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TESI DI LAUREA:

Expanded Newborn Screening: state of the art in the Veneto Region

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ABSTRACT

Newborn screening (NBS) was initiated in Europe in 1960's with the screening for phenylketonuria. Following the advancement in the technology and introduction of tandem mass spectrometry (MS/MS) in the 1990's the number of conditions that can be screened for using a single blood sample has risen to more than 40. With the increased availability of molecular technologies new conditions are continuously being added to the screening panels. This study presents an overview of the current status and critical issues of expanded newborn screening (eNBS) in Europe and Italy, more specifically Veneto Region. Data from Veneto Birth Registry was used to determine the place of birth and screening status of infants born in Veneto in last ten years (2012-2021). The information provision documents from Birth Centres of Padova, Verona, Emilia Romagna, Friuli Venezia Giulia, Tuscany, Lombardy and Piedmont were collected and compared in terms of the type of document available, languages used and knowledge aspects included.

The study showed that while the birth rate overall was decreasing, planned home births were increasing, especially with Italian women. Foreign women, however, were found to be around 30% of all women that gave birth in recent years. The study also showed that NSB program in Veneto is successful in terms of secondary prevention but suffers from a low positive predictive value which means high number of infants recalled for confirmatory testing. Parental education and information provision material analysed was found to be lacking many important knowledge aspects that would help parents wake an informed decision. We wrote an information provision document that covers all main knowledge aspects and can easily be adapted to any Region in Italy.

RIASSUNTO

Lo screening neonatale (NBS) ha origine nell' Europa degli anni '60 con lo screening per la fenilchetonuria. In seguito, grazie ai progressi tecnologici e all' introduzione della spettrometria di massa tandem (MS-MS) avvenuta negli anni '90, il numero di malattie rintracciabili da un singolo campione di sangue è salito a più di 40. Inoltre, il continuo e progressivo miglioramento di tali tecnologie permette di aggiungere un numero sempre maggiore di condizioni rilevabili alle procedure di screening neonatale. Questo studio si propone sia di fotografare la situazione attuale dello screening neonatale esteso (eNBS), sia di analizzarne i suoi punti critici, in Europa così come in Italia, e nello specifico nella Regione Veneto. Per determinare lo stato dell'arte sulle nascite e sullo screening neonatale in Veneto sono stati utilizzati i dati presenti nel Registro Nascita della Regione degli ultimi dieci anni (2012-2021). Le informazioni provenienti dai Centri nascita di Padova, Verona, Emilia-Romagna, Friuli-Venezia Giulia, Toscana, Lombardia e Piemonte sono state raccolte e confrontate in merito ai tipi di documentazione disponibili, lingue utilizzate, e aspetti informativi inclusi.

Lo studio mostra che, mentre il tasso di nascite è in fase di decrescita, i parti avvenuti in casa sono in aumento, specialmente tra le donne di nazionalità italiana. Altro dato significativo, il fatto che negli ultimi anni, circa il 30% delle donne che hanno messo al mondo un figlio sono straniere. Lo studio rivela inoltre che il programma di screening neonatale in Veneto risulta vincente nei termini di prevenzione secondaria, ma arranca per quanto riguarda la sua qualità predittiva; ciò comporta un elevato numero di infanti richiamati per accertamenti. Il materiale informativo e la comunicazione rivolti ai genitori sono risultati lacunosi in molti aspetti, e ciò si traduce in problematiche importanti per quanto riguarda il consenso informato degli stessi. Per questi motivi, nel presente studio abbiamo redatto un breve ma completo documento informativo rivolto proprio ai genitori ed adattabile a qualsiasi Regione italiana.

1. INTRODUCTION

Newborn screening (NBS) is a public health program with the goal of screening all newborns for various congenital and hereditary diseases for which an early diagnosis is proven or at least delays the disease manifestation. The testing is done in the first 24-48 hours of life using a bloodspot specimen from a heel-prick. If found to be positive, confirmatory testing will be done and the therapy started as soon as possible to guarantee the best outcome and quality of life. The aim of early disease detection (or secondary prevention) is to discover and cure conditions which have already produced pathological change in body, but which had not so far reach a stage at which medical aid is sought spontaneously[1].

1.1 DEFINITION OF SCREENING

The definition of screening most often quoted is from 1951 made by the United States Commission of Chronic Illness that describes screening as '*the presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic. Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment*.'[2]

Screening is a public health program that seeks to identify a population of apparently healthy people who carry a higher risk of developing a health problem and to whom an early-treatment or intervention can be offered. A person with a positive or suspicious screening test undergoes confirmatory testing before a diagnosis can be made, and followed up with either surveillance or treatment. Steps that lead from a screening test to a diagnosis can be seen in figure 1.

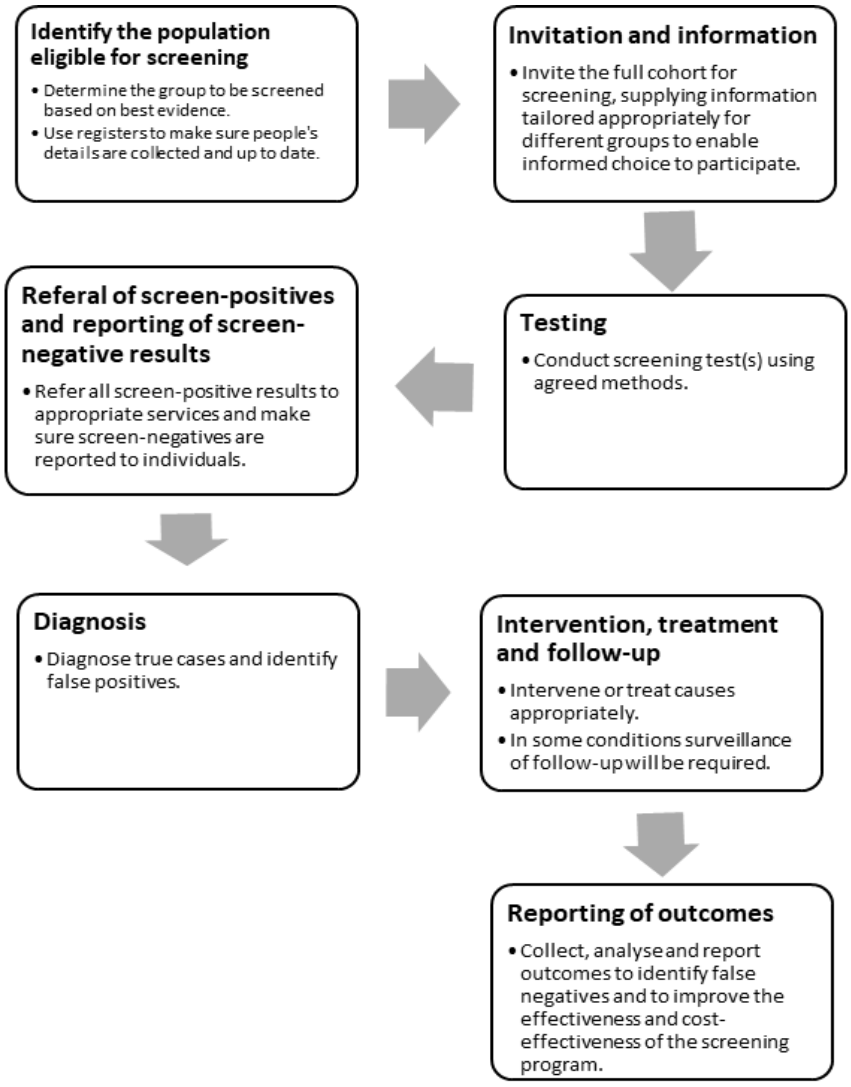


FIGURE 1 SCREENING FLOWCHART

Effectiveness of screening programs can be increased by targeting specific populations that are at a higher risk of a disease, based on considerations such as demographic factors, medical history or occupation'. [3]

An effective screening program is a cost-efficient public health tool; the diagnosis and treatment of a disease in its pre/asymptomatic phase is often more effective and less costly than treatment started after the person has developed symptoms (e.g., polypectomy of precancerous masses before it evolves in colorectal cancer).

1.2 CRITERIA FOR SCREENING

1.2.1 WILSON AND JUNGNER'S CLASSIC SCREENING CRITERIA

In 1968 J.M.G Wilson and G. Jungner have published the report '*Principles and practices of screening for disease'* in the World Health Organization's Public Health Papers series (n. 34), that outlined ten criteria to help guide the selection of conditions that would be suitable for screening[1].

Wilson and Jungner's list of screening criteria:

1. The condition sought should be an important health problem.
2. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
3. There should be a recognizable latent or early symptomatic stage.
4. There should be a suitable test or examination.
5. The test should be acceptable to the population.
6. There should be an agreed policy on whom to treat as patients.
7. There should be an accepted treatment for patients with recognized disease.
8. Facilities for diagnosis and treatment should be available.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a "once and for all" project.

For Wilson and Jungner, the importance of the disease is seen both on an individual and community level. Some diseases, like diabetes, are well suited for a screening program because of their high prevalence and, even though usually mild condition at the individual level, can have serious consequences for the community if not discovered and treated in time. On the other hand, phenylketonuria (PKU) is a rare

disease (1-5/10000) that warrants screening due to the extremely high cost to an individual's health and well-being if not discovered and treated very early in life.

The treatment is seen as the primary goal of any screening program; screening for a disease that cannot be treated may cause psychosocial issues for patients and people close to them. Even in some cases where treatment is available, an early intervention does not necessarily mean better prognosis or overall survival- many lung cancer screening programs have failed due to high false positive rates, overdiagnosis and unnecessary invasive testing[4]. People with positive screening results had lower quality of life, more aggressive interventions, spent more time hospitalized, or ill but did not have better survival rate compared to those who decided to seek medical help when symptomatic.

Further testing and treatment must be guaranteed for all of those who need it; if the infrastructure allows for only a small percentage of those tested positive to be followed-up on, the disease does not meet the inclusion criteria and should be excluded from the program.

The test administered must also be acceptable to the population, since an unpleasant or painful examination will lower the rate of people willing to undergo said screening. The acceptability can be influenced by raising public awareness about screening programs and its benefits. Informational material should be accessible, written in appropriate literacy level that ensures that most people will be able to understand it, published in different languages to help engage even marginalized communities.

The problem with deciding on who to treat as patients lies in defining strict lines of demarcation between a healthy population and a possibly ill one. In the years in which this paper was written doctors had a different role in decision making that we have today- healthcare workers were used to making decisions on behalf of the patient without necessarily consulting them. The paternalistic view of medicine that was held in the past (‘the Doctor knows best’) meant that ‘the Doctor’ could decide if a borderline result should be communicated to the patient and if the treatment should be started. Luckily, in the last 50 years the attitude has shifted- informing patients and asking for their consent is an integral part of any medical intervention today.

According to Wilson and Junger, implementing screening programs on a large scale can even lead to economic gain; automated methods that test for multiple conditions simultaneously mean that highly specialized professionals can dedicate their time to other, more complex tasks. The long-term economic gain is evident when, by preventing or treating early disease, the productive life of the population is lengthened which has an impact on overall economy. In the case of extended newborn screening (eNBS) one study found that each Euro spent on the screening program saved more than 25 Euros in health and social costs.[5]

1.2.2 WILSON AND JUNGNER CRITERIA IN THE NEW MILLENNIUM

Many authors have revisited Wilson and Jungner criteria in the last 50 years, some looking to adapt them as the technologies used for screening continue to evolve, others to discuss the merits of screening for the conditions that don't fit the classic criteria[6].

In a systematic review published in 2018,[7] Dobrow and colleagues discussed screening principles and their evolution since 1968. A Delphi consensus process that followed assessed the reviewed results and concluded that Wilson and Jungner's principles are remarkably enduring; however, there is a need for it to be modernized to best guide the population-based screening decision. Many emerging criteria reflect the broader change in Western medicine and society such as "*increased consumerism, the shift away from paternalism towards informed choice, a focus on evidence-based health care, and the rise of managed care models that emphasize cost-effectiveness, quality assurance, and accountability of decision-makers"*[8] The proposed list of updated screening principles focuses more on implementational and operational issues, coordination of screening program components and their integration in a broader health care system than their predecessor did.

TABLE I. FINAL REFINED SET OF CONSOLIDATED SCREENING PRINCIPLES BY DOBROW ET AL.

Domain	**Consolidated screening principles (after systematic review and modified Delphi consensus process)**
Disease/condition principles	**1. Epidemiology of the disease or condition**
	The epidemiology of the disease or condition should be adequately understood, and the disease or condition should be an important health problem (e.g., high or increasing incidence or prevalence, or causes substantial morbidity or mortality).
	2. Natural history of disease or condition
	The natural history of the disease or condition should be adequately understood, the disease or condition is well-defined, and there should be a detectable preclinical phase.
	3. Target population for screening
	The target population for screening should be clearly defined (e.g., with an appropriate target age range), identifiable and able to be reached.
Test/intervention principles	**4. Screening test performance characteristics**
	Screening test performance should be appropriate for the purpose, with all key components specific to the test (rather than the screening program) being accurate (e.g., in terms of sensitivity, specificity and positive predictive value) and reliable or reproducible. The test should be acceptable to the target population and it should be possible to perform or administer it safely, affordably and efficiently.
	5. Interpretation of screening test results
	Screening test results should be clearly interpretable and determinate (e.g., with known distribution of test values and well-defined and agreed cut-off points) to allow identification of the screening participants who should (and should not) be offered diagnostic testing and other post screening care.
	6. Post screening test options
	There should be an agreed-on course of action for screening participants with positive screening test results that involves diagnostic testing, treatment or intervention, and follow-up care that will modify the natural history and clinical pathway for the disease or condition; that is available, accessible and acceptable to those affected; and that results in improved outcomes (e.g., increased functioning or quality of life, decreased cause-specific mortality). The burden of testing on all participants should be understood and acceptable, and the effect of false-positive and false-negative tests should be minimal.
Program/system principles	**7. Screening program infrastructure**
	There should be adequate existing infrastructure (e.g., financial resources, health human resources, information technology, facilities, equipment and test technology), or a clear plan to develop adequate infrastructure, that is appropriate to the setting to allow for timely access to all components of the screening program. *
	8. Screening program coordination and integration
	All components of the screening program* should be coordinated and, where possible, integrated with the broader health care system (including a formal system to inform, counsel, refer and manage the treatment of screening participants) to optimize care continuity and ensure no screening participant is neglected
	9. Screening program acceptability and ethics
	All components of the screening program* should be clinically, socially and ethically acceptable to screening participants, health professionals and society, and there should be effective methods for providing screening participants with informed choice, promoting their autonomy and protecting their rights.
	10. Screening program benefits and harms
	The expected range and magnitude of benefits (e.g., increased functioning or quality of life, decreased decreased cause-specific mortality) and harms (e.g., overdiagnosis and overtreatment) for screening participants and society should be clearly defined and acceptable, and supported by existing high-quality scientific evidence (or addressed by ongoing studies) that indicates that the overall benefit of the screening program outweighs its potential harms.
	11. Economic evaluation of screening program
	An economic evaluation (e.g., cost-effectiveness analysis, cost–benefit analysis and cost–utility analysis) of the screening program, using a health system or societal perspective, should be conducted (or a clear plan to conduct an economic evaluation) to assess the full costs and effects of implementing, operating and sustaining the screening program while clearly considering the opportunity costs and effect of allocating resources to other potential nonscreening alternatives (e.g., primary prevention, improved treatments and other clinical services) for managing the disease or condition.
	12. Screening program quality and performance management
	The screening program should have clear goals or objectives that are explicitly linked to program planning, monitoring, evaluating and reporting activities, with dedicated information systems and funding, to ensure ongoing quality control and achievement of performance targets.

