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PRESENTATION OF AN INTEGRATED CARDIO–NEURO–GENETIC FRAMEWORK IN FETAL MEDICINE

Ph.D. Thesis

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Original publications related to the PhD thesis:

I. Elekes, T.; Csermely, G.; Kádár, K.; Molnár, L.; Keszthelyi, G.; Hozsdora, A.; Vizer, M.; Török, M.; Merkely, P.; Várbíró, S. Learning Curve of First-Trimester Detailed Cardiovascular Ultrasound Screening by Moderately Experienced Obstetricians in 3509 Consecutive Unselected Pregnancies with Fetal Follow-Up. Life 2024, 14, 1632.
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II. Elekes, T.; Ladanyi, A.; Pap, E.; Szabo, J.; Illes, A.; Gullai, N.; Varbiro, S. Second Trimester Ultrasound Diagnosis of External Hydrocephalus in Two Fetuses with Noonan Syndrome-Case Report Series. J. Clin. Med. 2025, 14, 3973.
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List of abbreviations:

AC	Abdominal circumference	**HLHS**	Hypoplastic left heart syndrome
ACMG	American College of Medical Genetics	**IVF**	In vitro fertilization
AVSD	Atrioventricular septal defect	**MRI**	Magnetic resonance imaging
BMI	Body mass index	**NB**	Nasal bone
BPD	Biparietal diameter	**NGS**	Next-generation sequencing
CARE	CAse REport guidelines	**NIPT**	Non-invasive prenatal testing
cfDNA	Cell-free DNA	**NS**	Noonan syndrome
CHD	Congenital heart defect	**NT**	Nuchal translucency
CGH	Comparative genomic hybridization	**PFO**	Patent foramen ovale
CMA	Chromosomal microarray analysis	**PFSR**	Prefrontal space ratio
CNS	Central nervous system	**PTPN11**	Protein tyrosine phosphatase non-receptor type 11
CNV	Copy-number variation	**SLE**	Systemic lupus erythematosus
CRL	Crown–rump length	**SNP**	Single nucleotide polymorphism
CVS	Chorionic villus sampling	**SOS1**	Son of Sevenless homolog 1
DNA	Deoxyribonucleic acid	**SUA**	Single umbilical artery
DV	Ductus venosus	**T18**	Trisomy 18
EH	External hydrocephalus	**T21**	Trisomy 21
EIF	Echogenic intracardiac focus	**TR**	Tricuspid regurgitation
FHR	Fetal heart rate	**VSD**	Ventricular septal defect
FMF	Fetal Medicine Foundation	**WES**	Whole-exome sequencing
GATK	Genome Analysis Toolkit		
HC	Head circumference		

1. Introduction

Fetal medicine has developed dramatically over the past century through major technological and genetic breakthroughs that have revolutionized prenatal diagnostics and improved perinatal outcomes. About 3–6% of fetuses have structural or genetic (or both) abnormalities, with CHD accounting for roughly one-third of all cases. Central nervous system malformations and genetic syndromes also represent a significant share. Even with combined use of karyotyping, CMA, and WES, a notable fraction of anomalies remain unexplained, emphasizing the importance of integrating detailed imaging with molecular genetics.

In Hungary, termination of pregnancy for severe fetal anomalies is legally permitted up to 23 + 6 weeks if the probability of serious fetal damage reaches 50%. Since diagnostic procedures must be completed early, rapid and accurate detection is critical.

2. Objectives

With such a dynamic development, the most important question for the clinician is how to deliver the most accurate and effective screening algorithm for pregnant patients to reassure them, or provide timely information of the best quantity and quality regarding the decision to proceed or not to proceed with the pregnancy, within the time frame allowed by law.

According to the candidate's opinion, the most reliable method for determining the characteristics of fetal abnormalities and predicting their prognosis is the simultaneous, systematic examination of the fetal heart and central nervous system, combined with staggered genetic evaluation, which can be achieved in the context of "cardio-neuro-genetic" framework.

In this thesis, I attempt to cover all aspects of this framework and therefore, I would like to study and present the following:

- learning curve of specialists in obstetrics and gynaecology in detailed (early) fetal echocardiography,

- our experiences with the staggered, three-level (karyotyping - microarray - WES) genetic diagnostics in Hungary and

- two cases illustrate the importance of routinely performed detailed neurosonography.

