Original Article

Journal of Inborn Errors of Metabolism & Screening
2016, Volume 4: 1–8

DOI: 10.1177/2326409816669027
iem.sagepub.com
SAGE

Incidence of Inborn Errors of Metabolism by Expanded Newborn Screening in a Mexican Hospital

Consuelo Cantú-Reyna, MD[1,2], Luis Manuel Zepeda, MD[1,2], René Montemayor, MD[3], Santiago Benavides, MD[3], Héctor Javier González, MD[3], Mercedes Vázquez-Cantú, BS[1,4], and Héctor Cruz-Camino, BS[1,5]

Abstract

Newborn screening for the detection of inborn errors of metabolism (IEM), endocrinopathies, hemoglobinopathies, and other disorders is a public health initiative aimed at identifying specific diseases in a timely manner. Mexico initiated newborn screening in 1973, but the national incidence of this group of diseases is unknown or uncertain due to the lack of large sample sizes of expanded newborn screening (ENS) programs and lack of related publications. The incidence of a specific group of IEM, endocrinopathies, hemoglobinopathies, and other disorders in newborns was obtained from a Mexican hospital. These newborns were part of a comprehensive ENS program at Ginequito (a private hospital in Mexico), from January 2012 to August 2014. The retrospective study included the examination of 10 000 newborns' results obtained from the ENS program (comprising the possible detection of more than 50 screened disorders). The findings were the following: 34 newborns were confirmed with an IEM, endocrinopathies, hemoglobinopathies, or other disorders and 68 were identified as carriers. Consequently, the estimated global incidence for those disorders was 3.4 in 1000 newborns; and the carrier prevalence was 6.8 in 1000. Moreover, a 0.04% false-positive rate was unveiled as soon as diagnostic testing revealed negative results. The most frequent diagnosis was glucose-6-phosphate dehydrogenase deficiency; and in the case of carriers, it was hemoglobinopathies. The benefit of the ENS is clear as it offers prompt treatment on the basis of an early diagnosis including proper genetic counseling. Furthermore, these results provide a good estimation of the frequencies of different forms of newborn IEM, endocrinopathies, hemoglobinopathies, and other disorders at Ginequito.

Keywords

incidence, expanded newborn screening, inborn errors of metabolism, inherited disorders, retrospective study

Introduction

Newborn screening for the detection of inborn errors of metabolism (IEM), endocrinopathies, hemoglobinopathies, and other disorders is a public health initiative that aims to identify these diseases in a timely manner, avoiding related complications that may arise if not detected on time. Early intervention is an important step in the management of these diseases, which are directly associated with affected patients' final outcomes. Newborn screening uses dried blood spots (DBSs) as samples, which should be obtained after the first 24 hours of life following previously established and standardized procedures.[1,2] The different screened disorders and the nomenclature used in this article, as suggested by Sweetman et al,[3] are provided in Table 1.

[1] Genomi-k SAPI de CV, Monterrey, Nuevo León, México
[2] Escuela de Medicina Tecnológico de Monterrey, Monterrey, Nuevo León, México
[3] Hospital de Ginecología y Obstetricia SA de CV Monterrey, Nuevo León, México
[4] Department of Biosystems Science and Engineering ETH Zurich, Basel, Switzerland
[5] Escuela de Biotecnología y Ciencias de la Salud, Instituto Tecnológico de Monterrey, Monterrey, México

Received April 14, 2016. Accepted for publication April 18, 2016.