**Components of a screening program include recruitment, testing, information access, diagnosis, referral, treatment, follow-up, patient education and support, staff training and program management and evaluation.*[7]

The authors of the review did not mention how the screening principles could be applied in the genomic age where there is an ever growing number of conditions that can be "*detected at the preclinical stage, and even in the pre-pathological stage, using molecular and non-molecular techniques"*[8]. The debate is spearheaded by various stakeholders with differing opinions on the topic – some lobbying to include genomic testing in neonatal screening process, others trying to sway the decision based on risk-benefit ratio. In the end, the decision whether to include certain diseases and technology used to screen for them is partly political, since screening and health care in general is a public health service and thus publicly funded.

1. 2 NEWBORN SCREENING

Newborn screening identifies conditions that can affect a child's long-term health or survival. Early detection, diagnosis, and intervention can prevent death or disability and enable children to reach their full potential .

NBS is more than testing; it is a coordinated and comprehensive system consisting of education, screening, follow-up, diagnosis, treatment and management, and program evaluation[9].

1.2.1 PHENYLKETONURIA AND THE HISTORY OF NEWBORN SCREENING

Neonatal or newborn bloodspot screening (NBS) started to be introduced in the 1960's when Guthrie and Susi have developed an effective and inexpensive method[10] that could be used to screen a considerable number of newborns by detecting elevated levels of phenylalanine in blood associated with phenylketonuria. PKU is a rare autosomal recessive disorder caused by phenylalanine hydroxylase deficiency, an enzyme responsible for breaking down phenylalanine, an amino acid found in food proteins, to tyrosine. This enzyme deficiency leads to the accumulation of phenylalanine, resulting in brain dysfunction and, if left untreated, severe intellectual disability, epilepsy and behavioral problems[11] . An early diagnosis and start of dietary treatment is proven to slow the evolution of the disease and symptoms as long as the treatment is continual and adequate.

The Guthrie test uses a type of filter paper (Guthrie card) to collect the blood sample from a heel-prick in the first 24-48 hours of life. In order to detect phenylalanine levels, initially a bacterial inhibition assay was used but today biochemical analysis is in use[12]. The filter paper containing the blood specimen and patient information (better known as dried bloodspot, DBS) is then shipped to a health laboratory for analysis. The result is usually communicated to the birthing center or primary care provider, who then contacts the parents in order to do ulterior confirmatory testing [13]. If a diagnosis has been made, the patient is referred to a specialized center that is responsible for treatment and long term follow-up of patients with rare diseases.

Before NBS, PKU was diagnosed by testing the urine of children who presented with intellectual disability and had already suffered from irreversible brain damage; placing them on a restrictive low protein diet could slow the progression of the disease but could not reverse the brain damage that has already occurred[14].

1.2.2. EXPANSION OF NEWBORN SCREENING

With the advancement of technology there was a possibility to screen for additional conditions, such as congenital hypothyroidism, cystic fibrosis and others. At that moment, there was no way to test for them using one method, as they required separate tests and separate blood filter paper specimens.

The introduction of tandem mass spectrometry (MS/MS) in the 1990's suddenly made testing for multiple metabolic diseases using a single bloodspot specimen a reality. In contrast to bacterial inhibition assay used by Guthrie that gives a qualitative result, tandem mass spectrometry detects the level of the metabolite present in DBS. Cut off values need to be established to differentiate between a normal and an abnormal screen result. "*These cut off values have to be determined by each laboratory, usually by measuring 1000-2000 normal NBS DBS samples, which allows the determination of normal ranges for all analytes. In our laboratory cut off values are usually 4-5 standard deviations above the mean for the analyte being measured."*[15].

MS/MS technology can be automated and used for reading a new panel every two-to-three minute, permitting the analysis of around 600 samples per 24 hours, with a very modest cost per sample[16], making it convenient to use in mass screening programs. The laboratory used for NBS should analyze a minimum number (30,000-50,000) of samples per year to maintain constant quality level and to obtain sufficient level of experience, especially on what to do in cases of uncertain results[17].

The results from MS/MS suffer from poor positive predictive values and high positive rates, a sacrifice that has been made as not to risk high negative rates due to too stringent cut-off[18]. DBS samples with a positive result get tested again

(second-tier testing) using more complex biochemical analysis or molecular genetics in order to reduce false positives and find true positives that will then be invited for confirmatory testing.

MS/MS made it possible to screen for a number of disorders for which the cure does not exist yet, thus not fully meeting the Wilson and Jungner criteria. The American College of Medical Genetics (ACMG) tried to bridge the gap between what is technologically possible and what the health experts recommend by publishing a list of proposed 29 core conditions (and 25 secondary conditions detected in the process of screening for the core 29) that are considered appropriate for extended newborn screening (eNBS) programmes[19].

TABLE II LIST OF CONDITIONS INCLUDED IN RUSP NEWBORB SCREENING PANEL

ACYLCARNITINES		AMINO ACIDS		
OA	FA	AA	HB Pathies	Others
CORE PANEL				
IVA	MCAD	PKU	Hb SS	CH
GA I	VLCAD	MSUD	Hb S/betaTh	BIOT
HMG	LCHAD	HCY*	Hb S/C	CAH*
MCD	TFP	CIT		GALT
MUT	CUD	ASA		HEAR
3MCC		TYR I		CF
Cbl A,B				
PROP				
BKT				
SECONDARY TARGET				
Cbl C,D*	SCAD	HYPER-PHE	Var HB*	GALK*
MAL	GA2	TYR II		GALE
IBG	M/SCHAD	BIOPT (BS)		
2M3HBA	MCKAT	ARG		
2MBG	CPT II	TRY III		
3MGA	CACT	BIOPT (REG)		
	COT IA	MET		
	DE RED	CIT II		

Codes are as follows: OA (disorders of organic acid metabolism), FAO (disorders of fatty acid metabolism), AA (disorders of aminoacid metabolism), Hb Pathies (hemoglobinopathies)

The ACMG group of experts reiterated that the NBS policy development should be primarily guided by what's in the best interest of the affected patient, with secondary consideration given to the interests of unaffected newborn's, families, health professionals and the public. The importance of having policies in place to ensure confidential storage and appropriate use of specimens are also mentioned-problems even more relevant today, in the age of genomic testing.

Inborn errors of metabolism (IEMs), better known as inherited metabolic diseases, are characterized by disruption of a metabolic pathway and subsequent accumulation of a toxic substrate that causes the disease. They are individually rare but collectively common. Many IEMs present with neurological symptoms in the neonatal period, e.g., encephalopathy, seizures, hypotonia or a combination of these findings[20]. Diagnosis and treatment are often delayed because of the sheer number of genetic disorders and heterogeneity of symptoms and phenotypes.

IEMs are detected by MS/MS and categorized into four groups: Amino acid disorders (AA), urea cycle disorders (UCD), organic acid disorders (OA) and disorders of fatty acid oxidation (FAO).

One of the first IEM to be included in NBS and has led to the expansion of NBS was medium-chain acyl-CoA dehydrogenase deficiency (MCADD), a fatty acid oxidation deficiency that is fatal in ¼ of cases if not treated[15]. Treatment of MCADD consists of avoidance of hypoglycemia as a consequence of prolonged fasting and supportive therapy in the first two years of life. As opposed to PKU where 100% of affected newborns will be symptomatic if not diagnosed and in the first days of life, a portion of children with MCADD will remain asymptomatic without treatment while others can die even before the diagnosis is possible due to fatal arrhythmias[21].

Not every eNBS program includes every IEM recommended by AMCG; certain conditions (e.g. SCADD) are caused by benign genetic variants with no clinical sequelae, and in others (e.g. LCHADD) mortality remains high since the effectiveness of the treatment is still unknown.

A new chapter in eNBS is being written by researchers who study lysosomal storage disease (LSDs), a type IEM resulting from more than 50 inherited disorders. LSDs are caused by the deficient or absent activity of a specific lysosomal enzyme or transporter, resulting in accumulation of uncatabolized, macromolecular substrates within lysosomes. If low enzyme activity is found in the DBS, a second blood sample is collected and the test is repeated. Biomarker identification can also be used as second-tier testing. If both tests are positive the patient is called in to do confirmatory testing. Diagnosis can be confirmed by biochemical analysis, clinical evaluation, or mutational analysis[2223]

The onset of symptoms vary- from presentation in utero to onset in late adulthood; most often it presents as a slowing of a child's developmental progress in disorders with central nervous system component, organomegaly or a dysmorphic facial appearance. "*Although the disorders are generalized, one organ or body system may be affected more than others. What is common is that all of the disorders are present from conception, all are progressive, many involve the central nervous system and finally treatment in many cases is palliative only*."[24]

Besides palliative care for symptom control, some disorders can be treated with hematopoietic stem cell therapy, enzyme replacement therapy (ERT) or small molecule chaperone therapy that can increase residual enzyme activity, thus converting the disease to a more attenuated form.

Seeing that innumerable new genes responsible for LSDs are being discovered in recent years, it should not be too surprising that the discussion on which ones to include in screening programs is ongoing. For example, there is a push to include Pompe disease, MPS I and MPS II in eNBS since many of the Wilson and Junger criteria are satisfied: a well-known natural history and prognosis, pre/asymptomatic stage present, available treatment. That said, the tests used today do not have the ability to differentiate between an early-onset form a late-onset phenotype. Even though patients with early-onset, rapidly progressive disease benefit from early diagnosis and intervention, those diagnosed with late-onset do not, as the therapy is started when first symptoms emerge.

Diagnosing a newborn with a late-onset disease can have a great impact on the parents' and children's lives: on one hand, a known diagnosis can help avoid

medical odyssey that people with rare disease often face, but on the other it can increase parent's anxieties and lead to 'vulnerable child syndrome', described as parental behavioral and psychological issues following a perceived health threat to a child[25]. In cases where recovery occurs, the problematic parental behavior can persist- parental overprotection, separation difficulty, poor school performance, challenges with limit setting, and preoccupation with somatic complaints like abdominal pain or headaches, frequent visits to emergency care or primary care provider. Such behavior can also be seen in cases of false positive screening results or identification of a heterozygous/carrier status.

To reduce false positive rates a second-tier test is done on the same DBS in the case of abnormal results. The combination of first and second tier testing reduces the number of infants that are called for confirmatory testing. In Northeastern Italy, a study of second-tier testing in DBS with low enzyme activity indicative of lysosomal storage disorders, largely eliminated false positive and helped achieve rapid diagnosis in cases of Gaucher disease and MPS I, while in Fabry disease and others more studies were needed[26].

Although the expanded use of MS/MS has ushered in a new era of NBS, Mutze recognized that the expansion of NBS has also revealed significant knowledge gaps: '*(1) incomplete understanding of the natural history and phenotypic variations of some screened diseases, (2) the unreliable early prediction of the individual disease severity, (3) the uncertainty about exact case definition, (4) the ambiguity concerning individual risk stratification and indication to treat, (5) the lack of clarity of long-term benefits of extended NBS program concerning individual health, health economics and the society'.*[27]

1.2.3 NEXT GENERATION SEQUENCING

Due to positive experiences with implementation of molecular genetic analysis into clinical practice, and spurred by the development of clinical treatment, there has been a push to use it as a tool for screening for conditions such as severe combined immunodeficiencies (SCID), spinal muscular dystrophy (SMA), X-linked adrenoleukodystrophy (X-ALD), lysosomal storage disorders, and others. Despite

growing technical possibilities, a number of medical, legal, ethical, psychological, and sociological concerns have been raised.

Next generation sequencing (NGS) can be used to sequence entire genomes or specific areas of interest, ranging from a whole exome to small number of individual genes[28]. There are three main approaches to NGS: sequencing of specific genes (targeted gene panels), sequencing of coding regions of all known genes (Whole exome sequencing, WES) and sequencing of whole genome (WGS).

In Danish NBS program[29], NGS has been applied as a second-tier testing, in case of an abnormal screening result, to reduce the false positive rate while simultaneously providing information of specific genetic variants that can inform treatment decisions, prognostic information, or simply differentiate from diseases that share common biomarkers. Certain studies propose using NGS as a confirmatory analysis in expanded NBS, especially as a way to explain abnormal metabolite concentration in blood samples; it could differentiate between affected patients and those with a heterozygotic variant.[30]

NGS could be used as a first-tier test for stratification of severe versus mild phenotypes of the same condition based on genotype or screening for treatable diseases where reliable biochemical markers or non-classical variants are often missed with biochemical analyses (e.g., intermittent MSUD).

In light of the fact that NGS is an unselective method used to sequence full genomes or exomes, it carries a risk of identifying secondary/incidental findings as well as variants of uncertain significance (VUS)[31] . Ambiguous results can be completely benign, but reporting on them may cause parental anxiety and medicalizing of the child in question. The interpretation of the sheer amount of information gathered is a time-consuming process that requires specialized professionals, such as geneticists, to make a final decision on whether the information is significant or insignificant. To help guide the decision the ACMG board of directors created a set of recommendations addressing incidental findings and a minimum list of conditions, genes and variants that are recommended to be returned whenever clinical sequencing is performed[32]. Such information should then be contextualized with the patient's clinical presentation and family history before reporting it to parents.

Best results from NGS screening are achieved for conditions with classic Mendelian inheritance and good phenotype-genotype correlation, with high penetration and solid knowledge on genetic variants and natural history[33]. However, it needs to be mentioned that there are still instances in which biochemical test performs better than genetic analyses in screening and diagnosing diseases (e.g., PKU)[34].

The public opinion on whether to report on non-actionable conditions, heterozygous carriers that won't develop any symptoms (e.g., sickle cell disease), late-onset conditions found in infants varies greatly from the opinion of experts. In a focus group study[35] parents were interviewed about their experience with NBS. Many supported the implementation of NBS for untreatable conditions presenting in infancy to help parents in terms of emotional preparation, support and research of the condition and would avoid 'diagnostic odyssey' often experienced by those with rare disease.

1. 3. INFORMED CHOICE

1.3.1. INFORMED CONSENT

Informed consent is a process in which a health care provider educates a patient about the risks, benefits and alternatives of a given procedure or intervention. The patient must be competent to make a voluntary decision about whether to undergo the procedure or intervention.[36] In the case where parents decide on behalf of the child the term proxy-consent can be used.

Hargreaves and colleagues[37] (?) in their article outlined elements of informed choice for newborn screening:

1. Decisions are made voluntarily
2. By parents who have considered and understood information about the screening, including benefits and risks
3. In line with their own values, beliefs, wishes or priorities
4. To accept or decline screening for all or some of the conditions
5. To accept or decline test that identify carriers' status
6. To accept or decline follow-up screening or diagnostic tests
7. To accept or decline receiving invitations to participate in research related to newborn bloodspot screening programme
8. The extent of parent choice can be offered with regard to use of DNA analysis as part of screening, and the use of residual blood spots for wider public health purposes and research remain limited.

1.3.2. PARENTAL EDUCATION

The issue of informed consent cannot be separated from the issue of parental education about NBS. In countries where NBS was made mandatory, parents are often informed about NBS minutes before the heel-prick. NBS is often seen by parents as a standard part of the birthing process, with limited information given

and assumed to be mandatory[38]. Numerous parents recount that they have no memory at all of their child being screened, as it happens when the parents are tired, overwhelmed and stressed. [39] In places with mandatory testing, parents may be able to opt-out of the program for religious or personal reasons. The voluntary approach entails an informed decision made by parents or legal guardians. The proponents of mandatory screening claim the welfare of the child supersedes parental right to refuse medical intervention and that *'parental refusal of screening or treatment unjustifiably poses a grave, if remote, risk of harm to the child'*[40]. On the other hand, proponents of the voluntary approach maintain that parents should have broad discretion for making health care decisions for their child.