3. Methods

3.1 Learning curve of first-trimester fetal cardiac screening

In this retrospective study we evaluated the learning curve of first-trimester fetal echocardiography performed by obstetrician-gynecologists at the RMC Fetal Medicine Center (2010–2015). The study was approved by the Hungarian Medical Council (2013/EKU-588/2013). Routine extended first-trimester scans followed FMF guidelines for aneuploidy and preeclampsia screening using Accuvix V20 (Samsung-Medison) and Voluson S8 (GE) ultrasound systems with 4–8 MHz convex probes. Initially, three FMF-certified obstetricians with moderate experience in abnormal cardiac anatomy conducted the scans; later, an FMF-audited technician joined for prescreening. A standardized protocol (from 2011) required documentation of situs, cardiac position, four-chamber and three-vessel views, aortic and ductal arches, and optional pulmonary and venous connections. Examinations were comprehensive when all structures were recorded; no time limits were applied. Abnormal or high-risk cases were re-evaluated by a pediatric cardiologist, and genetic counselling was offered when abnormalities were detected. Patients with positive aneuploidy screenings underwent chorionic villus sampling or amniocentesis. Follow-up data were collected via email, institutional records, and phone interviews. Statistical analysis was performed with the chi-square test ($p < 0.05$) using GraphPad Prism 6.0.

3.2 Chromosomal microarray and whole-exome sequencing in Hungary

A diagnostic workflow was applied: (i) karyotyping, (ii) CMA, and (iii) WES if previous results were negative.

CMA analyses were performed using the GeneChip System 3000 instrument (Affymetrix, Santa Clara, CA, USA). For hybridization, we employed the GeneChip Hybridization Oven 645 (Thermo Fisher Scientific, Waltham, MA, USA); washing steps were carried out on the GeneChip Fluidics Station 450 (Thermo Fisher Scientific), and signal intensities were measured using the GeneChip Scanner 3000 7G (Thermo Fisher Scientific). The assay is based on an SNP-based comparative genomic hybridization method.

Next-generation sequencing (NGS) was conducted to determine the complete human exome sequence in 42 prenatal samples. Sequencing was performed on IonTorrent (Thermo Fisher Scientific) and Illumina (Illumina, San Diego, CA, USA) platforms. Following sequencing, read alignment to the reference genome (hg37) and subsequent variant calling were

carried out using the GATK v4.1.4.1 software suite. Variant annotation and interpretation were performed using 'Franklin by Genoox'.

All patients provided written informed consent for genetic testing, and retrospective data processing was conducted in an anonymized manner.

3.3. Presentation of two clinical cases

Two prenatal cases of Noonan syndrome demonstrated second-trimester external hydrocephalus associated with SOS1 and PTPN11 gene variants. To our knowledge, this represents the first report of SOS1-related external hydrocephalus diagnosed prenatally and these are the earliest ultrasound-detected cases of Noonan syndrome in the second trimester. The study followed the CARE guidelines. External hydrocephalus defined at the Sylvian fissure level when exceeding the 95th percentile according to Alonso et al. These cases underscore the importance of detailed mid-trimester neurosonography in detecting subtle genetic syndromes and guiding subsequent molecular testing.

4. Results

4.1. Learning curve of first-trimester fetal cardiac screening

Between January 2010 and January 2015, 4,769 fetuses (4,441 singletons, 155 twins, 6 triplets, Table 1.) underwent first-trimester cardiac ultrasound screening, identifying 42 congenital heart defects (CHD, 0.88%).

Table 1. Prenatally observed abnormal hearts during the learning curve.

	2010	2011	2012	2012	2014	Total
# of 1st-trim. examinations	228	586	872	1228	1855	4769
# of abnormal hearts (1st-trim. Dx)	2 (1)	8 (7)	6 (5)	15 (14)	11 (9)	42 (36)
HLHS		2		1	1	4
AVSD	1	1	1	1	3	7
AVSD + HRH		1				1
AVSD + HLH			1		1	2
AVSD + Heterotaxia		1				1
VSD		1	2	3	1	7
VSD + LV < RV				1		1
ASD + LV < RV			1			1
LV > RV					1	1
LV < RV					1	1
HLH + VSD				1		1
HRH + VSD		1				1
Aortic stenosis + HLH + AVSD				1		1
Aortic atresia	1	1				2
Pulmonary valve regurgitation					1	1
Pulmonary atresia + HRH				1		1
Septal fibrosis + VSD				1		1
Ventricular fibro-elastosis				1		1
Rhabdomyoma					1	1
TGA					1	1
Tetralogy of Fallot				1		1
Isolated pericardial fluid			1	1		2
CHD—non evaluated				2		2

Abbreviations: number , number; Dx, diagnosis; HLHS, hypoplastic left heart syndrome; AVSD, atrio-ventricular septal defect; HRH, hypoplastic right heart; HLH, hypoplastic left heart (LV << RH); VSD, ventricular septal defect; LV, left ventricle; RV, right ventricle; ASD, atrial septal defect; TGA, transposition of great arteries; CHD, congenital heart defect.