Corresponding Author:
Consuelo Cantú-Reyna, Genomi-k SAPI de CV, Av Cerro de las Mitras 2411 Col Obispado, Monterrey, Nuevo León, México, 64060.
Email: cocantu@genomi-k.com

Table 1. The IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders Detected in the Ginequito's ENS Program.[a]

Detection Method	Condition	Nomenclature
MS/MS	Fatty acid oxidation disorder conditions	
	Carnitine acylcarnitine translocase deficiency	CACT
	Carnitine palmitoyltransferase type I deficiency	CPT IA
	Long chain L-3-hydroxyacyl CoA dehydrogenase deficiency	LCHAD
	2,4-Dienoyl-CoA reductase deficiency	DE RED
	Medium-chain acyl-CoA dehydrogenase deficiency	MCAD
	Glutaric acidemia type II	GA2
	Carnitine palmitoyltransferase type II deficiency	CPT II
	Short-chain acyl-CoA dehydrogenase deficiency	SCAD
	Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	SCHAD
	Trifunctional protein deficiency	TFP
	Very long-chain acyl-CoA dehydrogenase deficiency	VLCAD
	Organic acid disorder conditions	
	3-Hydroxy-3-methyglutaricaciduria	HMG
	Glutaric acidemia type I	GA
	Isobutyrylglycinuria	IBG
	Acute isovaleric acidemia	Acute IVA
	Chronic isovaleric acidemia	Chronic IVA
	2-Methylbutyrylglycinuria	2MBG
	3-Methylcrotonyl-CoA carboxylase deficiency	3-MCC
	3-Methylglutaconicaciduria	3MGA
	Methylmalonyl-CoA mutase deficiency (MUT 0 type)	MUT
	Methylmalonyl-CoA mutase deficiency (MUT type)	MUT
	Cobalamin disorders	Cbl A, B, C, D
	β-ketothiolase deficiency	β KT
	Acute propionic acidemia	Acute PROP
	Chronic propionic acidemia	Chronic PROP
	Holocarboxylase synthase deficiency	MCD
	Malonic acidemia	MAL
	Argininemia	ARG
	Acute argininosuccinic aciduria	Acute ASA
	Chronic argininosuccinic aciduria	Chronic ASA
	5-Oxoprolinuria	5OXOPRO
	Citrullinemia type I	CIT
	Citrullinemia type II	CIT II
	Homocystinuria	HCY
	Hypermethioninemia	MET
	Hyperornithinemia–hyperammonemia–homocitrullinuria	HHH
	Classic maple syrup urine disease	MSUD
	Intermediate maple syrup urine disease	MSUD
	Classic phenylketonuria	PKU
	Benign hyperphenylalaninemia	H-PHE
	Biopterin defect in cofactor biosynthesis	BIOPT(BS)
	Biopterin defect in cofactor regeneration	BIOPT(REG)
	Transient neonatal tyrosinemia	TNT
	Tyrosinemia type I	TYR I
	Tyrosinemia type II	TYR II
	Tyrosinemia type III	TYR III
Mixed technologies	Other conditions	
	Complete biotinidase deficiency	BIOT
	Partial biotinidase deficiency	BIOT
	Salt-wasting congenital adrenal hyperplasia	CAH
	Simple virilizing congenital adrenal hyperplasia	CAH
	Primary congenital hypothyroidism	CH
	Cystic fibrosis	CF
	Galactokinase deficiency	GALK
	Classic galactosemia	GALT
	Galactoepimerase deficiency	GALE
	Glucose-6-phosphate dehydrogenase deficiency	G6PD
	Hemoglobin S disease	Hb S
	Hemoglobin SC disease	Hb S/C
	Hemoglobin S-β thalassemia disease	Hb S/βTh
	Hemoglobin C disease	Hb C
	Hemoglobin E disease	Hb E

Abbreviations: IEM, inborn errors of metabolism; MS/MS, tandem mass spectrometry.
[a]Nomenclature adopted from Sweetman et al.[3]

Thanks to the benefits of tandem mass spectrometry (MS/MS), nearly 40 diseases can be diagnosed.[4] Detection programs, such as the one described in this report, began in the 1960s using DBSs for phenylketonuria (PKU) detection. A few years later, maple syrup urine disease (MSUD), homocystinuria (HCY), tyrosinemia, galactosemia, and congenital hypothyroidism (CH) were added.[2,4]