Some concerns have been raised about the consequences of letting the parents decide: would leaving the decision up to parents impact the number of newborns screen and influence, not only singular child's life, but also have unintended effect on the effectiveness of the newborn screening system[41]? One solution is to make the screening for conditions which meet the standard of direct benefit to the infants mandatory, but require parental consent for screening where the direct-benefit is not met[42]. However, some studies[43] concluded that education of the public and asking for parental consent did not lower the percentage of newborns screened, as the parents remain overwhelmingly supportive of NBS when informed adequately on risks and benefits.

One thing that many parents have an issue with is DBS storage and use. After clinical screening is complete, DBS cards are stored in archives in order to be available for quality improvement and research purposes. DBS cards are an important source of population-representative genetic samples, and their use could be a great source for population-based genomic studies. Even when DBS have been unidentified the concerns about privacy persist. The fear of potential misuse or unintended harm from genetic data, such as genetic discrimination or possession of genetic information by a government agency will have to be addressed.

DBS storage and use varies, but it is important to ensure appropriate protection of sensitive personal information. Information about how DBS is stored and used for research is severely lacking for EU countries[44]; in 2021 less than 50% of countries

had not mentioned storage and use of residual samples in the informational material provided to parents. Furthermore, 2/3 of countries studied failed to ask for consent regarding storage and secondary use. Nonetheless, all countries store DBS samples- some for as little as 3 months, some for 1000 years[45].

Parental education is a sore spot for many NBS programs- often rushed or neglected, rarely made mandatory. Studies have shown that few parents remembered that the newborn screening was performed and even fewer the reason behind it[4647].. Frequently the information about NBS is given just before the heel-prick is done, when parents are physically exhausted and emotionally overwhelmed, making it difficult for them to learn and retain new information. The education of parents often falls onto the shoulders of birth attendants or other hospital staff who haven't been educated themselves on how to rely on this information or simply, do not have the time to do it properly. With an inadequate understanding of NBS, parents can be adversely affected if they receive a false-positive or indeterminate result.[48]

An expert opinion document was formulated by the European Union Network of Experts on Newborn Screening (EUNENBS) that contained recommendations on best practices including several recommendations regarding the provision of information about NBS[49].

The general consensus among parents confirms EUNENBS recommendations- that receiving information about NBS would be more useful if it were done prenatally, preferably in the third trimester of the pregnancy[50] with a verbal reminder in the post-natal period shortly before sampling[51]. Informational material, such as booklets or brochures, could form the basis of the educational approach but it is not a substitute to face-to-face discussion with a healthcare provider[52].

General Medical Council (GMC) published a list of knowledge aspects for making an informed decision about participation in NBS program:

1. The purpose of screening;
2. The likelihood of positive and negative findings;
3. The possibility of false positive and false negative findings;
4. The uncertainties and risks attached to the screening process;
5. Medical implications of screening (conditions and treatment);

6. Social implications of screening;

7. Financial implications of screening;

8. Follow-up plans including the availability of counselling and support services.

Depending on the interpretation of GMC's list, some may view it as lacking; how the parents will be notified, the importance of responding and how to respond to a positive result, how the samples will be stored and used (research) and the process for refusing the screening (if applicable) should be addressed as well.[53]

1.3.3 Informed consent for genetic testing

In 2013 the ACMG board of directors published a document 'Points to Consider for Informed Consent for Genome/Exome Sequencing'[54] that focuses on the need for informed consent before clinical application of genome and exome sequencing for germ-line testing. Parents should be informed about the possibility of incidental findings, about benefits and risks of using NGS, and the potential implications for family members.Genetic counseling should be offered before any testing has begun. If NGS is to be used for NBS the model of presumed consent cannot be valid anymore and an informed consent will be required.

In the era where genetic testing is readily available and relatively affordable, some ethical issues need to be examined; genetic information is familial, therefore, the results of one person has direct health implications for others who are genetically related. If information about possible genetic conditions or predisposition for an illness is uncovered, should those relatives be informed so they can decide whether to get tested themselves?

Genetic testing for illnesses or conditions that do not follow strict Mendelian inheritance can have limited predictive power; the interaction between our genes and the environment is difficult to predict and explains why the severity and manifestation can differ even with people carrying the same mutations.

Psychological risk associated with genetic testing is high and can manifested as anxiety, self-esteem issues, social stigma, insurance, and employment discrimination, just to name a few. To help mitigate these issues, some authors propose counseling before and after the test, giving relevant information to parents and obtaining consent and a guarantee of confidentiality and privacy.

As for the introduction of new tests and maintenance of the established ones, IOM report[55] recommends following 3 principles to aid the decision making:

1. Identification of the genetic condition must provide a clear benefit to the child;
2. A system must be in place to confirm the diagnosis;
3. Treatment and follow-up must be available for affected newborns.

In 2001 the AAP's Committee on Bioethics published a set of recommendations on genetic testing in pediatrics that put the emphasis on continual review and evaluation newborn screening tests, importance of informing and counseling the parents and obtaining informed consent but did not support the broad use of carrier testing or screening for adult-onset conditions[56].

1. 4 LONG-TERM FOLLOW-UP

Newborn screening programs are a complex system of education, screening, diagnosis and referral (short-term follow-up, STFU), treatment and care management (long-term follow-up, LTFU) and ongoing evaluation of effectiveness of all components. Some countries, such as the US, prefer the STFU model of NBS, where once a patient is referred to a specialist the they are no longer tracked by the system[57]. After referral for diagnosis the public health mandate for most US states ends and the responsibility rests with pediatricians and other clinicians.

The role of long-term follow-up (LTFU) is care coordination and health management over the course of lifetime, provision of evidence based therapeutic and rehabilitative care, age appropriate preventive care throughout the life, and new knowledge discovery. Tracking health outcomes is crucial for improving the knowledge base of the newborn screening disorders; sharing the collected data and opening communication channels with families, public health officials, medical subspecialists, primary care physicians, researchers, and policy makers is useful to build a more successful NBS[5859].

Research in German eNBS program showed more than 95% of children diagnosed with IEMS attended regular kindergarten or primary school and have age-appropriate development quotients or intelligence quotients.[60] As the goals of NBS are optimal physical, developmental and social outcomes with NBS conditions, children demonstrating normal growth and development are examples of indicators of program's success.

In the article *'Newborn screening and Long-Term Outcomes'*[61] Powell proposed creation of national registries and flow of data from electronic and medical record systems to public health NBS programs to optimize the ability to track patient outcomes, establish evidence-based best practices, improve quality of care and assess the needs of patients and family. Data from registries can be used to asses traditional metrics of mortality and morbidity, proxy measures such as preventable health care utilization (hospitalization, emergency department visits), measures of harm associated with treatment, developmental and social-emotional outcomes and family experience of care, and identify potential disparities in these outcomes

among individual groups[62]. Using registries to study natural history of conditions, assess genotype-phenotype relations, track clinical and laboratory information as children grow, can fill gaps in evidence bases left by the lack of randomized controlled studies and prospective observational cohort studies on rare disease.

One of the issues in LTFU is the lack of coordination within the public health program: all of the conditions identified through NBS are chronic and therefore require medical care and other service throughout the patient's life. The patient often interacts with specialists, primary care doctors, nurses, clinical psychologists, genetic counselors, occupational and physical therapists, education specialists, social workers, pharmacists, and others. These figures should ideally work together to offer the best quality of life for the patient, but between the lack of resources, time and knowledge this is a difficult task to achieve. Unfortunately, LTFU activities are often low priority for funding compared with activities related to screening and diagnosis.

1.5 LEGAL FRAMEWORK FOR NEWBORN SCREENING

1.5.1 NBS IN THE EUROPEAN UNION

There is significant heterogeneity between European Member States (MS) in terms of health care systems, and as a consequence NBS programs differ as well. MS have complete autonomy in how to structure and deliver health service and medical care as they see fit. EU health policy serves to complement national policies, help modernize and lessen disparities between MS healthcare systems by proposing legislation, providing financial support and facilitating the exchange of best practices between health experts from different states.

The Commission Communication on Rare Diseases: Europe's challenges (2008)[63] wrote about NBS as follows:

Section 5.8 *"Screening practices Neonatal screening for Phenylketonuria and congenital hypothyroidism is current practice in Europe and proved highly efficient in preventing disabilities in affected children. As technology evolves, many tests can now be performed, including those by robots, at low cost for a wide range of rare diseases, especially metabolic disorders and genetic conditions in general. It is recommended to encourage cooperation in this area to generate evidence on which decisions should be based at Member States level. An evaluation of current population screening (including neonatal screening) strategies for rare diseases and of potential new ones, will be conducted by the Commission at EU level to provide Member States with the evidence (including ethical aspects) on which to base their political decision. The Commission will consider such support as a priority for action."*

In 2009, following a European Tender ('Evaluation of population of newborn screening practices for rare disorders in Member States of the European Union')[64], European Union Network of Experts on Newborn Screening (EUNENBS) was established to support activities and creation of its outputs. EUNENBS included experts from competent national constitutions of all MS, healthcare professionals and scientific organizations involved in NBS. The Tender included a decision-making matrix for implementing NBS and a report on the status quo in 2011, noting

that the number of screened disease in EU countries at the time varied from 2 to 29; 37 European countries (including 27 MS) screened for congenital hypothyroidism, 33 for phenylketonuria/hyperphenylalaninaemia (PKU/HPA). Conditions most frequently included in screening programs of MS are : congenital adrenal hyperplasia (CAH), cystic fibrosis (CF), Medium chain acyl-CoA dehydrogenase deficiency (MCADD), and sickle cell anemia(SCD)/beta thalassemia in Mediterranean countries or those with migrant populations[65]. Notably, the number of screened diseases was not correlated with the country's GDP.

In 2013 EUCERD proposed a list of topics for potential European collaboration based on the Tender from 2011[66]. The topics include:

- Production of Standard Operating Procedures for the organisation and management of NBS process;
- Production of good practice guidelines for the management and follow-up of patients, for each screened disease;
- Adoption of Standard Operating Procedures for the communication with parents
- Production of Informational material for prospective parents and the public, and for parents whose child was screened positive but whose diagnosis is not yet confirmed;
- Adoption of Standard Operating Procedures for the training of health professionals involved in the screening process;
- Organisation of European training schemes;
- Networking between laboratories to ease collaboration and resource sharing in order to improve the quality and cost-efficiency of national operations;
- Establishment of shared databases between NBS laboratories and centres of expertise in charge of the follow-up of patients to gain better knowledge of the screened diseases and to assess the benefit of the screening strategy;
- Discussion on the Wilson and Jungner criteria and other criteria to be used when considering any expansion of NBS, as view diverge in many countries on this issue;

- Common assessment of the new proposal for NBS, between MS wishing to do so, when new technologies allow for such a consideration, via EEnetHTA (The European Network for Health Technology Assessment);
- Establishment of public health key indicators for the continuous evaluation and monitoring of the screening programs.

Majority of MS report to already have a body which oversees NBS; some are devoted to newborn screening only while others have broader tasks in health prevention, public health, insurance, and rare disease. In 2011 about half of the countries surveyed, reported having laws and regulations in place to regulate NBS: from mandating the implementation of screening programs, without obliging the parents to use it; informed consent or dissent; to the obligation for parents to have their newborn screened.[67]

An Expert Opinion[68] document published in 2011 stated that General principles that should be adopted in each MS, according to the EU Council Recommendation on rare diseases are: identification of limited number of centres of expertise for diagnosis and definition of treatment in order to ensure appropriate concentration of expertise[69]; participation of such centres in EU reference networks and in registries for exchange of expertise and consultation.

At EU level NBS committees should be involved in scientific evaluation, horizon scanning, prioritisation, assessment of technology and evaluation of cost-effectiveness and ethics of potential screening possibilities. EURORDIS- a non-governmental patient-driven alliance of organisations and individuals active in the field of rare diseases in Europe published its own set of principles that should be adopted at the national level to achieve greater synergy at EU level. EURORDIS highlighted the need for NBS to have clearly defined roles, responsibilities, accountabilities and communication pathways that are embedded into the national health care system. European-wide standards addressing the timing, sample collection methods, follow-up, and information shared with parents are needed to guarantee uniformity and quality throughout the process. In addition, legal guidelines should also be put in place to specify how, when and who is responsible for information provision about the screening process. Unfortunately, only ten countries surveyed in one study[70] have made it obligatory to inform parents in

prenatal period, some (e.g., Croatia and Cyprus) have not made it obligatory to inform parents at all[71].

Data collected in 2019 by Resource on the State of the Art of Rare Disease Activities in Europe (SotAR)[72] painted a picture that was not too dissimilar to the 2011 one: number of conditions included in the screening programs ranged from 1-26, with certain regions in Italy offering screening for at least 58 diseases.

TABLE III. NUMBER OF DISEASES INCLUDED IN NBS PROGRAMS IN EU MEMBER STATES IN 2019

EU MS	Number of Diseases in NBS Program
Austria	25
Belgium	11-13
Bulgaria	3
Croatia	2
Cyprus	2
Czech Republic	19
Denmark	No data
Estonia	No data
Finland	21
France	4 + sickle cell anaemia for those at risk
Germany	15
Greece	No data
Hungary	26
Ireland	8
Italy	3-58
Latvia	2
Lithuania	4
Luxembourg	5
Malta	2
Netherlands	19
Poland	No data
Portugal	26
Romania	2
Slovak Republic	23
Slovenia	18
Spain	7
Sweeden	24
UK	9

ERNs are European networks of Health Care Providers- Centres of Expertise, healthcare professionals and laboratories- that offer a frame of reference to help navigate through complicate landscape of healthcare for those with rare disease; ERNs aim to improve collaboration efforts between experts from different countries, accumulate and integrate knowledge and offer it to those who need it, particularly in countries where such expertise is lacking. [73]

1.5.2. ITALY

Italian NBS program is second only to the United States (62) for the number of rare diseases included in newborn screening[74] and is internationally recognized for its strong organizational model of regional disease networks, excellence of several centres of competence and existence of several institutional helplines of reference for rare disease, the accessibility of medicinal products and the surveillance and monitoring system implemented on regional/interregional and national basis.

In 1978 the Italian government established a National Health Service (Servizio Sanitario Nazionale, SSN), a public health care service funded by taxes and meant to provide universal coverage to the whole population, irrespective of income or location. Health is considered a fundamental right of every person and thus, human dignity, health needs and solidarity were set out as the guiding principles of the SSN. The SSN is organized under the Ministry of health and is administered on a regional basis, giving each of the 20 regions significant independence and flexibility to determine its own priorities and goals, resulting in 20 separate health care systems with varying levels of access and quality of health services. To help synchronize and provide a more equal healthcare across the country a core benefit package called Essential Levels of Care (LEA) was released*;* LEA are the services and benefits that the SSN is required to provide to all citizens, in every region. free of charge or upon payment of a participation fee. The services provided are divided into three categories: collective prevention and public health, district assistance and hospital assistance.

As a consequence of the decentralized healthcare system before 1992, NBS was offered in some Italian regions while not in others. In 1992, Law. 104/1992, Decree n. 548/1993 made newborn screening for the identification and early treatment of congenital hypothyroidism, phenylketonuria and cystic fibrosis mandatory for every infant born on Italian territory. Certain regions started pilot projects for early diagnosis of IEMs, that have been included in the list of rare diseases since 2001. The National Plan for Rare Diseases 2013-2016 emphasized both primary and secondary prevention for rare disease patients and their families[75].

