports it is even stated that air quality is good).

-2. ***Some data from official public sources are incomplete***. Example: In the report from the Ministry of the Environment, the *data* from the air quality measurement stations related to *Ilfov county are assimilated to Bucharest*. For *Călăraşi county*, the *reports* from recent years no longer indicate the actual values measured for O3, PM10 and NO2, but *only the number of violations of the limit values* etc.

-3. ***The time allocated*** for the collection of the database necessary for the study of the PhD thesis is ***insufficient*** to be able to capture a clear trend of the quality of the surrounding air on the product of conception. At the same time, on the territory of Romania, during the period subjected to the statistical analysis (2017-2020 respectively), *no devastating natural or human-caused events*, which could have had an immediate visible impact on the population, were reported. It should not be overlooked that, *usually the impact of pollution on public health* does not have immediate repercussions but *over the course of time*. Probably, in order to study the effect of the environment during 2017-2022, *I should have studied the years 2012-2017 in terms of air quality*, however, even in this case, the *attribution of cause and effect would have been quite difficult to achieve*, since there are numerous variables and barriers (incomplete official public data for the years 2012-2016, multiple personal events and illnesses in the mothers' lives, their regional mobility, changes in the workplace, changes in lifestyle and diet etc.) ***Proposal***: For the years following, studies on the ***Effect of the SARS-CoV-2/COVID Pandemic on public health and on the fetus.***

-4. **Territorial/regional mobility of mothers**: some may have changed their residence and come from completely different counties (where there may be other values of the pollution indicators); some mothers live abroad (values of pollution indicators being different from those from Romania); some mothers commute to work (and so, although they may live in a less polluted environment, they spend much of their time at work in big cities). In the statistical

analysis, *for a greater veracity of the data*, the mother's environment of origin was changed with her current residence; probably, it would have been the same, possibly even better, *to submit to the analysis also the place of birth*, thus being able to follow the *effect of population dynamics on health and quality of life*. At the same time, *in order to facilitate data processing and due to the lack of official data on localities*, the study used the county/rural environment/urban environment as the mother's residence, and not a specific area/locality (**N.B**.: Some areas of the county are more polluted than others).

-5. The addresses most frequently found in the database were: Bucharest, Ilfov, Giurgiu and Călărași. *There were no differences between the groups* (control group and the group with malformation cases) regarding the distribution in percentage of the subjects. ***Observation***: *The similarity of the county distribution of neonates from both groups* can be an argument to support the results obtained: Following the *statistical analysis between the number of cases of malformations and the air quality indicators* in the counties of origin, ***no relevant correlations were highlighted***. In conclusion, ***a relatively uniform population was included in the study***, *which was submitted essentially to the same environmental factors*, covering a *small area of the country*, namely Bucharest and neighboring counties. ***Observation***: *Probably, from a complex, uneven database (containing mothers from all regions of the country* → mining areas, areas with iodine deficiency, areas with refineries/industrial areas, forest areas etc.*) correlations between the number of cases of malformations and air quality indicators in the counties of origin would have been obtained.*

-6. ***Uniformization of the population*** in the database *not only according to the domicile, but also the lifestyle, diet etc.* (Examples: The emergence of *hypermarkets and supermarkets* has led to the current decrease in consumption from their own harvest among the population; *Population dynamics; Information sources from the mass media*/radio, television, Internet, multimedia complexes, etc.)

-7. *Romania is not a highly industrialized country compared to other Western countries*; the main sources of pollution throughout the country remain *road traffic* and *improper burning of waste*.

-8. During the collection of the database (2017-2020 respectively), ***no devastating events of natural or human causes were reported*** on the territory of Romania, which could have had an immediate visible impact on the population.

-9. ***Insufficient number of cases*** to be able to observe any influence of air pollution on the number of cases with malformations.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Final conclusions

The paper aims to determine the *risk factors, other than genetic ones*, that contribute to the occurrence of *congenital anomalies in the neonatal population*; in fact, *a primary prophylaxi*s is wanted in terms of their prevention.