Nowadays, the diseases included in newborn screening programs, detected by MS/MS, vary widely among countries. In the United States, the implementation of MS/MS is covered in almost all states, each state being in charge of the program management. Similarly, it occurs in Canada, where each province or territory is responsible for its own program. Until July 2008, all Canadian provinces and territories carried out a screening for PKU by MS/MS; however, heterogeneity is still present for the detection of other IEM.[4]

In the case of Europe, the newborn screening for CH and PKU constitutes an obligatory requirement for all programs, and in recent years there has been an increased interest for expanding the number of screened diseases. For example, in Spain, the only IEM detected in all communities is PKU. Since 2001, the MS/MS is being used in Galicia to identify nearly 30 IEM. This technique is spread across the country; and in 2007,

Murcia—for analyzing 15 diseases—and the Basque Country—for analyzing only PKU and medium-chain acyl-CoA dehydrogenase (MCAD)—adopted it.[4]

Asian countries, such as Singapore and Taiwan, have recently incorporated MS/MS in their newborn screening programs; while China, South Korea, India, Japan, Malaysia, and Thailand are in the implementation phase.[4]

The situation in Latin America is also diverse; some countries (Chile, Costa Rica, Cuba, and Uruguay) have well-established programs supervised by their own health authorities, with a coverage of over 98% in the diagnosis, treatment as well as follow-up regarding positive cases.[4] Other countries, such as Mexico, Brazil, and Argentina, are expanding their coverage; some others are in the implementation stage (Colombia, Paraguay, Venezuela, Nicaragua, Peru); others such as Guatemala, Panama, and Ecuador have isolated activities concerning this matter; and others are not carrying out at all newborn screening activities (El Salvador, Honduras, and Haiti).[5]

Mexico initiated a newborn screening program in 1973 that was aimed at detecting PKU, galactosemia, MSUD, homocystinuria, and tyrosinemia. Unfortunately, the program ended in 1977; nevertheless, it was in 1986 that another program was initiated for the detection of PKU and CH.[6] By 1988, the Health Department published the Official Mexican Technical Norm, which stated that screening for CH was mandatory within all health institutions providing services for newborns. This official norm was obligatory in nature until 1995.[7]

A few years later, in October 2003, the Official Mexican Standard for the prevention and control of birth defects was established (NOM-034.SSA2-2002), which subsequently modified (NOM-034.SSA2-2010 and NOM-034.SSA2-2013) to include more IEM, endocrinopathies, hemoglobinopathies, and other disorders.[8-11] The differences between the type and number of diseases screened in newborns vary considerably worldwide and even within each country. This has been a huge problem and has represented a global concern, given that each nation has different regulations, economic resources, and disease frequencies.[8,12]

With the aim of standardizing and establishing an expanded newborn screening (ENS) guidelines among states, the Department of Health and Human Services of the United States commissioned the elaboration of these recommendations to the American College of Medical Genetics (ACMG). As main proposal, the ACMG determined a 29-condition core panel and other 25 secondary targets, suggesting the employment of the MS/MS with mixed technologies as detection methods.

The diseases considered in the core panel had basic principles to be met, such as: a clinical diagnosis cannot be made within the neonates' first 24 to 48 hours of life; tests featuring enough sensitivity and specificity; moreover, there should be proof that early detection offers beneficial outcomes and that early diagnosis leads to a specific treatment with a more favorable outcome.

On the other hand, the secondary targets included diseases that can be detected collaterally as part of the differential diagnosis of the core panel conditions and are clinically relevant in spite of the lack of an effective treatment.