The need to harmonize the regional situation was addressed in 2016, when the Italian Parliament issued the Law no. 998 "Dispositions for neonatal diagnostic tests required for the prevention and treatment of inherited metabolic diseases", which grants the expanded neonatal screening to all Italian Regions. In October of the same year, a ministerial decree defined the screening program as a system articulated into four main functions (the screening laboratory, the laboratory for confirmatory diagnosis, the clinical centres, and the regional coordination/supervision). It also defined the panel of screening conditions, the timing for specimen collection, the screening methodology, the confirmatory tests and clinical follow up. The same year, Law n. 167/2016, raised the number of IEMs included in eNBS to 40[76]. In 2016 and updated in 2017, NBS was included in the new Essential Levels of Care (LEA), guaranteeing a free screening, follow up and treatment (where appropriate) to any newborn in Italy. In the 2019, Budget Law expanded the eNBS to include additional IEMs, hereditary neurological disorders, congenital immune deficiencies, lysosomal storage disorders along[77]. Even before 2019 multiple regions started to screen for conditions such as congenital adrenal hyperplasia (CAH), severe congenital immunodeficiency (SCID), Guanidinoacetate methyltransferase (GAMT) deficiency, hemoglobinopathies, in addition to conditions mentioned above[78].

The core panel is to be reviewed and updated every two years. There are still differences in the number of conditions newborns are screened for in different regions.

Today the core panel includes 47 conditions: 36 IEMs detected by MS/MS, 2 (biotinidase defect, galactosemia) are detected by other methods, and 9 metabolic

conditions that enter in differential diagnosis with the 36 IEMs detected by MS/MS, as they share the same biomarkers.

TABLE IV. CORE PANEL AND SECONDARY CONDITIONS INCLUDED IN DM 13.10.2016

	CONDITION			
	AA	**OA**	**UCD**	**FAO**
Core Panel DM 13.10.2016 (MS/MS)	PKU	GA I	CIT I	CUD
	HPA	IVA	CIT II	CPT Ia
	BIOPT (BS)	BKT	ASA	CACT
	BIOPT (REG)	HMG	ARG	CPT II
	TYR I	PA		VLCAD
	TYR II	MUT		TFP
	MSUD	Cbl A		LCHAD
	CBD	Cbl B		MCAD
	MTHFR	Cbl C		M/SCHAD
		Cbl D		GA II/MADD
		2MBG		
		MAL		
		MCD		
DD with Core Panel (MS/MS)	TYR III	3MGCA		SCAD
	GNMT	3MCC		
	MAT	2M3HBA		
	SAHH	IBG		

TABLE V CONDITIONS INCUDED IN DM 13.10.2016, NOT DIAGNOSED WITH MS/MS

	Disorder of carbohydrate metabolism	Disorder of AA and other OA metabolism
IEMs not diagnosed with MS/MS	GALT	BTD

In 2017, only 8 regions (FVG, Marche, PA Trento, Piedmont, Puglia, Tuscany, Umbria, Veneto) had accomplished to adhere completely to DM 13.10.2016 and screen for all 47 conditions; the remaining regions had varying level coverage. In

2021, all Regions report to be completely adhering to the DM16 and offer the screening for all 47 conditions, with the exception of Calabria which is collaborating with Campania to expand screening efforts on its territory.

Regions are free to start additional screening programs in form of pilot projects- Veneto Region has started to screen for Aromatic L-amino acid decarboxylase (AADC) deficiency, Friuli Venezia Giulia for mucopolisaccaroidosis type I (MPS I), Fabry disease, Pompe disease, Gaucher disease; Lazio and Liguria added severe combined immunodeficiency to their screening programs and Tuscany started screening for Spinal muscular atrophy (SMA); PA Trento initiated a pilot project to screen for lysosomal storage disease (Fabry, Gaucher, MPS I, Pompe).

At the end of 2021, the eNBS is reported to be active in all Regions and Autonomous Provinces, as is audiological and ophthalmological screening (with the exception of one Region in case of ophthalmological screening where it is being implemented). However, not every Region had updated the panel of pathologies codified by Law n 167/2016.[79]

TABLE VI. STATE OF IMPLEMENTATION ENBS ON 30.09.2019. ADAPTED FROM MONITORARE REPORT 2019.

REGION/PA	STATUS OF IMPLEMENTATION OF ENBS on 30.06.2019	PRESENCE OF ONE OR MORE REGIONAL REFERAL FOR SCREENING	PRESENCE OF RARE DISEASE COORDINATING CENTER FOR SCREENING
ABRUZZO	Active	Yes	Yes
BASILICATA	Active, in collaboration with other Regions	Yes	Yes
CALABRIA	On the way to implementation with collaboration with other Regions	Yes	No
CAMPANIA	Active	Yes	No
EMILIA ROMAGNA	Active	Yes	Yes
FRIULI VENEZIA GIULIA	Active, in collaboration with other Regions	Yes	Yes
LAZIO	Active	Yes	Yes
LIGURIA	Active	Yes	Yes
LOMBARDY	Active	Yes	Yes
MARCHE	Active	Yes	Yes
MOLISE	Active, in collaboration with other Regions	No	No
PA BOLZANO	Active, in collaboration with other Regions	Yes	No
PA TRENTO	Active, in collaboration with other Regions	Yes	No
PIEDMONT	Active	Yes	No
PUGLIA	Active	Yes	Yes
SARDEGNA	Active	No	No
SICILY	Active	Yes	Yes
TUSCANY	Active	Yes	Yes
UMBRIA	Active, in collaboration with other Regions	Yes	No
VALLE D'AOSTA	Active, in collaboration with other Regions	Yes	No
VENETO	Active	Yes	Yes

In 2017, Istituto Superiore di Sanità (ISS) founded Neonatal Screening Coordinating Centre (Centro di Coordinamento degli screening neonatali- CCSN) with the purpose of standardizing screening programs between different Regions. CCSN put in place guidelines concerning catchment area of each Coordinating Centre, codification of collected information, standardization of levels of care, therapy, and follow-up. In addition, CCSN standardized the timing and mode of collection and handling of blood samples and instituted an archive for storage of DBS.

TABLE VII. NUMBER OF CONDITIONS SCREENED IN 2019, 2020 AND 2021

REGION/PA	NUMBER OF CONDITIONS INCLUDED IN NBS ON 31.12.2019		NUMBER OF CONDITIONS INCLUDED IN NBS ON 31.12.2020		NUMBER OF CONDITIONS INCLUDED IN NBS ON 31.12.2021			
	INCLUDED IN CORE PANEL (DM 13.10.2016)	NOT INCLUDED IN CORE PANEL (DM 13.10.2016)	INCLUDED IN CORE PANEL (DM 13.10.2016)	NOT INCLUDED IN CORE PANEL (DM 13.10.2016)	CONGENITAL HYPOTHYROIDISM	CYSTIC FIBROSIS	INCLUDED IN CORE PANEL (DM 13.10.2016)	OTHER (EXTRA-LEA)
ABRUZZO	40	0	40	0	YES	YES	40	0
BASILICATA	40	-	40	0	YES	YES	40	0
CALABRIA	-	-	-	-	YES	YES	-	-
CAMPANIA	41	0	41	1	YES	YES	45	0
EMILIA ROMAGNA	40	1	44	0	YES	YES	47	1
FRIULI VENEZIA GIULIA	40	0	40	0	YES	YES	40	0
LAZIO	41	0	41	0	YES	YES	48	2
LIGURIA	36	0	40	0	YES	YES	49	0
LOMBARDY	40	1	40	0	YES	YES	47	3
MARCHE	40	-	40	0	YES	YES	47	0
MOLISE	37	0	37	9	YES	YES	37	9
PA BOLZANO	40	3	40	3	YES	YES	40	3
PA TRENTO	44	1	41	3	YES	YES	47	5
PIEDMONT AND VALLE D'AOSTA	41	3	41	3	YES	YES	40	3
PUGLIA	40	0	40	0	YES	YES	40	1
SARDEGNA	39	-	40	0	YES	YES	40	0
SICILY	40	0	40	8	YES	YES	39	16
TUSCANY	40	5	40	4	YES	YES	40	5
UMBRIA	40	0	40	0	YES	YES	40	0
VENETO	40	4	40	4	YES	YES	40	8

After the health professional collects the blood specimen, the sample is sent to one of 16 regional or interregional reference Screening Centres within 24/48 hours. The DBS will be analysed on the same day or the following day.

In 2021, 19 total screening laboratories were active in 14 Regions, 8 of which have an interregional catchment area that covers the territory of 7 Regions that are lacking.

In 2020, there were 18 laboratories that offered biochemical confirmatory testing. distributed in 14 Regions; the same number of laboratories were involved in confirmatory testing using molecular genetics.

FIGURE 2 MAP OF ITALIAN REGIONS AND LABORATORIES PERFORMING ENBS.

Map of Italian regions. The blue dots represent laboratories performing expanded Newborn Screening in Italy. The red dot marks the only regional laboratory not performing NBS tests by MS/MS in the years 2019–2020. Regions without a dot have established interregional agreements with neighbouring regions.

Before the sample is taken, the parents should be informed about the NBS and given privacy policy to read and sign. Informing the parents about the diseases screened for, preservation methods of the DBS and possible use of data it contains. Parental informed consent is not required when screening for conditions included in the core panel, only for the additional ones not yet enlisted in the panel.

In case of a positive result parents are contacted by the birth centre, invited to return for confirmatory testing and referred to a Centre of Expertise where they can receive appropriate treatment and care.

Regional Rare Disease Coordinating Centres are responsible for the management and upkeep of the Registry of Rare diseases, exchange of information with other Centres and organizations on national and international level, work on educating and consulting with healthcare professional about rare diseases and treatments available, organize awareness and education campaigns for the general public.Patients diagnosed with a rare disease are referred to Rare Disease Centres of Expertise that have documented history of diagnosing and treating patients with rare disease and infrastructure necessary to handle such patients. There were 223 Centres of expertise active on Italian territory, around 3.7 Centres for every 1 million people, similar ratio to those in other EU Member States.

A total of 1.586.578 infants born in Italy were screened in the period between January 2017 and December 2020.

Between 2019 and 2020, the coverage of eNBS in Italy has reached 97.5% of the total neonatal population[80]. Aminoacidemias were the most common inborn error of metabolism detected.

1.5.3 VENETO REGION

Veneto Region has always been on the forefront of medicine and science so it is not out of the ordinary that the first newborn screening program has been implemented in Veneto in 1975, at first only for cystic fibrosis, and later for congenital hypothyroidism as well.

Veneto Region today has two active screening centres: one in Padova (Azienda Ospedale Università di Padova- AOUP), and one in Verona (Azienda Ospedaliera Integrata di Verona- AOUIVR), each with its own interregional catchment area (Table VIII). Catchment area for AOUP includes provinces of Belluno, Treviso, Padova, Venezia, Friuli Venezia Giulia Region and PA Trento. AOUIVR screens the population from provinces of Verona, Vicenza, Rovigo and PA Bolzano.

In addition to the conditions found in the Table VIII, AOUP screens for severe congenital immunodeficiency (SCID), Niemann-Pick A/B, Zellweger syndrome, X-linked adrenoleukodystrophy, as a part of a pilot project, aromatic L-amino acid decarboxylase deficiency (AADC).

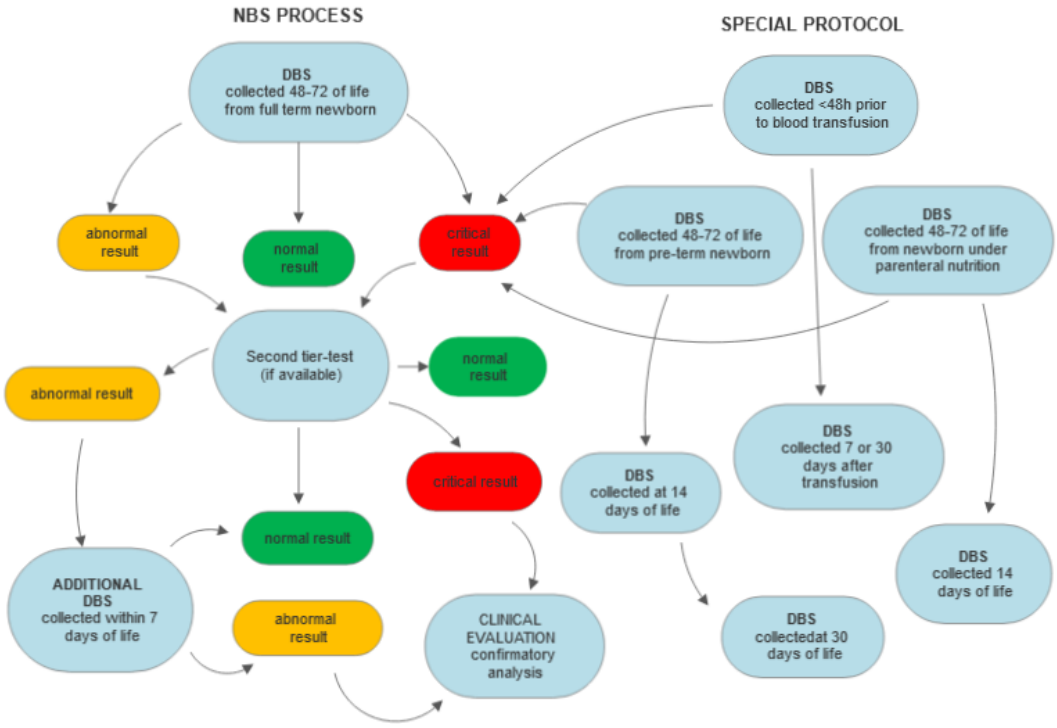


FIGURE 3 NBS FLOWCHART

TABLE VIII CONDITIONS OBJECT OF SCREENING AND CATCHMENT AREA FOR AOUP AND AOUIVR, VENETO REGION, YEAR 2019

Region/PA	Veneto West *Verona, Vicenza and Rovigo*	Veneto East *Padova, Treviso, Belluno, Venezia*	PA Trento	Friuli-Venezia Giulia	PA Bolzano
IEMs core panel DM 13.10.2016 (MS/MS)	AOUIVR	AOUP	AOUP	AOUP	AOUIVR
Galactosemia	AOUIVR	AOUIVR	AOUP	AOUP	AOUIVR
Biotinidase deficiency	AOUIVR	AOUIVR	AOUP	AOUP	AOUIVR
LSD (Fabry, Pompe, Gaucher, MPS I) (NeoLSD Kit)	/	AOUP	AOUP	AOUP	/
Congenital hypothyroidism	AOUIVR	AOUIVR	AOUP	AOUP	AOUIVR
Congenital adrenal hyperplasia	AOUIVR	AOUIVR	AOUP	/	AOUIVR
Glucose-6-phosphate dehydrogenase deficiency	AOUIVR	AOUIVR	AOUIVR	/	/
Cystic fibrosis	AOUIVR	AOUIVR	AOUIVR	/	AOUIVR

2. OBJECTIVES

2.1 GENERAL OBJECTIVES

The present study aims at providing the state of the art of expanded newborn screening at European, national and in the Veneto region (Italy) highlighting strengths and areas of improvement of what has been implemented so far. The analysis considers the state of art of screening in Veneto region and drivers for possible future changes, such as the global trend of births at regional level, modifications to the legislation in force especially regarding the panel of screened conditions, and the new technologies available for testing. Possible areas of improvement are identified, with particular attention to: the definition of a common information document to be used at regional level and a defined screening pathway in case of home births.