The special part of the thesis (Personal contributions) includes Study 1- *Non-Genetic Risk Factors Associated with Congenital Anomalies/ Particular Phenotypic Traits in the Neonatal Period* and *Study 2- Environmental pollution/ influences on the fetus*.

The broad objectives of Study 1 were achieved:

1. The results obtained in the study were compared with the data from the specialized literature;

2. Following the statistical analysis of the database, it was possible to predict the predominant profile of the pregnant woman predisposed to having a neonate with a congenital anomaly/ particular phenotypic trait;

3. Following the statistical analysis of the database, the predominant profile of the neonate with a certain malformation could be predicted.

Limitations of Study 1:

1. *Type of study:*
   The study of this PhD thesis is of *case-control type*, thus providing less evidence of causality compared to a randomized controlled trial. The case-control study only determines a probability ratio (odds ratio, abbreviated OR), which has a lower association power compared to the relative risk (RR).
2. *The relatively small number of subjects* included in the database.
3. Since the topic of the PhD thesis was the identification of risk factors, a *holistic approach was applied in collecting and compiling the database*. However, *the population included in the study was quite uniform*, mostly representing mothers/new mothers from Bucharest and neighboring areas (Ilfov, Giurgiu, Călărași). For this reason, the results of the study do not refer to all of Romania, but only to the region stated above. Thus, the predominant

profiles obtained from the statistical analysis for mothers and neonates should be verified if they also apply to a larger population and/or to a larger area of the country.

4. *Difficulties and possible errors that may have occurred in the inclusion/exclusion of neonates* in the study. (Examples: underage mothers; mothers who do not know how to give details about the pregnancy, personal physiological antecedents, personal pathological antecedents, heredo-collateral antecedents; uninvestigated pregnancies; mothers who refuse to include the neonate in the study; data provided incompletely or incorrectly by the mothers; the annual leave period of the undersigned; the period March 2020-2021, when the Bucur maternity hospital became Covid support unit etc.)

Future directions:

-I wish to continue the *ultrasound screening of neonates at risk for congenital anomalies*.

-I propose to check/validate the *odds (OR/Odds Ratio*) *obtained following the statistical analysis*.

-I propose to verify/validate the *predominant profile of pregnant women predisposed to having a neonate with a congenital anomaly/particular phenotypic trait* (possibly on a different population, from other maternity hospitals in Bucharest or in the country).

-I propose to validate/verify the *predominant profile of the neonate with a certain malformation* (possibly on a different population, from other maternity hospitals in Bucharest or in the country; possibly even on the pediatric population in hospitals).

-I plan, following the veracity of the results obtained in the study on a more extensive and non-homogeneous population, to create *Standardized Worksheets*: Algorithm for maternal risk factors and Algorithm for the neonate with malformation risk.

Suggestions:

- creation of a UNIQUE NATIONAL REGISTER OF NEONATAL MALFORMATIONS;

- the formation of *multidisciplinary teams* aimed at the diagnosis and treatment of these patients with congenital anomalies/rare diseases (some of them with special needs);

-making *informative notes* (with precise and local data, from the country)/ renewing existing informative notes and recommendations for public use, as well as NGOs for helping patients with particular phenotypes and/or congenital anomalies;

-***Observation***: The database of this PhD thesis was developed for the most part in the period preceding the SARS Cov2 pandemic. ***Suggestion***: New studies, including subjects from the pandemic period and the post-pandemic period, as well as a parallel between the three large groups (before, during and after the pandemic), could bring additional information regarding the action of external factors on the fetus.