The incidence of different IEM in Mexico is unknown or uncertain due to the lack of nationwide ENS programs.[13] The Instituto Nacional de Pediatría and the Universidad Autónoma de Nuevo León have undertaken studies pertaining to ENS, which clarify the incidence within the Mexican population. Although limited data are available from these investigations, Mexican health providers and researchers still use this information and records from different countries to establish their cutoffs for a positive diagnosis.[2,12,14-16]

Out of all diseases detected via the newborn screening program in Mexico, the most frequent one, according to the results of previous studies, is CH, with an incidence of 1/2000 newborns. Moreover, cystic fibrosis (CF) has a reported incidence of 1/3721, and the incidence of sickle cell disease ranges from 1/3721 to 1/5000.[2,13] Significant variations arise depending on the region and studied population.[12,13]

The retrospective study presented in this work was carried out at the Hospital de Ginecología y Obstetricia SA de CV (Ginequito), which was founded in 1976 and was initially dedicated to address women's needs throughout their lives and to provide newborn care. It is a general hospital that offers medical care for the whole family, mainly for individuals from Nuevo Leon, Mexico; women and their newborns account for 85% of this hospital's patients. Moreover, Ginequito launched an ENS program in July 2011, which included the detection of over 50 IEM, endocrinopathies, hemoglobinopathies, and other disorders, applying mixed technologies.

Aim

The purpose of this article is to present the local incidence of IEM, endocrinopathies, hemoglobinopathies, and other disorders in Nuevo Leon based on the first 10 000 newborns screened at Ginequito starting from January 1, 2012, to August 9, 2014. This new information will broaden the knowledge of the incidence of diseases detected by ENS in this northern Mexican population.

Methods

Ginequito, in collaboration with Genomi-k SAPI de CV, implemented an ENS program for the early detection and intervention of more than 50 IEM, endocrinopathies, hemoglobinopathies, and other disorders (Table 1). This is a retrospective study that included the evaluation of medical records and ENS results from all 10 000 screened newborns, starting January 1, 2012, at the hospital. Blood samples were taken by venipuncture between the neonates' first 24 and 48 hours of life; the samples were then placed on filter paper.[17] The DBSs were processed by PerkinElmer Genetics Laboratories in Bridgeville, Pennsylvania.

The technologies applied in the screened disorders detection were MS/MS, biochemical assays, isoelectric focusing,

and molecular studies. The first technology was used for studying the amino acid and acylcarnitine profiles; biochemical assays were performed in order to detect the principal biomarker related to congenital adrenal hyperplasia (CAH), CH, galactosemia, biotinidase deficiency (BIOT), and CF. Moreover, hemoglobinopathies were detected by isoelectro focusing, and glucose-6-phosphate dehydrogenase deficiency (G6PD) was diagnosed by a molecular study.

The protocol put into practice during this ENS program consisted of a first sample, in which the technologies mentioned were performed; however, if the biomarker was reported out of the reference value, a second-tier analysis was carried out, or in the absence of a second step, a second sample was requested. The disease, its marker, the test, and the tier analysis are shown in Table 2.

In addition, a newborn screening result is considered false positive when a negative result in a diagnostic test is obtained.

Results

From January 2012 to August 2014, a total of 10 000 newborns were screened, of which 9812 were normal and 188 were abnormal. The summary of the results concerning the first sample are shown in Table 3. The newborns diagnosed directly from molecular studies done within the ENS included 26 having G6PD (26 [A-] phenotype) and 1 having a homozygous delta-F508 mutation. Furthermore, 1 newborn female was identified having heterozygous G1388A mutation for G6PD and 4 having delta-F508 mutation carriers for CF. Moreover, 64 newborn carriers with hemoglobin (Hb) disorders were detected: 7 Bart Hb, 38 Hb S, 3 Hb E, 1 Hb DLos Angeles, and 15 others with unidentified variants. On the other hand, for the 96 newborns presumed to be positive, the medical protocol was carried out.