To ensure consistent methodology, fetuses examined before 2011 (n = 228) were excluded due to the absence of a standardized protocol, and those from late 2014 as those pregnancies were still ongoing. Thus, 3,372 pregnancies (3,509 fetuses) screened between from 2011 to mid-2014 formed the analytical cohort, with a 93% follow-up rate (3,142/3,372).

The mean maternal age was 33.9 years (range 17–45); 46.9% were > 35 years. Among pregnancies with severe CHD, maternal age averaged 35.8 years and the ≥ 35 years group represented 71.0% ($\chi^2 = 6.642$, $p = 0.0099$). Mean fetal CRL was 64.0 mm (45–84 mm).

NT, DV, and TR measurements showed that 56.1% of NT were above the median, 4.9% above the 95th percentile. Yet 54% of CHD fetuses had NT < 3.5 mm, and 46% < 2.5 mm. TR occurred in 0.6% of normal and 29% of CHD fetuses ($\chi^2 = 308.486$, $p < 0.05$); abnormal DV flow in 4.3% of all fetuses vs. 51% with CHD ($\chi^2 = 168.282$, $p < 0.05$).

Of the 3,270 fetuses, 34 were labeled as “abnormal” by moderately experienced obstetricians. After pediatric cardiology review, 32 CHDs were confirmed (6 minor, 26 major). CHD was defined as “major” if requiring cardiac surgery in the first year of life. Two minor cases normalized by the second trimester; one initially minor (pericardial fluid) was later reclassified as major (complex pulmonary atresia).

Of the 3,238 hearts initially classified as normal, four additional defects were identified later (3 minor VSDs, 1 severe aortic stenosis with endocardial fibroelastosis). A case with family history showed rhabdomyomas in the third trimester after normal earlier scans.

Among 3,270 fetuses, 3,191 (97.6%) were liveborn; 79 (2.4%) were lost (stillbirth or early death). Eighteen spontaneous abortions (0.5%) occurred without major CHD. Fifty-three pregnancies (1.6%) were terminated for genetic reasons, 29 of which involved CHD.

Postnatally, 13 minor heart defects were found among newborns (10 innocent murmurs and 2 PFOs excluded from analysis, Table 2.). One case of complex pulmonary atresia was re-classified as major. Altogether, 49 heart defects (1.49%) were identified in the cohort.

Of these, 71.4% (35/49) were diagnosed prenatally and 28.6% (14/49) postnatally. Severe CHDs were diagnosed prenatally in 96.4% (27/28) and already suspected at first trimester in 89.2% (25/28).

The learning curve showed rapid improvement: in 2011–2012, 11 major CHDs were found in 1,458 cases (9 first-trimester detections). By 2013–2014, all severe CHDs were detected in the first trimester except one rhabdomyoma.

Table 2. Postnatally recognized cardiac findings.

Type of Cardiac Anomaly	Number of Fetuses
Ventricular septal defect (2–5 mm)	8
Atrial septal defect II	3
Aortic valve stenosis min. grade	1
Bicuspidal aortic valve + PFO	1
Complex pulmonary atresia	1
TOTAL	14
(PFO + innocent cardiac murmurs)	(2 + 10)

Abbreviation: PFO: patent foramen ovale.

4.2. Our experiences with chromosomal microarray (CMA) and whole-exome sequencing (WES) in Hungary

A total of 252 prenatal cytogenetic tests were performed. In 92.8% of cases, CMA was indicated due to structural ultrasound abnormalities. WES was applied after negative karyotype and CMA or in cases with suspected genetic etiology. Common ultrasound anomalies (Table 3.) included hydrops, cystic hygroma, and thick nuchal fold (40.6%), cranio-spinal/facial (20.5%), and cardiovascular abnormalities (17.9%).

Table 3. CMA and WES examinations due to structural fetal ultrasound abnormalities.