-***Observation***: Only the neonatal population is included in the study of the present work; the PhD thesis does not refer to stillbirth or abortion. ***Suggestion***: The expansion of the study group can be considered in the future (the search for risk factors responsible, along with the primary genetic factor, for the appearance of congenital anomalies) in pregnant women/new mothers who have as personal pathological antecedent spontaneous abortions/stillbirths/dead children with severe malformations/ children with congenital anomalies/ particular phenotypes under their care. The anatomical-pathological study of the placenta together with the product of conception (when applicable) is performed after each birth. In the case of the presence of malformations in the fetus, genetic studies can also be done; - however, they can be expensive, which is why they usually cannot be performed. Carrying out such a study is expensive, requiring material support (sponsors to settle the cost of genetic tests).

*The results of **Study 2** were not relevant. The reasons for this and the limitations of Study 2 are detailed in the sub-chapter "7.4. Discussions" (**page 285**).*

Suggestions:

- ***Digital applications*** for citizens, regarding the quality of the surrounding air in real time (See the air monitoring platform in Bucharest and Ilfov: ***https://aerlive.ro/importanta-indicilor-si-indicatorilor-de-calitate-a-aerului-2);***

- awareness of the danger of pollution, by the general population, through the mass media and through medical professionals (family physicians, internal medicine physicians, cardiologists, nutritionists, obstetricians etc.);

- awareness of the danger of pollution, an unhealthy lifestyle and eating mistakes in schools;

-promotion of “Friends of nature” actions with the help of Local Town Halls, NGOs, schools etc.

- promoting a healthy lifestyle and correct and balanced nutrition through the media, NGOs and medical professionals.

Personal Contributions

-*The thesis has a holistic approach*, includes numerous information from different fields (ecology, psychology, genetics, cardiology, ultrasound, neonatology), which gives a touch of originality to the thesis, which makes the subject treated to be approached in a personal way.

-*The design of the study is my own: I designed the inclusion criteria, the exclusion criteria, the classification of malformations by degrees of severity, the division of ultrasound results by degrees of severity.*

-*I drafted the questionnaire addressed to the mother to detect the risk factors in the production of congenital anomalies in the fetus.*

-*The ultrasounds were completely performed by the holder of this PhD thesis*, pediatrics - neonatology specialist physician with competence in general ultrasound and cardiac ultrasound, a physician employed by the Emergency Clinical Hospital “St. Ioan” – Bucur Maternity Hospital, Bucharest, Neonatology Department. In the situations of complex cases, a second opinion was also involved (example: ultrasounds were performed in state clinics such as the Emergency Clinical Hospital for Children „M.S. Curie” - Bucharest, I.N.S.M.C. (National Institute for Maternal and Child Health) “Alessandrescu – Rusescu” - Bucharest, the Clinical Emergency Hospital for Children “Grigore Alexandrescu” - Bucharest or „Dr. Victor Gomoiu” Children's Clinical Hospital - Bucharest). Of course, depending on the situation, imaging investigations were supplemented with radiological ones (thoracic-abdominal x-rays; limb x-rays; skull x-rays). *N.B.:* During the ultrasounds, emphasis was placed on creating a friendly and quiet environment for the babies.

-Following the statistical analysis of the study, ***we found prediction rates/OR***, which can help us *in the selection of mothers at risk of malformed fetus*:

Exemplification: *The odds (Odds Ratio/ OR) were found for*: ***single umbilical artery****, mothers who have* ***heredo-collateral antecedents (cancer, genetic diseases, diabetes)****,* mothers who

have ***personal pathological antecedents (mild genetic diseases, mental diseases, neurological diseases)***, ***viral infections and maternal infections (usually cervical and urinary infections) during pregnancy, alcohol consumption, exposure to a toxic environment, Roma ethnicity.*** (***N.B.***: A possible cause includes the existence of *marriages between blood relatives*, *pregnancies not properly managed* and the higher frequency of *underage mothers* in this ethnic group etc.).

-Following the statistical analysis of the study, ***I found the predominant profile of pregnant woman predisposed at having a neonate with a congenital anomaly/particular phenotypic trait***.

-Following the statistical analysis of the study, ***I found the predominant profile of the neonate with a certain malformation***.

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