2.2 SPECIFIC OBJECTIVES

- to provide a general description of births trends in the Veneto region, with particular regard to elements of importance for the screening programme
- to describe the phenomenon of planned home births and its management within the regional screening programme
- to describe Veneto region screening programme, in terms of screened diseases, screened newborns, and newborns tested positive at first and second tier tests
- to compare the contents of the information documents in use in the two regional screening Centres, in other Italian regions (Emilia-Romagna, Tuscany, Lombardy, Friuli Venezia Giulia and Piedmont) and considering also the one proposed as a national model by the National Institute of Health (ISS).
- to formulate a proposal of a common information document, to be adapted at local level, providing information to parents/guardians on the expanded newborn screening programme for diseases included in the national panel

- to identify some aspects that could represents drivers for the improvement of the global functioning of the expanded screening programme ongoing in the Veneto region

3. MATERIALS AND METHODS

For the description of the state of the art of newborn screening we have used the following data sources: the Veneto region birth registry and a survey carried out annually by the National Institute of Health filled by screening Centres and the regional coordination level for the screening programme, based at the rare diseases regional Coordinating Centre.

3.1. REGIONAL BIRTH REGISTRY

Veneto region is located in the north-east of Italy and has a population of 4.854.633 inhabitants (ISTAT, 2021). The Veneto region birth registry, ongoing since 2002, collects and analyses data from the "Certificate of Delivery Care (CeDAP)"[81] .

The data collection has been established per Law at national level to provide epidemiological information on births and newborns, to serve public health purposes. The data collection on births was established through the Ministerial Decree no. 349/2001. Data collected are divided into six sections; each one includes specific information referring to the birthplace, parents sociodemographic characteristic and health conditions, pregnancy course, childbirth, newborn/s, presence of congenital malformations and/or neonatal mortality causes.

The Veneto Region birth registry maintains the data collection through a web-based system connecting via a protected network all the maternity/birth Centres of the region. Maternity/birth Centres are located in the main hospitals of the region. Their number has decreased in recent years, passing from 42 in 2002 to 36 in 2021. The birth registry collects data on all the births (including stillbirths) occurring in the regional monitored area. Information is recorded at and following delivery by the attending midwife and by the paediatrician in charge of the care of the newborn, each one for the sections of competence. Collected information includes parental, pregnancy, delivery, and newborn (or stillbirth) characteristics. Data quality controls are regularly performed by the regional registry and, once checked, data are transferred every six months to the Ministry of Health.

For the present study we have used aggregated data on births occurring in the Veneto region during the period 2012-2021. We have considered in particular the variable “place of birth” to distinguish between births occurring at hospital and outside the hospital i.e. at home or elsewhere (either planned or unplanned births).

3.2. NEWBORN SCREENING DATS

The RD coordinating Centre is responsible for the collection of data on newborn screening which are annually sent to the National Committee on newborn screening established at the National Institute of Health since 2017. Data are collected at regional level and local level thanks to the involvement of the regional coordinating Centre and of the two newborn screening Centres active in the region, based at the Verona University Hospital and at the Padua University Hospital. Data collected at regional level are then transferred to the national level for evaluation purposes and comparison between screening programmes ongoing in the Italian regions and Autonomous Provinces.

Data collected are referred to multiple aspects, corresponding to the different moments and settings in which the screening programme takes place, from the information provided to parents before the test is performed, their consent to the procedure, the collection of the dried spots, the laboratory activity (including first and second tier tests), the results disclosure and the referral to a Centre of expertise part of the regional RD care network in case a hereditary metabolic disease is identified.

Data from Birth Registry and Rare Disease Registry was used to determine the prevalence of inborn errors of metabolism at birth and the coverage rate of newborn screening in Veneto and the positive predictive value of the screening.

3.3. INFORMATION PROVISION

For the present study we have collected the information documents in use in 2021 in the two Centres of the region and in other Italian regions (Emilia-Romagna, Friuli Venezia Giulia, Emilia-Romagna, Lombardy, Tuscany and Piedmont). A comparison of the contents has been carried out in terms of type of information document available, languages used, knowledge aspects as described in the work carried out at European level by Ijzebrink et al. (2021)[82]. The investigated aspects are the following: purpose of NBS; positive or negative findings; false positive or negative findings; uncertainties and risks; medical implications; social implications; financial implications, follow-up plans.

On the basis of this analysis, a proposal of a common information document on expanded newborn screening to be used to inform parents/guardians on the screening programme in order they can express their informed consent has been formulated for evaluation and adoption at regional level.

4. RESULTS

4.1 BIRTH RATES IN VENETO REGION

The birth rates in Italy are decreasing: in 2020 the rates have dropped to 404,000 births, over 30% less than the ones recorded only 12 years ago (n=577,000).

The reproductive rates sank to 1.24 children per woman (the lowest since 2003). Numerical and structural changes of the female population had seen fertility on the decline and median age on the rise.

The declining birth rates due to the Covid-19 pandemic have been particularly noticeable in the last few months of 2020: the drop (-2,5% in the first 10 months of 2020) was accentuated in November (-8,3% than the same month in 2019) and December (-10,7%), months that reflect the decrease in conception started at the beginning of the pandemic.

4.1.1 THE NETWORK OF BIRTH CENTRES IN VENETO AND THE PLACE OF BIRTH

In 2020, Veneto had 34 active Birth Centres (BC) distributed in the Region (Fig 1.). The impact of the pandemic can be seen on the Centers transformed in Covid Hospitals in an effort to cope with the increasing numbers of Covid-19 patients in need of treatment. Certain Birth Centres had ceased their operations for different lengths of time during the year, some caused by Covid-19 restrictions (BC Dolo, Schiavonia, Villafranca), other for unrelated reasons (BC AOUI Verona). Two Birth Centres (BC Vittorio Veneto, BC Asiago) have been permanently closed since March 2020.

The network counts 13 Birth Centres, including two University Hospitals, that register more than 1,000 births/year; 14 Birth Centres , including 1 private

institution, that register between 500 and 1000 births/year; and 7 Birth Centres that register less than 500 births/year. (tab1)

Almost the totality (99.5%) of Births in Veneto occurs in a hospital (public or private) and only a limited number in different settings. In 2020 145 (0.5%) births that occurred outside the hospital setting were registered: 115 at home, 6 in an alternative care facility, and 24 in other settings (road, ambulance, car…). Planned home births that were assisted by a midwife accounted for 52.2% (n=60), while the remaining 55 home births were considered accidental.

Home births interested mainly Italian women (93%), with a median age of 32.9 years. In most of the cases these women have a higher education or University degree (63%), are employed (73%) and pluriparous (73%).

4.1.2 Fertility and fecundity

On the 01/01/2020, the number of fertile women residing in Veneto was 987,653. The number of births registered was 32,.055, for a total of 32,493 newborns (1.3% of births are twin births). The resulting birth rate is calculated to be 6.6 ‰, while the standardized fecundity rate is 33.8 ‰.

The birth rate calculated is somewhat smaller than the national average (6.8 ‰), placing Veneto, together with Lazio, between Abruzzo and Emilia-Romagna, while the Regions from the South of Italy, Trentino and Lombardia report the highest birth rates in Italy.

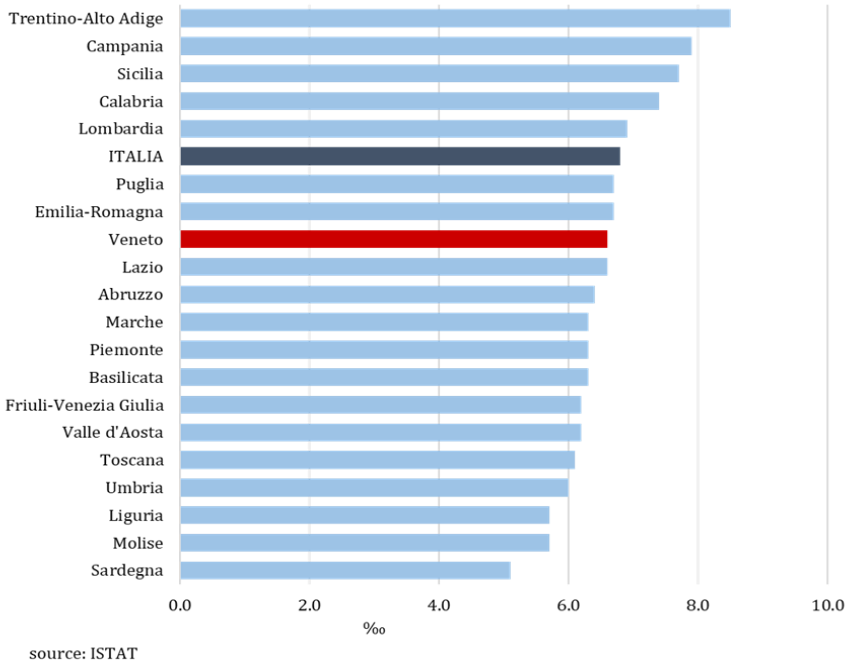


FIGURE 3 DISTRIBUTION OF BIRTH RATE BY REGION

The trend of birth rates in the Veneto region has been on the decline in the 1970's and 80's, followed by a steady increase in years 1978-2008, and an eventual downward turn in the last decade.

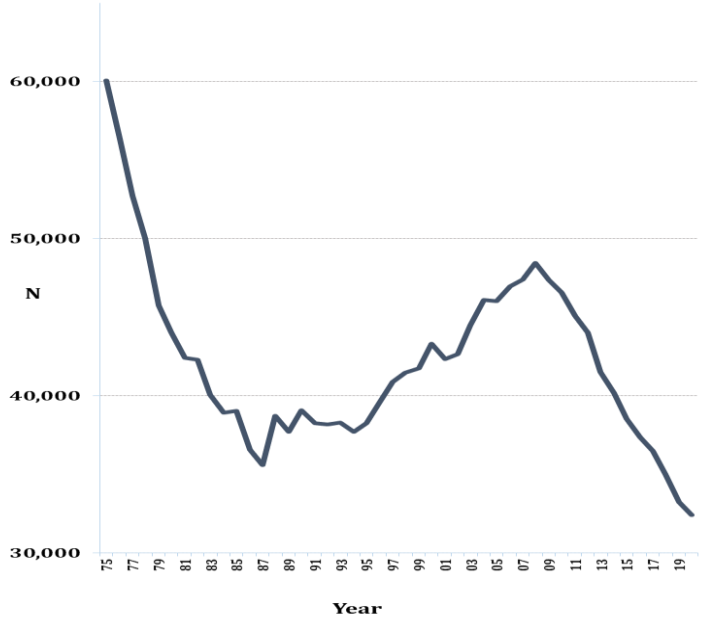


FIGURE 4 NUMBER OF LIVE BIRTHS IN VENETO, 1975-2020.

In 2020, the lowest number of births in the last 45 years was recorded. This corresponds to a decrease of 2.5% compared to 2019, and about a third of what it was 10 years ago.

The median number of children per woman in Veneto is 1.25, close to the national average of 1.24, the lowest since 2003.

The highest natality and fecundity in Veneto is reported in Verona (7.7 ‰ and 37.4 ‰, respectively), the lowest in Rovigo.

TABLE IX CORE INDICATORS. VENETO 2020

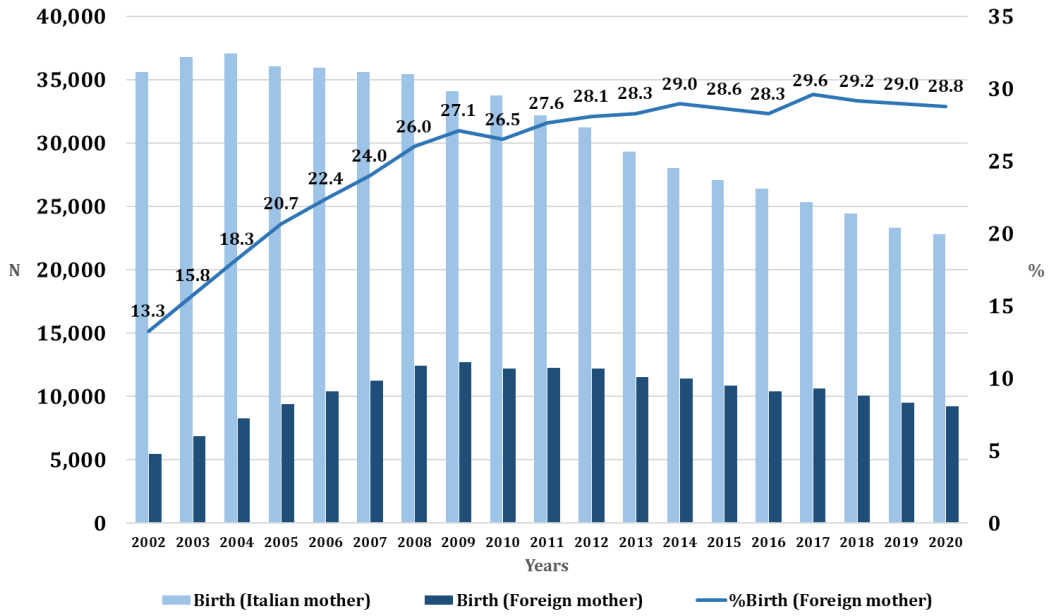
Province	Birth rate (‰)	Crude fecundity	Standardized fecundity rate* (‰)	Average n. of chilren per woman	Average age	C-sections (%)
Veneto	**6.6**	**33.1**	**33.8**	**1.25**	**32.16**	**24.50**

* population with residence in Italy on 01.01.2020

Source: Veneto Region Birth Registry

An additional factor that is implicated in the lowering of the birth rates is the dwindling number of foreigners women giving birth in Italy. Between the years 2002 and 2009 a rapid growth of births was recorded (+113%); the trend reached the plateau in 2010-2011 before beginning to decline in 2012-2020, dropping from 12,204 to 9,225 births. Nonetheless, births by foreigner mothers represent 28,8% of the total number of births in Veneto, a figure higher than those recorded at the national level (20.4%).

FIGURE 5 NUMBER OF BIRTHS PER NATIONALITY. VENETO, 2020



The distribution of births by mother's nationality has been constant through the years: the most prevalent groups are mothers from East Europe (13.5%), Africa (7.6%), and Asia (5.2%).

The bulk of Eastern European mothers were from Romania (5.6%), Albania (2.2%), and Moldavia (2.0%). African mothers were primarily from Morocco (3.9%).

Confronting the data about nationality and place of birth the number of foreigners grows to 32%.

Table X **DISTRIBUTION OF NUMBER OF BIRTHS BY NATIONALITY. VENETO, 2020**

Nationality	N	%
ITALIAN	**22.825**	**71,2**
FOREIGN:	**9.225**	**28,8**
East Europe	4.325	*13,5*
Europe (other)	111	*0,3*
North Africa	1.398	*4,4*
Africa (other)	1.040	*3,2*
Middle East	170	*0,5*
RPC	397	*1,2*
Suoth East Asia	105	*0,3*
Asia (other)	1.176	*3,7*
America	502	*1,6*
Oceania	1	*0,0*
TOTAL	**32.050**	**100**

Noi declared = 5

Median age of an Italian mother in Veneto is 32,9 years, the same as the national average, and 30,2 for foreigners; 70% of Italian women are at least 30 years old, and a third of them are over 35.

The labour and delivery in those who are younger than 20 and older than 35 years suffers from a higher level of complication and may require additional assistance during birth.

In the last 20 years, the number of women over 35 giving birth has risen from 25% to 31.4% in 2020, and more than doubled for those aged 41-44 (from 3.5% to 8.2%). The number of under-aged mothers remains stable (0.15%).

Women who gave birth in Veneto in 2020 generally report medium/high levels of education: 45,9% graduated from high school, and 35,4% had a University degree.