Organ system	Cases (n)	%
Fetal hydrops, cystic hygroma, thick nuchal fold	95	40.60
Craniospinal and craniofacial abnormalities	48	20.51
Cardiovascular abnormalities	42	17.95
Other thoracic abnormalities	6	2.56
Abdominal wall and abdominal abnormalities	7	2.99
Urogenital abnormalities	8	3.42
Limb anomalies and ossification disorders	14	5.98
Other structural ultrasound abnormalities	14	5.98
Total	234	100

Abbreviations: CMA = chromosomal microarray analysis; WES = whole exome sequencing.

CMA detected chromosomal imbalances in 105 cases (42%), 22% pathogenic and 2% trisomies (5 T21, 1 T18). Table 4. presents the most common abnormalities and their chromosomal locations. Balanced parental translocations causing unbalanced fetal CNVs were found in three families [e.g., t(9;20), t(4;5), t(4;8)]. In one compound heterozygous case, CMA revealed a 4.138 Mb deletion (12q12), while WES identified a single-base KIF21A deletion on the paternal allele, causing severe fetal asphyxiated thorax syndrome.

CMA from three fetuses after intrauterine death identified trisomy 18 and partial trisomy 9 in two cases. Subsequent WES was performed in 42 fetuses, revealing pathogenic variants in 9 (21.4%), including PTPN11 (2 cases), SOS1 (1 case), EBP (1 case), and TNNT3 (1 case shared with mother). Two complex heterozygous variants were identified in CC2D2A causing autosomal recessive ciliopathy and fetal akinesia. Variants in CBL and TEK genes matched phenotypes but lacked definitive pathogenic classification. Eighteen secondary findings were classified as (patho)genic or likely pathogenic per ClinVar/ACMG (Table 5.).

Table 4. The most common copy number variations.

Copy number variation	Description	Cases (n)	%
16p11.2	Known LCR/SD region / VUS	23	30.26
3q24q29	Known LCR/SD region*	8	10.52
8p22p24.3	LOH polymorphic region**	8	10.52
16p13.11p12.3	Known LCR/SD region	7	9.21
Xq21.31q21	VUS	7	9.21
15q11.2q13.3	Known BP-(1–3) region*	6	7.89
4p16.3p16.2	Telomer region	5	6.58
9p24.3p24.3	Telomer region	4	5.27
22q11.21	DiGeorge region	4	5.27
20q13.33q14.33	Telomer region	4	5.27
Total		76	100

*LCR = low copy repeat; **LOH = loss of heterozygosity
BP = breakpoint; LCR = low copy repeat; SD = segmental duplication; VUS = variant of uncertain significance

Overall, the stepwise approach (karyotype → CMA → WES) yielded a significant increase in diagnostic accuracy and highlighted the need for integration of molecular and imaging data in prenatal genetics.

Table 5. Presumed incidential findings in the prenatal WES examination group.

Gene	Variant	ClinVar classification	ACMG classification
ATAD3A	c.229C>G p.Leu77Val	P / VUS	LP
ANO5	c.2272C>T p.Arg758Cys	P / LP	LP
CFI	c.772G>A p.Ala258Thr	P / LP	LP
ROBO4	c.190C>T p.Arg64Cys	P	P
PROS1	c.701A>G p.Tyr234Cys	P / LP	LP
ANO5	c.692G>T p.Gly231Val	P / LP	LP
GATA4	c.487C>T p.Pro163Ser	P / VUS	LP
GATA4	c.34G>C p.Gly12Arg	VUS	LP
RPS7	c.298A>T p.Ile100Phe	–	LP
PAH	c.506G>A p.Arg169His	LP	P
F2	c.*97G>A	P / VUS	LP
ADGRV1	c.9623+1G>A	LP	P
F11	c.1693G>A p.Glu565Lys	LP / VUS	LP
BSCL2	c.974dupG p.Ile326fs	P / LP	P
PUS3	c.212A>G p.Tyr71Cys	P / VUS	LP
GJB2	c.35del p.Gly12ValfsTer2	P	P
CAPN3	c.550del p.Thr184ArgfsTer36	P	P
TPM3	c.253G>T p.Glu85Ter	–	LP