A minority (20%) of women report having lower education: 16,.1% completed middle school, and 2.6% attended only elementary school or report having received no education at all.

The percentage of Italian mothers with lower education levels has dropped from 36,2% in 2003 to 10,1% today, a number much lower than that for foreign mothers (30%).

Overall, over 65% of women were employed at the time of birth, Italian mothers were usually employed (80% of the cases), while foreign mothers in a minority of cases (28%).

In the monitored population, births usually occur in a hospital setting (public or private). Only a small amount of births occurred at home. These can be divided into four categories: accidental home births, planned home births, births in other care facilities, and others (i.e. road, ambulance, car).

Although the number of home births can be considered residual compared to the ones occurring in the hospital setting, a steady increase in terms of absolute numbers of home births can be observed during the considered period.
In 2012, 43,426 births were registered through the Veneto region Birth registry. A very small proportion (n=85) occurred outside the hospital: 29 were planned home

births and 56 accidentals. In 2019, whilst the global number of births registered a marked decrease compared to 2012 (n=32,845), an increase in the number of non-hospital births was observed (n=114). Of these, 98 occurred at home and 16 in other settings. 62 (63%) of all the home births were planned events assisted by a private midwife, whilst the others were accidental home births (n=42). Women who experienced a planned home birth were mostly Italian (98,4%), pluriparous (68%) and with a mean age of 32 years and. 65% had a university or higher education degree and 71% were employed. Compared to the previous year, in 2020 a further decrease in the global number of births was recorded (n=31,910), mainly due to the pandemic effect. 145 (0,4%) births occurred outside the hospital: 115 (79,3%) at home and 30 (20,6%) in other settings (i.e. road, ambulance, car). Overall, 73 (63,5%) home births were planned and 42 (36,5%) occurred at home unexpectedly.
In 2021, 32,169 births were registered in the Veneto region, corresponding to a slight increase compared to 2020. 31.987 (99,4%) births occurred in a hospital setting and 182 (0,56%) outside. Home births accounted for 152 (83,5%) and 30 (16,5%) occurred on the road, in ambulance, car, etc. 96 (63,1%) of the home births were planned and 56 (36,8%) can be considered accidental home births.

TABLE XI DISTRIBUTION OF BIRTHS BY PLACE OF BIRTH IN THE VENETO REGION (2012-2021)

Distribution of births by place of birth in Veneto (2012-2021)						
	hospital (public or private)	accidental home birth	other facility	other (road, ambulance, car...)	planned home birth	Total
2012	43.341	37	0	19	29	43.426
2013	40.851	35	0	22	33	40.941
2014	39.529	44	0	17	41	39.631
2015	37.849	41	1	20	56	37.967
2016	36.646	46	2	25	53	36.772
2017	35.840	48	2	24	61	35.975
2018	34.380	46	3	24	54	34.507
2019	32.731	36	3	13	62	32.845
2020	31.910	42	6	24	73	32.055
2021	31.987	56	7	23	96	32.169

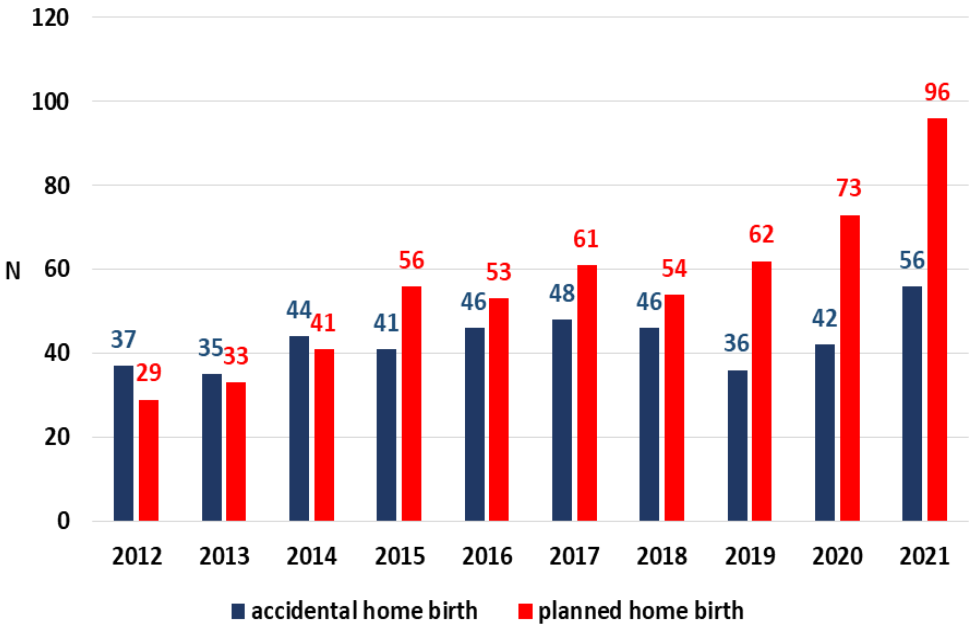


FIGURE 6 DISTRIBUTION OF HOME BIRTHS IN ACCIDENATL AND PLANNED. VENETO 2012-2021.

TABLE XII DISTRIBUTION OF BIRTHS BY PROVINCE – VENETO REGION (2012-2021)

Place of birth	Province of birth							Totale
	Verona	Vicenza	Belluno	Treviso	Venezia	Padova	Rovigo	
hospital (public or private)	80.27	63.169	14.58	66.588	52.653	75.668	12.136	**365.064**
home birth	90	92	7	91	71	70	10	**431**
other care facility	3	14	0	2	0	5	0	**24**
other (road, ambulance, car…)	47	29	5	59	34	31	6	**211**
planned home birth	132	194	19	77	47	86	3	**558**
Total	**80.542**	**63.498**	**14.611**	**66.817**	**52.805**	**75.86**	**12.155**	**366.288**
% total home births	*0,28*	*0,45*	*0,18*	*0,25*	*0,22*	*0,21*	*0,11*	***0,27***
% planned	*0,16*	*0,31*	*0,13*	*0,12*	*0,09*	*0,11*	*0,02*	***0,15***

Whilst the total number of births is steadily declining in the period studied, the number of home births is on the rise, with a significant uptick in 2020 and 2021. Veneto Region has two Screening Centres, one in Padova (AOUP) and one in Verona (AOUI VR); both Screening centres reported the percentage of blood samples collected after the 72-hour mark in hospital setting; Verona SC reported 0.35%, Padova SC 0.98%. For the samples collected from home birth the timing seems to be respected as well.
The birth registry collects data from public and private hospital birth units as well from midwives operating privately. In 2020 12 private midwives assisted with home births, other midwives work with associations that assist with home births or organizations that offer a place for mothers to labour and deliver in a non-hospital setting (the so called "case maternità").

TABLE XIII REPORT THE DISTRIBUTION OF BIRTHS BY PROVINCE AND PLACE OF BIRTH FROM 2012 TO 2021.

Organizations	Sede (Provincia)						
	Verona	Vicenza	Belluno	Treviso	Venezia	Padova	Rovigo
Mamastè		X					
Melograno	X			X			
Cuna Spazio Ostetrico				X			
Kirikù Spazio Ostetrico		X					
Le Ostetriche					X		
Mammanin fea	X						
Private midwives	**Bacino (Provincia)**						
1	X	X		X		X	
2		X	X	X	X	X	
3		X				X	
4				X	X		
5		X		X			
6					X	X	
7	X	X				X	
8						X	
9							
10			X	X	X		
11		X	X	X			
12							
Case maternità	**Sede**						
Dora Luce		X					
Mammanin fea	X						

Either private midwives or organizations supporting home births are operating in every province of the region, except in the Rovigo area.

4.2 NEWBORN SCREENING DATA 2019-2020

TABLE XI SCREENING DATA FROM VERONA SCREENING CENTER. 2019.

	Screening Center	VERONA		
	Catchement area	Provinces: Verona, Vicenza, Rovigo		
	Year	2019		
GROUP	Condition	N. of positives on 1st DBS (1st and 2nd tier test)	n. of confirmed positives (biochemical/colecular confirmatory testing)	n. of confirmed positives for secondary conditions
AMMINOACIDEMIAS	PKU/HPA	56	6	0
	Biopterin regeneration deficiency	4	0	0
	Biopterin biosynthesis deficiency	3	0	0
	TYR I	0	0	0
	TYR II	33	0	0
	MSUD	7	0	1 Hh MSUD
	CBS	4	0	0
	MTHFR	2	0	0
ORGANIC ACIDEMIAS	GAI	1	0	0
	IVA	1	0	0
	BKT	0	0	0
	HMG	2	0	0
	PA	1	0	0
	MMA-mut	0	0	0
	CblA	1	0	0
	CblB	0	0	0
	CblC	4	0	3 maternal deficit vit B12
	CblD	3	0	4 maternal deficit vit B12
	2-MBG	0	0	0
	MA	4	0	0
	MCD	8	0	1 3MCC, 2 Hh 3MCC
UREA CYCLE DISORDERS	CIT I (citIa-burlina)	0	0	0
	CIT II	15	0	0
	ASA	3	0	0
	ARG	7	0	0
FATTY ACID OXIDATION DISORDERS	CUD	82	0	0
	CPT I	0	0	0
	CACT	0	0	0
	CPT II	1	0	0
	VLCAD	5	1/2 (heterozygosity synergic with GA2)	0
	TFP	1	1	0
	LCHAD	1	1	0
	MCAD	5	0	0
	M/SCHAD	0	0	0
	GA2/MADD	13	1/2 (heterozygosity synergic with VLCAD), 1 GA2	0
	tot	267	8	11

In 2019, 32,845 births were registered in the Veneto region. 33,232 dried blood spots were collected and tested within the screening programme. 398 (1,2%)

newborns tested positive for inherited metabolic diseases (IEMs) in first and second tier tests. Infants tested positive underwent a confirmatory testing (biochemical or molecular). Out of 398 infants, a minority (n=19; 4.8%) were confirmed to have one of the metabolic diseases included in the screening panel. The rate of newborns diagnosed with IEM through the screening programme in the Veneto region was 0.057%.

TABLE XII SCREENING DATA FROM PADOVA SCREENING CENTER. 2019.

	Screening Center	PADOVA		
	Catchement area	Provinces: Belluno, Padova, Venezia, Treviso		
	Year	2019		
GROUP	Condition	N. of positives on 1st DBS (1st and 2nd tier test)	n. of confirmed positives (biochemical/colecular confirmatory testing)	n. of confirmed positives for secondary conditions
AMMINOACIDEMIAS	PKU/HPA	8	2	0
	Biopterin regeneration deficiency	0	0	0
	Biopterin biosynthesis deficiency	0	0	0
	TYR I	0	0	0
	TYR II	27	2	0
	MSUD	0	0	0
	CBS	0	0	0
	MTHFR	0	0	0
ORGANIC ACIDEMIAS	GAI	1	1	0
	IVA	2	0	0
	BKT	0	0	0
	HMG	6	0	1
	PA	0	0	0
	MMA-mut	1	1	0
	CblA	0	0	0
	CblB	0	0	0
	CblC	8	0	0
	CblD	0	0	0
	2-MBG	0	0	0
	MA	4	0	0
	MCD	0	0	0
UREA CYCLE DISORDERS	CIT I (cit la- burlina)	9	1	0
	CIT II	0	0	0
	ASA	0	0	0
	ARG	2	0	0
FATTY ACID OXIDATION DISORDERS	CUD	42	0	1
	CPT I	6	0	0
	CACT	0	0	0
	CPT II	4	0	0
	VLCAD	6	0	0
	TFP	0	0	0
	LCHAD	0	0	0
	MCAD	2	2	
	M/SCHAD	0	0	0
	GA2/MADD	3	0	0
	tot	131	9	2

In 2019 in the screened population, aminoacidemias were the most frequent group of IEMS diagnosed, corresponding to 10 cases (52,6%). Most common aminoacidemia was phenylketonuria/hyperphenylalaninaemia, diagnosed in 8 newborns (41% of total IEMs diagnosed new-borns). Remaining aminoacidemias were the 2 cases of tyrosinaemia (type II).

Mitochondrial fatty acid beta-oxidation deficits were diagnosed in 6 cases (31,6%). Furthermore, the following disease cases were found: 2 multiple carboxylase deficiency (10.5%), 1 glutaric acidemia type II/multiple acyl-CoA dehydrogenase deficiency (5.25%), one trifunctional protein deficiency case (5.25%), one long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (5.25%) and one patient with a synergic heterozygosity of very long chain acyl-CoA dehydrogenase deficiency and glutaric acidemia type II (5.25%).

Two organic acidemias were diagnosed: one case of glutaric aciduria type I and one of methylmalonic acidemia caused by methylmalonyl-CoA mutase deficiency. Only 1 case of urea cycle disorder, having citrullinemia type 1 (CIT1) (5.25%) was diagnosed in 2019.

Most common secondary condition diagnosed was maternal vitamin B12 deficiency (7 cases).

TABLE XIII SCREENING DATA FROM VERONA SCREENING CENTER. 2020.

	Screening Center	VERONA		
	Catchement area	Provinces: Verona, Vicenza, Rovigo		
	Year	2020		
GROUP	Condition	N. of positives on 1st DBS (1st and 2nd tier test)	N. of confirmed positives (biochemical/colecular confirmatory testing)	N. of confirmed positives for secondary conditions
AMINOACIDEMIAS	PKU/HPA	53	3	0
	BIOPT (BS)	5	0	0
	BIOPT (REG)	4	0	0
	TYR I	0	0	0
	TYR II	57	0	0
	MSUD	3	0	0
	CBS	1	0	0
	MTHFR	0	0	0
ORGANIC ACIDEMIAS	GAI	5	0	0
	IVA	1	0	1 Hh IVA
	BKT	0	0	0
	HMG	0	0	0
	PA	1	1	0
	MMA-mut	0	0	0
	CblA	1	1	0
	CblB	0	0	0
	CblC	3	0	3 maternal deficit vit B12
	CblD	0	0	0
	2-MBG	0	0	0
	MA	5	0	0
	MCD	5	0	1 Hh 3MCC
UREA CYCLE DISORDERS	CIT Ia	0	0	0
	CIT II	18	0	0
	ASA	0	0	0
	ARG	5	0	0
FATTY ACID OXIDATION DISORDERS	CUD	40	0	0
	CPT I	0	0	0
	CACT	0	0	0
	CPT II	0	0	0
	VLCAD	4	0	0
	TFP	0	0	0
	LCHAD	0	0	0
	MCAD	10	0	0
	M/SCHAD	0	0	0
	GA2/MADD	5	0	0
	tot	226	5	5

TABLE XIV SCREENING DATA FROM VERONA SCREENING CENTER. 2020.

	Screening Center	PADOVA		
	Catchement area	Provinces: Belluno, Padova, Treviso, Venezia,		
	Year	2020		
GROUP	Condition	N. of positives on 1st DBS (1st and 2nd tier test)	N. of confirmed positives (biochemical/colecular confirmatory testing)	N. of confirmed positives for secondary conditions
AMINOACIDEMIAS	PKU/HPA	10	2	0
	BIOPT (BS)	0	0	0
	BIOPT (REG)	0	0	0
	TYR I	3	0	0
	TYR II	56	0	0
	MSUD	1	0	0
	CBS	0	0	0
	MTHFR	0	0	0
ORGANIC ACIDEMIAS	GAI	0	0	0
	IVA	1	0	0
	BKT	0	0	0
	HMG	3	1	0
	PA	0	0	0
	MMA-mut	0	0	0
	CblA	0	0	0
	CblB	0	0	0
	CblC	24	0	0
	CblD	0	0	0
	2-MBG	2	2	0
	MA	7	0	0
	MCD	0	0	0
UREA CYCLE DISORDERS	CIT Ia	16	1	0
	CIT II	0	0	0
	ASA	1	1	0
	ARG	3	0	0
FATTY ACID OXIDATION DISORDERS	CUD	47	0	2
	CPT I	2	0	0
	CACT	0	0	0
	CPT II	1	0	0
	VLCAD	9	1	0
	TFP	0	0	0
	LCHAD	0	0	0
	MCAD	2	1	0
	M/SCHAD	0	0	0
	GA2/MADD	7	1	0
	tot	195	10	2

In 2020, 32,055 births were registered, for a global amount of 32,440 screening tests performed. First and second tier tests identified 429 (1,3%) newborns. After confirmatory testing, 15 (3,5%) cases were confirmed. The rate of newborns diagnosed with inborn error of metabolism through the screening programme in the Veneto region was 0,047%. Both in 2019 and in 2020 no false positive results were reported.

The same year, 5 aminoacidemias (33.3%) and 5 organic acidemias (33.3%) were diagnosed, the remaining 5 cases are 3 fatty acid beta-oxidation deficiencies (20%) and 2 urea cycle disorders (13.3%). All 5 aminoacidemias diagnosed in 2020 were phenylketonuria/hyperphenylalaninaemia, representing 33.3% of all the inborn errors of metabolism diagnosed in 2020.

Organic acidemias diagnosed in 2020 were two 2-methylbutyryl-CoA dehydrogenase deficiencies, one case of propionic aciduria, one of cobalmin A defect, and one 3-hydroxy-3-methylglutaryl- CoA lyase deficiency. Urea cycle disorders found were: one citrullinemia type 1, and one argininosuccynic aciduria.

One each of mitochondrial fatty acid beta-oxidation disorders were diagnosed: very long chain acyl-CoA dehydrogenase deficiency, medium chain acyl-CoA dehydrogenase deficiency, glutaric acidemia type II/multiple cyl-CoA dehydrogenase deficiency.

Three cases of maternal maternal vitamin B12 deficiency were found, making it the most frequent secondary condition diagnosed.

Following newborn screening, a total of 34 newborns with errors of metabolisms were identified over two-year period 2019- 2020: 15 aminoacidemias (44%), 9 fatty acid oxidation disorders (26.5%) 7 organic acidemias (20.6%), and 3 urea cycle disorders (8.8%) (fig. 7).

The positive predictive value (PPV), or the percentage of newborns with abnormal results on the first or second-tier test (827) that have a confirmed diagnosis (34), was observed to be 4,11%.

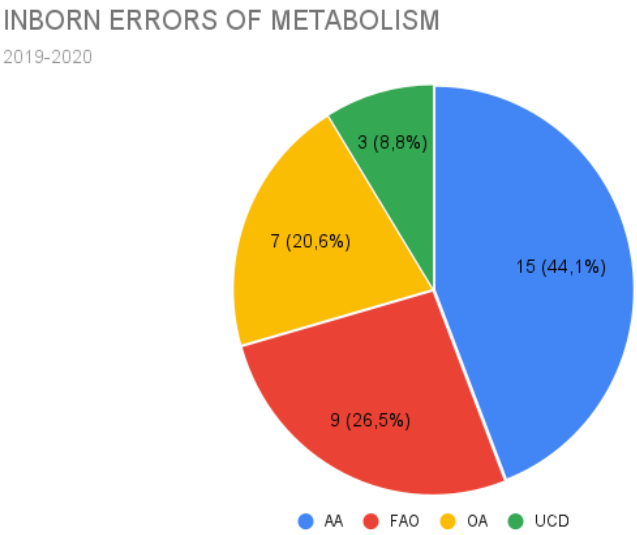


FIGURE 7 INBORN ERRORS OF METABOLISM DIAGNOSED IN 2019-202'. VENETO

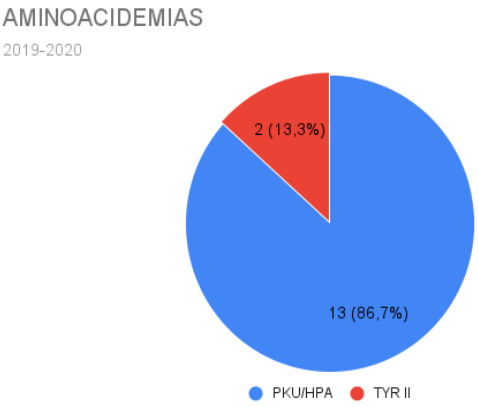


FIGURE 8 AMINOACIDEMIAS DIAGNOSED IN 2019-2020. VENETO

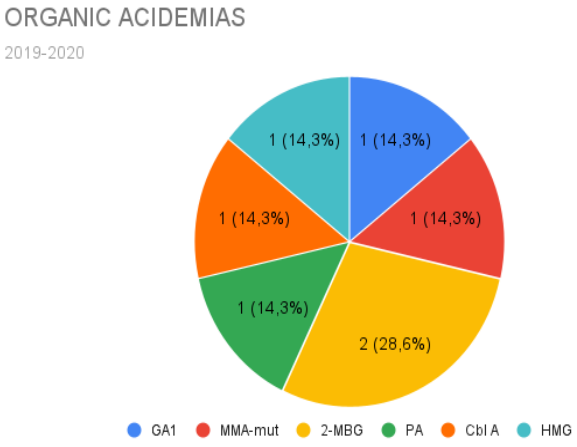


FIGURE 9 ORGANIC ACIDEMIAS DIAGNOSED IN 2019-2020. VENETO

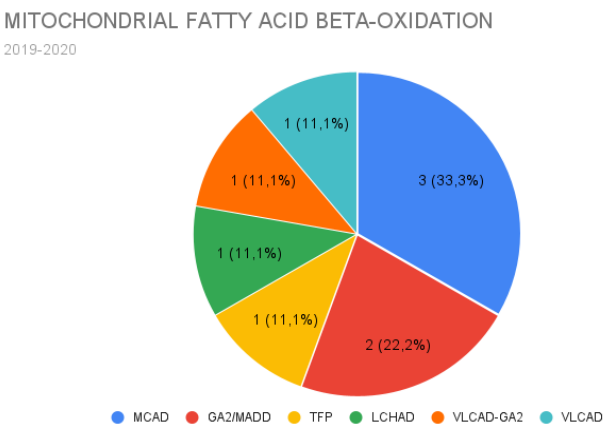


FIGURE 10 MITOCHONDRIAL FATTY ACID BETA-OXIDATION DIAGNOSED IN 2019-2020. VENETO

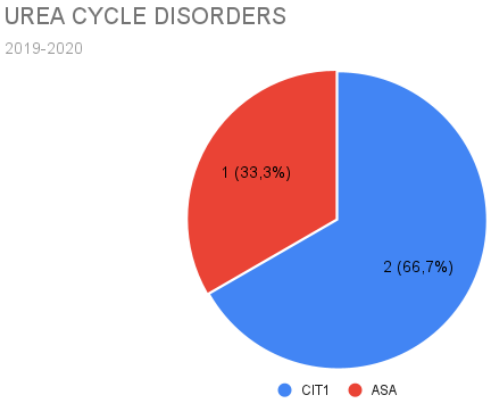


FIGURE 11 UREA CYCLE DISORDERS DIAGNOSED IN 2019-2020. VENETO

4.3 COMPARISON ANALYSIS OF NBS INFORMATION DOCUMENTS

A comparison analysis of the contents of different information documents in use in the two newborn screening Centres in Veneto, located at Padua University Hospital and Verona University Hospital and in other 5 Italian regions (Emilia-Romagna, Friuli Venezia Giulia, Lombardy, Tuscany and Piedmont) was performed.

Based on the work by IJzebrink et al[83]. and adapted for the purposes of the present study we have analysed the contents of the available information documents checking the presence of information related to 3 main domains (general information provided, aspects of knowledge and availability of additional information). The domain "aspects of knowledge" has been further divided into the following 8 subdomains: purpose of newborn screening, positive and negative findings, false positive and false negative findings, uncertainties and risks, medical implications, social implications, financial implications, and follow-up plans.

Table XVIII summarizes the results of our analysis.

5 out of 8 information products were in the form of an information sheet (1-3 A4 pages), one was a leaflet (1 A4 page, folded into thirds) while the remaining 2 were printed as a booklet (8-12 pages).

Knowledge Aspects in Parental Information Products:

TABLE XVIII ANALYSIS OF ASPECTS OF KNOWLEDGE IN INFORMATION PROVISION MATERIAL .

	Screening Center	Padova	Verona	Emilia Romagna	Friuli Venezia Giulia	Lombardy	Piedmont	Tuscany	ISS
General information	Document type	Information sheet	Leaflet	Booklet	Information sheet	Information sheet	Information sheet	Booklet	Information sheet
	Pages	2	3 +2 (genetic testing)*	8	3	3	1	12	3
	Langauage (Italian)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Langauage (other)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
GMC's aspects of knowledge	1. Purpose of NBS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	2. Positive or Negative Findings	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3. False Positive/False Negative Findings	Yes / No	Yes / Yes	Yes / No	Yes / No	Yes / No	No / No	No / No	Yes / No
	4. Uncertanties and Risks	No	Yes (geneti analysis)	No	No	Yes	No	No	No
	5. Medical Implications	Yes (list of conditions)	Yes (list of conditions)	Yes	Yes (list of conditions)	Yes	Yes (list of conditions)	Yes	Yes (list of conditions)
	6. Social Implications	No	Yes (genetic counselling)*	Yes (genetic counselling)	Yes (genetic counselling)	Yes	No	No	No
	7. Financial Implications	Yes	No	No	Yes	Yes	No	No	Yes
	8. Follow-up Plans	No	No	Yes	No	Yes	No	Yes	Yes
Additional information	Sample collection	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	DBS storage	Yes (3 years)	No	No	Yes (3 years)	Yes	No	Yes (10 years)	Yes (at least 5 years)
	Privacy and data treatment	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

***** *Two separate documents were provided; uncertainties and risks are mentioned in genetic analysis informational material.*

(1) The purpose and importance of NBS is discussed in all eight information products, as are the (2) possible positive or negative results of NBS. The probability of such findings was not mentioned in any document, apart in one document, presenting this issue in general terms i.e. that a positive result is rare:

'*The probability of identifying a condition is very low, as the conditions included in screening programmes are rare*.'- *Emilia Romagna document*

Description of the timing and collection of blood sample was included in every document analysed:

'*A blood drop from a heelprick is collected in second or third day…Dried bloodspot is sent to a screening laboratory where the testing is done.' -ISS*

Six out of eight products explored the topic of (3) and false positive results, warning that a positive screening result does not equal a diagnosis, only that more testing should be done to exclude false positives:

'*A positive result does not represent a diagnosis: if the result is negative we can exclude the presence of a disease; a positive result is not evidence of disease just an indication that more testing needs to be done'- Padova*

Only one document states that a false negative finding is possible:

'…it is important to remember that even when the neonatal screening results negative there is still a very small chance of disease in the future.'- Verona

Two documents don't discuss the possibility of false positive/false negative result at all.

(4) The possibility of finding uncertainties and risk of inconclusive test results were mentioned in two documents (Verona, Lombardy); in addition to conditions included in the core panel/additional panels secondary conditions with uncertain meaning could be found:

'…unexpected information that can offer benefit in terms of treatment/prevention or family planning will be communicated.'-Verona

(5) The conditions included in the screening programs were listed in every document. In 4 (50%) of them, certain conditions objects of screening are described in detail and treatment for each of them was mentioned:

'Congenital hypothyroidism (CH): Primary CH is the most common endocrinopathy found in paediatric population (1:2.500 live births). CH is caused by altered or null thyroid activity that determines severe physical and mental issues. Health problems can be avoided with daily supplementation of the missing hormone…' -Lombardy

In the remaining 4 conditions included in the screening panel were listed, but were not described, and treatment was mentioned as possible, but only in very general terms:

'Early diagnosis and treatment can prevent serious health complications …'- Padova

(6) Social Implications were discussed in four documents as it pertains to parents' health and future reproductive choices. Genetic counselling is offered to parents of diagnosed newborns.

'Genetic consultation and further information for relatives are available whenever a carrier is identified.' -Verona

Four out of 8 products state that (7) neonatal screening is covered by the national health system and offered to all newborns:

'…bloodspot screening, which is offered by SSN to every newborn, free of charge.' -ISS

(8) Only three (37.5%) documents explain the follow-up process:

'If the diagnosis has been confirmed, doctors from Clinical Centre will communicate the diagnosis to the parents and patient's paediatrician. The paediatrician will be put in contact with the USL of residence. A health professional (case manager) will be assigned to the case; the role of case manager is to coordinate the long-term follow-up process, build report with patient's family, paediatrician and Centres of expertize specialized in treating the patient's condition.'- Emilia Romagna

Remaining documents do not mention what follows the diagnostic testing.

5. DISCUSSION

5.1 BIRTH RATES IN VENETO REGION

In 2020, demographic changes already visible in the previous years have been clearly outlined by the Sars-CoV-2 outbreak and related health emergency. The Italian Institute of Statistics (ISTAT) has recently published updated data confirming and highlighting the continuous demographic decline observed in recent years, stemming from a steady drop in population, increasing divergence between the number of births and deaths, and slowing of the migration flows.

The cause cannot be assigned to a single factor; an amalgam of economic crisis, lower numbers of fertile women, Covid-19 epidemic, and personal reproductive choices all play a role in the decline of natality.

As a consequence of low birth rates recorded in 1970's and 1980's ,the population of women of child-bearing age in Veneto is smaller than the population of women who were born in the decades before or after. In addition, Italian women who choose to have children often have it later in life, with the average age being 32,9.

In 2000's there was a rapid expansion of birth rates due to foreign women giving birth in Italy, before it plateaued in late 2010's and is on the decline today, the same as with Italian women. This phenomenon can be explained by better integration of foreign women into Italian society: life styles and reproductive choices, such as having fewer children and having them later in life (average 30 years) match those of Italians. Another factor is represented by the Sars-CoV-2 pandemic and the economic crisis that followed: immigration flow was redirected from Italy to other countries in the European Union in order to seek better jobs and opportunities elsewhere.

Data analysed in this study show that foreign women that give birth in Veneto are often younger and less educated than their Italian counterparts. They are often unemployed or have a role of a stay-at-home parent, making them more isolated and difficult to reach with educational efforts. Cultural differences can make it more difficult to approach topics such as newborn screening and rare disease since

in some culture having a disease can be seen as ‘divine punishment’ or the mother’s fault and the person can be ostracised by their community[84]

However foreign women should never be left out of the discussion as they represent around 30% of all women that gave birth in Veneto in recent years.

The Veneto region birth registry collects information both on births occurring in the hospital setting and outside the hospital, including planned and unexpected home births. When a neonatal screening programme is in place it is very important to consider and identify pathways in case of home births, although their number is limited compared to the births occurring in birth wards.

The Italian Ministerial Decree 13.10.2016 on expanded newborn screening clearly defines responsibilities during the collection process of the dried blood spots and their arrival to the newborn screening Centre. The timing and modalities of the collection are a highly regulated part of the screening programme. The collection of the samples is in charge to appropriately trained healthcare professionals working in public or private hospitals where births take places[85]. In the case of home births, the midwife who assisted with the birth must collect and deliver the sample to the birthing Centre or directly the screening Centre of competence. The sample should be collected from every live birth, including those who passed in the first 48-72 hours for which a peri-mortem sample is necessary and the occurrence communicated promptly to the screening laboratory.