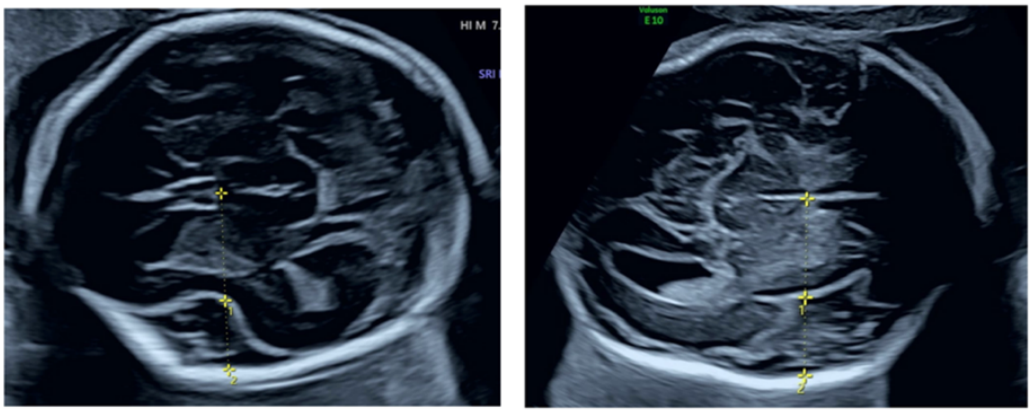
ACMG = American College of Medical Genetics and Genomics variant classification system (Franklin platform); ClinVar = ClinVar database classification based on current knowledge; LP = likely pathogenic; P = pathogenic; VUS = variant of uncertain significance; WES = whole exome sequencing.

4.3. Presentation of two neurosonography cases of Noonan-syndrome

4.3.1. Case 1

A 46-year-old woman with an IVF pregnancy (egg donor of less than 25 years) with antiphospholipid syndrome and hypothyroidism underwent first-trimester screening at 12 + 5 weeks showing NT 2.4 mm (85th percentile) and otherwise normal scan.

In the second trimester, mild polyhydramnios appeared; BPD and HC were >90th percentile. Posterior horn of the lateral ventricle measured 9 mm, ambient and quadrigeminal cisterns were enlarged and Sylvian fissure depth was 10.5 mm (>95th percentile). Subsequent scans at 27 and 34 weeks confirmed progressive external hydrocephalus.



(a) (b)

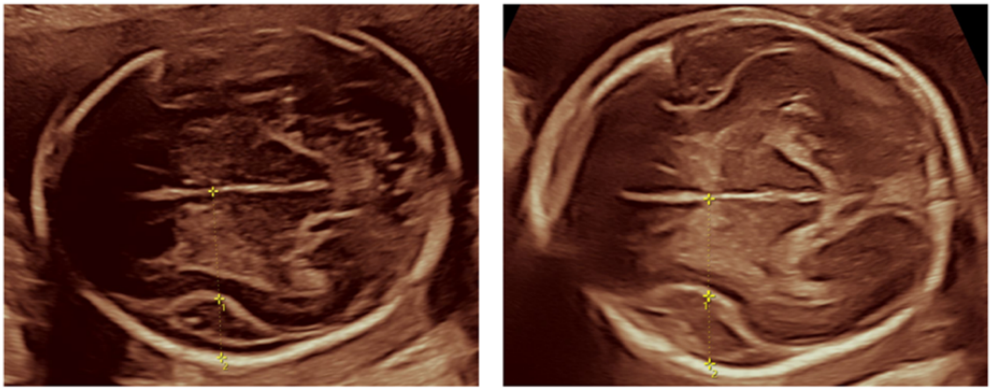
Figure 1. Measurements of the Sylvian fissure. Measurements marked with 1 indicate the insular depth, measurements marked with 2 indicate the Sylvian fissure depth. (**a**) The depth of the Sylvian fissure was measured 10.0 mm at the 20th week; (**b**) and 17.4 mm at the 27th week. Both measurements exceeded the 95th percentile according to the database published by Alonso et al. in 2010.

The cfDNA-test was normal; amniocentesis (46XY) and array CGH revealed a 2 Kb microdeletion in RAB6C and SMPD4. WES identified a heterozygous pathogenic SOS1 variant (c.1644T>A, p.Ser548Arg), confirming Noonan syndrome 4.

A male newborn was delivered at 38 weeks (3530 g, Apgar 9/10). Follow-up showed mild supravalvular pulmonary stenosis and minimal psychomotor delay requiring rehabilitation.

4.3.2. Case 2

A 32-year-old woman was referred due to an NT of 3.4 mm and absent ductus venosus. Additional findings included single umbilical artery (SUA), echogenic focus in right ventricle, and frontal bone depression. Follow-up at 16–22 weeks confirmed DV agenesis and small subaortic VSD with septal aneurysm. Neurosonography revealed Sylvian fissure depths of 8.0 mm and 10.8 mm at 19 and 22 weeks (>95th percentile).



(a) (b)

Figure 2. Measurements of the Sylvian fissure. Measurements marked with 1 indicate the insular depth, measurements marked with 2 indicate the Sylvian fissure depth. (**a**) The depth of the Sylvian fissure was measured 8.0 mm at the 19th week; (**b**) and 10.9 mm at the 22nd week. Both above the 95th percentile according to the database published by Alonso et al. in 2010.

Amniocentesis and SNP microarray were normal; WES detected a heterozygous PTPN11 variant (c.124A>G, p.Thr42Ala), confirming Noonan syndrome 1. After genetic counseling, the pregnancy was terminated.

5. Conclusions

Our study demonstrates that when suitable equipment, quality assurance, and motivation are provided, first-trimester fetal cardiovascular ultrasound screening performed by moderately experienced obstetricians can achieve up to 90% diagnostic efficiency in detecting severe congenital heart defects (CHD) in an unselected population. Early fetal echocardiography not only reassures the majority of pregnant women but also significantly contributes to the early recognition of chromosomal abnormalities. Most CHDs identified in the first trimester are complex and severe, allowing parents to make informed reproductive decisions earlier and thereby reducing maternal risks and the psychological burden associated with second-trimester terminations. Key first-trimester cardiac markers - nuchal translucency

(NT), tricuspid regurgitation (TR), and ductus venosus (DV) - serve as important indicators of both major and minor CHDs. Their integration into early screening enables the planning of perinatal cardiac management, improving neonatal morbidity and mortality.

The study also highlights the importance of collaboration between obstetricians and pediatric cardiologists: second-opinion fetal echocardiography refines uncertain diagnoses, supports genetic counseling, and alleviates parental anxiety caused by diagnostic ambiguity. Over a five-year learning curve, the research team conducted nearly 4,800 first-trimester fetal cardiac scans, identifying over 40 fetal heart abnormalities. Physicians' skills in visualizing the four-chamber view, outflow tracts, and great vessels improved significantly. It is estimated that in Hungary, if 10–15% of experienced obstetric sonographers received focused training in early fetal echocardiography, universal access to this service could be achieved nationwide. A structured, quality-controlled education program could therefore incorporate detailed cardiac screening into routine first-trimester assessments.

Alongside ultrasound innovations, chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) have become indispensable tools in modern prenatal diagnostics. CMA is particularly valuable following abnormal ultrasound or NIPT results, providing precise characterization of chromosomal imbalances, identifying unbalanced rearrangements in fetuses of parents with balanced translocations, and analyzing samples unsuitable for cell culture after miscarriage.

CMA provided additional diagnostic information in 22% of 252 cases, while WES yielded new findings in 21.9% of 42 cases beyond conventional cytogenetics - values consistent with global data. Combined use of G-banding, CMA, and WES explained approximately 35% of fetal abnormalities, with the remaining cases likely linked to teratogenic or multifactorial causes.

The study emphasizes the need for a standardized national diagnostic protocol integrating CMA and WES, harmonized with international standards but adapted to national clinical practice. In advanced pregnancies or complex phenotypes, CMA and WES should be initiated simultaneously; where a single-gene disorder is suspected, WES may precede CMA. Nonetheless, the residual risk of undetected rare disorders persists, and genetic counseling must always address these limitations transparently.

This thesis proposes a cardio–neuro–genetic framework, integrating early echocardiographic, neurosonographic, and genetic data into a unified model of fetal diagnostics. This approach bridges the gap between phenotype and genotype, improving diagnostic precision, accelerating decision-making, and enabling personalized prenatal care. When extended echocardiography is performed under standardized conditions, its linkage to genetic testing reveals the molecular mechanisms underlying structural abnormalities. Similarly, advanced neurosonography expands the detectable prenatal phenotype, facilitating recognition of subtle cerebral changes, as demonstrated in Noonan syndrome cases.

Beyond its scientific value, the cardio–neuro–genetic concept also has educational and ethical implications. It promotes cross-disciplinary learning, the standardization of diagnostic protocols, and transparent, evidence-based prenatal counseling. Implementing the cardio–neuro–genetic paradigm promises more efficient national screening programs, earlier and more accurate diagnoses, and ultimately, better perinatal outcomes for families.