Based on the data from the last available ISS Report on the state of the eNBS in Italy (citare fonte in biblio[86]) the need for a better delineation of roles and responsibilities emerged at national level in the context of planned home births.

The ISS Report is based on data gathered from questionnaires sent to referral persons for the screening programme in all Italian regions. The Italian situation seems fairly homogenous and in compliance with the guidelines, in particular considering the timing of collection and delivery of the sample to the laboratory.

5.1.1 THE IMPACT OF SARS-COV-2 PANDEMIC

The number of home births has been increasing in recent years; the Sars-CoV-2 pandemic made the numbers skyrocket; the measures put in place to prevent the spread of Sars-CoV-2 limited the number of people that could assist during and following the delivery[87] and added stress on the parent. it has been demonstrated, even before the pandemic, that going through labour and delivery without a partner or other supportive figures had a detrimental effect on the mother[88].

An Italian study looked at how the pandemic impacted the maternity experience of 575 women by exacerbating anxiety, symptoms of depression and PTSD both in expecting and new mothers[89]. The combination of limitations put in place to control the spread of Sars-CoV-2 and the fear of being admitted to the hospital gave way to discussions of demedicalizing the pregnancy and delivery[90]. At the moment there is no way to predict how this will affect home birth numbers in the future.

An Australian study by Australian College of Midwives found that the number of request for home birth was rising during the pandemic even as the midwives worked less since PPI was difficult to obtain, especially at the beginning of 2020[91].

In any case, a rise in home births made issues with blood sampling more evident to the Screening Centres; samples taken by midwives were found to be often inadequate and thus had to be repeated, timing was not always respected, and guidelines not always followed.

The evolution of knowledge, diagnostic methods and ever-increasing number of conditions screened for highlighted the need for further education of midwives and those who assist with home births or births in alternative settings. Those who assist with labour and delivery have a responsibility to inform and educate parents about the NBS before the blood sample is taken.

For these reasons it is important to quantify the phenomenon of home births that happen and inquire into concerns that arise from it.

Contrarily from other European countries, Italian law requires that the births in a non-hospital setting (planned or accidental) get sent to the regional birth registry.

The percentage of non-hospital birth varies from Region to Region, with some Regions offering reimbursement for the cost of home birth.

Although the number of home births is low when compared with the total number of births in a year, it is crucial to pay attention to the process as lack of quality control can have an impact on the coverage and efficacy of the screening process.

Using information collected by the birth registry to find freelance midwives who offer assistance with home birth and offering them education and training on NBS could be key for expanding the coverage of eNBS to the population that is otherwise left out of the program.

5.2. NEWBORN SCREENING DATA

A total of 65672 newborns were screened in Veneto in 2019-2020. This study looked at disorders included in eNBS core panel (DM 13.10.2016.) identified by tandem mass spectrometry (MS/MS). In total 827 (12.6%) newborns had a positive result on first/second tier tests and were invited to return for confirmation testing. In the end only 34 (4%) have a confirmed diagnosis with inborn error of metabolism. Amminoacidemias, or more specifically phenylketonuria/hyperphenylalaninaemia., were found to be most common inborn error of metabolism in Veneto. Organic acidemias were second most frequent condition diagnosed, closely followed by fatty acid beta-oxidation defects. The least frequent conditions were those included in urea cycle disorders. to the national data for the same time period (2019-2020), we can see that the results from our study are similar to those the national level: around 50% of positive results were due to aminoacedemias and almost all were reported to be hhenylketonuria/hyperphenylalaninaem

National data for 2019-2020 reported 516 newborns with metabolic disorder- the 0,06% of the total newborn population (806,770 live births screened between 2019 and 2020). According to Burlina et al. [92] amminoacidemias (AA) were the most common IEM in Italy, representing 52% of positive results. Moreover, equal percentages of patiens were diagnosed with organic acidemias (OA) and mitochondrial fatty acid beta-oxidation defects (FAO). Of note, 95% of all aminoacidemias were due to phenylketonuria or hyperphenilalaninemia..

In Italy, 12.5% of NBS positive cases were considered secondary conditions, such as maternal conditions, almost 90% of which were related to maternal vitamin B12 deficiency. While in Veneto the percentage of maternal vitamin B12 deficit was lower (50%) it was still the most frequent secondary condition diagnosed. Relatively high frequency of low maternal levels of vitamin B12 could be due, at least in part, to the increase in popularity of the vegan diet. Adding vitamin B12 supplementation to expecting mothers' diets and educating them about the issue could lower those numbers and reduce the risk for neurological sequelae in the future.

In general, the percentage of newborns screened in Veneto is high, meaning that almost all infants born in the Region have been screened for conditions included in eNBS. The high coverage is evidence that even those who are born at home are not left out of the programme.

Out of 827 newborns that were recalled and asked to return for confirmatory testing, only 34 were actually diagnosed with a condition, and almost half of those were diagnosed with phenylketonuria or hyperphenylalaninemia, a condition that first one to be implemented in newborn screening in 1992, even before the invention of MS/MS technology.

The advancement of technology makes it possible to screen for and diagnose rare conditions soon after birth. The decision on whether to include a disease in a screening programme should not be based on what is technically possible but on whether the screening of such a condition is advantageous to the newborn. The same could be said about innovative treatments that are being researched and released by pharmaceutical companies every day. Between the cost and the lack of knowledge about rare diseases , it is difficult to justify the continual expansion of screening programmes just because it is possible.

During the years considered in this study no false positives were reported; however false positive cases take years to be uncovered: a child has to become symptomatic and has to be correctly diagnosed after a clinical visit. Children with rare diseases often have to go through a '*medical odyssey'* where they keep being tested, probed and poked for years before a diagnosis could be made. Not to mention the fact that the timing between becoming symptomatic and getting a diagnosis for the conditions included in eNBS, grows even longer since the condition could easily be dismissed by a clinician as the child has been screened at birth..

False negative rates could be calculated by studying Screening and Rare Disease Registries, but that research was not an object of this study.

5.3. INFORMATION PROVISION

Providing knowledge and transparency to parents raises the public support of NBS. Even though the information about NBS can be found if one looks for it, the biggest problem is that parents are not even aware of the any testing is being done[93]. Having been informed briefly only minutes before the blood is sampled, in a moment where parents are exhausted and stress levels are high, information retention is very low. Studies on the topic concluded that the best time to inform and educate parents on the topic of NBS is during the third trimester of pregnancy, and briefly before the testing is done[94].

In a study done by IJzebrink et al[95], the Authors analysed the informational material given to parents in 36 European countries based on GMC's eight knowledge that are deemed important for informed consent for screening. In addition to the eight knowledge aspects, authors noted that additional aspects are frequently mentioned, such as storage of NBS material (DBS), privacy and confidentiality, parental consent, choice, how the heel-prick is performed, and stakeholders involved in NBS (policy-makers, health professionals, specialists).

The question of storage of dried blood spots is very complex: certain countries, such as the UK and Australia, do not ask for parental consent to store DBS cards, while others (e.g., Netherlands) offer the possibility of dissent to the storage and use of DBS.

In this study similar themes were noted: half of the documents analysed DBS storage and its potential use in research. All of the documents viewed had specified where the screening data will be collected (e.g., rare disease registry, birth centre, ISS), and who will be able to access it (not accessed by third parties).

In Italy, Art. 2-8. of DM 13.10.2016 states that the information products given to parents must be written in language that is easily understandable for the majority of the population and translated in languages spoken on the territory. Offering the information material only in the native language leaves out an already marginalized group of people.

The purpose of the screening, the timing and method of sample collection were described in all eight documents analysed in a very succinct and clear way. Words like “rare disease”, “low probability”,”infrequent”, but not percentages and ratios, were used to characterize the probability of receiving a positive result. The emphasis was put on differentiating a positive result and confirmed diagnosis. Parents are briefly informed that in the case of a positive result they will be contacted and invited for confirmatory testing.

Most often, the materials provided a list of conditions included in the screening, without any description on what the parents should expect in case of a diagnosis or whether the treatment is available. Uncertain findings were either not discussed, or, in 2 cases, very briefly mentioned mostly as they pertained to genetic testing: genetic tests may yield information of uncertain significance that will be returned to the patient only if the probability of having an effect on health is relatively high. Perhaps authors of informational materials concluded that discussing these findings could confuse and overwhelm parents, and, since it’s a relatively rare occurrence, decided to omit it.

There was a missed opportunity to include more detail on who is the health professional responsible for the recall, the timing in which such call can be expected, and where the testing will be done. To alleviate the parental anxiety, detailed description of the follow-up should be included: information about Screening Centres, Centres of expertise and health professional figures (e.g., case manager) involved. Having a step-by-step guide to consult could make the parents feel more calm and taken care of in this critical period. The social implication of inherited disease is an important topic to consider for the parents: a definition of how the conditions are inherited and assurance that the genetic counselling is available to parents and blood relatives.

Emilia Romagna, for example, managed to fit a brief description and treatment for each condition. It is understandable that as the number of conditions included in the eNBS grows the description of every one would turn a booklet into a book, but a QR code or indication on where to find more information could be useful to the parents.

The most complete information provision materials were the 2 booklets, printed by Emilia Romagna and Tuscany. The number of pages were 8 and 12, respectively. Other documents were much more brief, with the length ranging from one to three pages. Obviously, much more information can be provided with a booklet than with an information sheet. Nonetheless, booklets managed to be easier to read and understand. Using colours, pictures and bigger font to emphasize could facilitate the understanding of what the important information is at a glance.

No document analysed in this study covered every aspect of knowledge which is considered needed to make an informed decision regarding screening programmes.

The information provision material needs to be brief and easy to understand for the general population, but should not be the only way of providing information about NBS to parents. Written documents, such as pamphlets, should be used as an aid in parental education, something that parents could consult at any moment, preferably after the NBS has been discussed with their primary physician or in prenatal classes. Using multimedia tools could also be a valuable way to deliver information to parents, especially more detailed information about conditions, treatments and DBS. However, more research is necessary to confirm the effectiveness of digital tools.

6. CONCLUSION

The findings of this study show that, even though newborn screening in Veneto is a successful public health programme there are some critical issues that need to be resolved.

As the percentage of foreign women who give birth in Veneto is around 30%, more effort should be put into reaching out to them. These women are often somewhat younger and less educated than their Italian counterparts and many of them are housewives. Organizing prenatal classes and offering information provision material in different languages, as well as being respectful of different cultures would be a big step to help make programs such as newborn screening more fair and equally accessible.

This study analysed information material from various Italian regions: while some were better than others, almost all lacked crucial information about false positive and false negative rates or uncertain findings. The material often failed to describe the screening as mandatory for cystic fibrosis, congenital hypothyroidism and phenylketonuria, but voluntary for conditions included in expanded NBS. Parents should be informed about the program and given the option to opt-out of it if they prefer. Knowing what to expect and having better access to pertinent information could decrease parental anxiety in the case of a false positive or real positive result.

We analysed the screening data from Veneto Region for the years 2019 and 2020 and found that positive predictive value was only 4.11%, meaning that many newborns require follow-up and a period of uncertainty and anxiety in parents until the condition is ruled out. The anxiety and stress the parents feel can manifest in 'vulnerable child syndrome'[96] where the child is treated as though are sick even when they are perfectly healthy. In the long run, such parental behaviour can cause real psychological problems.

Almost 50% of infants diagnosed with inborn error of metabolism have been diagnosed with either phenylketornuria or PHA, a condition that has been in NBS

before the introduction of MS/MS. More emphasis should be put on the evaluation of the conditions included in eNBS. Nevertheless, an increasing and somehow alarming number of diseases get proposed every year; just because the technology allows us to screen for a condition it does not mean that we should, as more conditions diagnosed does not mean more health overall.

Does the benefit of the few outweigh the stress and anxiety of the rest?

Better parental education and information provision, and larger community outreach to closed of communities are the first step to making the NBS more successful.

ABBREVIATIONS:

2M3HBA, 2-Methyl-3-Hydroxybutyric Acidemia;

2-MBG, 2-methylbutyryl-CoA dehydrogenase deficiency;

3MCC, 3-Methylcrotonyl-CoA Carboxylase deficiency;

3MGCA, 3-methylglutaconyl-CoA hydratase deficiency;

ARG, argininemia;

ASA, argininosuccynic aciduria;

BKT, beta-ketothiolase deficiency;

CACT, Carnitine-acylcarnitine translocase deficiency;

CblA/B, Cobalamin A/B defect;

CblC/D, Cobalamin C/D defect;

CBS, cystathionine beta-synthase deficiency;

CIT I, citrullinemia type I;

CIT II, citrullinemia type II;

CPS, Carbamoyl phosphate synthetase I deficiency;

CPT I, Carnitine palmitoyltransferase I;

CPT II, Carnitine palmitoyltransferase II;

CUD, carnitine uptake defect;

GA I, glutaric aciduria type I;

GA2/MADD, Glutaric Acidemia Type II/Multiple Acyl-CoA Dehydrogenase Deficiency;

GNMT, Glycine N-methyltransferase deficiency;

HHH, hyperornithinemia–hyperammonemia-homocitrullinuria;

HMG, 3-Hydroxy-3-methylglutaryl- CoA lyase deficiency;

HPA, hyperphenylalaninaemia;

IBG, Isobutyrylglycinuria;

LCHAD, Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency;

M/SCHAD, medium/short chain L-3-hydroxyacyl-CoA dehydrogenase; MAT, Methionine adenosyltransferase deficiency;

MCADD, medium chain acyl-CoA dehydrogenase deficiency;

MCD, Multiple Carboxylase deficiency;

MMA-MUT, methylmalonic acidemia caused by methylmalonyl-CoA mutase deficiency;

MSUD, maple syrup urine disease;

MTHFR methylenetetrahydrofolate reductase deficiency;

IVA, isovaleric academia;

PA, propionic aciduria;

PKU, phenylketonuria;

SAHH, S-adenosylhomocysteine hydrolase deficiency;

SCAD, Short-chain acyl-CoA dehydrogenase deficiency;

TFP, trifuncional protein deficiency;

TYR I, tyrosinaemia type I;

TYR II, tyrosinaemia type II;

TYR III, tyrosinaemia type III;

VLCADD, very long chain acyl-CoA dehydrogenase deficiency.

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ATTACHMENT A:

Proposed information provision model

Format: booklet, brochure, preferably in colors

Newborn screening (NBS)

Information about newborn screening (NBS) to help parents make an educated decision

Dear parents,

Newborn screening (NBS) is offered by SSN to every newborn, free of charge. NBS is regulated at the national level by Law 167/2016, and DGRV 1308/2016 at the regional level.

The purpose of screening it to identify if newborns have an inherited condition, diagnose them and start treatment before they show any symptoms. Without early intervention these conditions can cause irreversible harm and could even lead to death.

The procedure:

In the 48-72 hours after birth a midwife or nurse will take a drop of blood from the newborn's heel (heelprick). The drop will be collected on a special type of filter paper that contains information about the newborn and parents. This is called dried bloodspot (DBS) card and will be sent to the laboratory to be tested. In some cases the test will need to be repeated:

- On the 15th day if the newborns was born prematurely, with low weight at birth or was provided with parenteral nutrition (I.V. feeding);
- On the 15th day if the mother is in treatment with corticosteroids;
- On the 3rd-5th day if the newborn has been dismissed or transferred before 48 hours;
- Before a therapy (e.g., therapy) is started.

There are no risks associated with the procedure.

If the laboratory found anything out of the ordinary on the test, it will report such findings to the Birth Center, which will then contact the parents in around 20 days and ask them to bring the baby for more testing. The positive result only means that additional tests need to be done before saying if the baby has or has not an inherited condition. A positive test is no reason for alarm; many newborns are called in for