In 6 of those 96 cases, a diagnostic test was requested due to the high analyte concentration and/or the biomarker encountered. Of that, 1 newborn was diagnosed with CAH due to 21-hydroxylase deficiency, 1 was ruled out for CH following a normal thyroid function test, and 1 was analyzed for further mutations after detecting 1 delta-F508 copy mutation, having a positive identification of (TG)12-5T/(TG)10-9 T. For the other 3 heterozygous for delta-F508 mutation, an additional molecular study could not be performed.

For the remaining 90 newborns presumed to be positive, a second DBS sample was requested. Of those, only 84 samples were processed (Table 4); 5 samples were not obtained due to a change of address or the provision of incorrect contact information, and 1 newborn had died due to a factor VII deficiency before his screening was concluded.

Of the 84 newborns who had a second sample taken, 76 had normal results and 8 remained abnormal (Table 4). Of the 8 abnormal cases, 2 had fatty acid oxidation defects that were confirmed with organic acid analysis of urine; 1 was a female with 3-methylcrotonyl-CoA carboxylase deficiency (3-MCC) that did not have the common 518insT mutation but had substantially elevated 3-OH-isovaleric acid levels in her urine. The second case featured a female with methylmalonic acidemia that did not show the 2 common mutations (*N219Y* or *G717*); nevertheless, methylmalonic acid was markedly elevated in her urine. Additionally, 2 other newborns showed positive elevated tyrosine; 1 patient was diagnosed with TYR I by plasma amino acid quantification, urine organic acid analysis, and positive succinylacetone, while the other case was ruled out by a normal plasma amino acid quantification. Another newborn was suggestive of MSUD; although the second-tier testing did not show a positive mutation (*Y438 N*), it was considered positive by plasma amino acid and organic acid analysis. Two other newborns showed a significantly elevated IRT (immunoreactive trypsinogen) value suggestive of CF; both were ruled out—1 by a negative sweat test and the other by a significant drop in the IRT values between her first and second samples. Finally, a newborn with presumptive positive BIOT was confirmed by the detection of a homozygous *D444H* mutation. However, even with the presence of a homozygous genotype, it was not enough to classify this case as a partial deficiency since the enzymatic activity in the newborn's serum was 82%.

It should be noted that 29/84 newborns had a positive amino acid profile on their first sample, while 14 were diagnosed with transient neonatal tyrosinemia (TNT) when their tyrosine levels returned to normal in the second sample; 9 of those cases (64%) consisted of premature infants (24-36.6 weeks).

In summary, during the ENS program, the estimated global incidence for IEM, endocrinopathies, hemoglobinopathies, and other disorders was 34 newborns diagnosed of the total 10 000 screened. There were 26 diagnosed with G6PD, 2 with CF,1 newborn with a 3-MCC, 1 case of methylmalonic acidemia, 1 newborn with CAH, 1 case of MSUD, 1 newborn with TYR I, and 1 newborn with BIOT (Table 5). Sixty-four newborns were identified with a hemoglobinopathy heterozygous mutation, 3 with *CFTR* heterozygous mutations, 14 with TNT, and 1 female carrier of G6PD.

In this study, the results of the ENS were obtained by applying mixed technologies that established a presumptive positive rate of 1.88% after analysis of the first sample. According to the adopted protocol, it was necessary to request a second sample for 90 cases, of which only 84 samples were obtained.

Furthermore, this study suggested that there was a false-positive rate of 0.04%; this value is related to the healthy newborns in whom no disease was detected after the diagnostic tests performed. On the other hand, the false-negative rate was assumed to be close to 0%. Therefore, the positive predicted value for detecting IEM, endocrinopathies, hemoglobinopathies, and other disorders is close to 89.5% among the screened newborns.

Discussion and Conclusions

The ENS program has the advantage of detecting over 50 IEM, endocrinopathies, hemoglobinopathies, and other disorders, presenting an estimated incidence of 3.4:1000 and a carrier

Table 2. The IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders Detected Markers and Methodologies Within the ENS.

IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders	First Tier		Second Tier	
	Marker	Test	Marker	Test
CF	IRT	Fluoroimmunoassay	39 common *CFTR* gene mutations and 4 polymorphisms	AS-PCR (Allele-specific Polymerase Chain Reaction)
CH	TSH	Fluoroimmunoassay	NA	NA
Hemoglobinopathy				
β (S, C, E, and D variants)	Hemoglobin	Isoelectric focusing	Most common mutations associated with those types	AS-PCR
α (Bart trait)	Hemoglobin	Isoelectric focusing	NA	NA
CAH	Total 17-OHP (17-hydroxyprogesterone)	Fluoroimmunoassay	Organic extraction of 17-OHP	Quantification
BIOT	Biotin	Colorimetric assay	*BTD* gene mutations: *G98: d7i3*, *D444H, R538C*, *Q456H*, *A171T*, *D252G*, *R157H*, and *F403V*	AS-PCR
G6PD	*G202A, A376G, C563T, G1376T*, and *G1388A*	AS-PCR	NA	NA
GALT	Total galactose (galactose plus galactose-1-phosphate) quantification GALT enzyme activity	Fluorometry assay	If total galactose is over the limit, free galactose is quantified. If GALT activity is under the limit, *GALT* mutations are tested: *N314D*, *Q188R*, *S135L*, *K285N*, and *L195P*	AS-PCR
Amino acid profile				
MSUD	Valine, leucine, isoleucine	MS/MS	*Y438N* mutation	AS-PCR
Other amino acid–related diseases (eg, tyrosinemia)	Amino acids	MS/MS	If the Tyr is out of range, succinylacetone is quantified	Quantification
Acylcarnitine profile				
MCAD	C6, C8, C10, C10:1; C8/C2 and C8/C10 ratios	MS/MS	*985A>G*, *199T>C*	AS-PCR
LCHAD	C16-OH, C16:1-OH, C18-OH, C18:1-OH, and C16-OH/C16 ratio	MS/MS	*1528G>C*	AS-PCR
Glutaric acidemia type I	Glutaric acid bound covalently to carnitine, C5DC/C5-OH, C5DC/C8, and C5DC/C16 ratios	MS/MS	*A421V*, *R402W*	AS-PCR
Propionic acidemia	C3-acylcarnitine, C3/C2 and C3/C16 ratios	MS/MS	*E168K*, *1218del14/ins12*, *1170insT*	AS-PCR
Methylmalonic acidemia	C3-acylcarnitine, C3/C2 and C3/C16 ratios	MS/MS	*N219Y*, *G717V*	AS-PCR
Isovaleric acidemia	C5-acylcarnitine, C5/C0, C5/C2 and C5/C3 ratios	MS/MS	*932C>T* (*A282V*)	AS-PCR
3-MCC	C5-OH acylcarnitine, C5-OH/C8 and C5-OH/C0 ratios	MS/MS	*517insT*	AS-PCR

Abbreviations: BIOT, biotinidase deficiency; CAH, congenital adrenal hyperplasia; CH, congenital hypothyroidism; CF, cystic fibrosis; ENS, expanded newborn screening; G6PD, glucose-6-phosphate dehydrogenase deficiency; GALT, galactosemia; IEM, inborn errors of metabolism; IRT, immunoreactive trypsinogen; LCHAD, long chain L-3-hydroxyacyl CoA dehydrogenase deficiency; 3-MCC, 3-methylcrotonyl-CoA carboxylase deficiency; MS/MS, tandem mass spectrometry; MCAD, medium-chain acyl-CoA dehydrogenase deficiency; MSUD, maple syrup urine disease; NA, not applicable; TSH, thyroid stimulating hormone.

detection of 6.8:1000. This proves the importance of establishing a nationwide screening program for every newborn in Mexico. This finding is higher than expected, considering other previous reports.[2,18] A study undertaken in Nuevo Leon, Mexico, showed an IEM incidence of 1 in 5000, considering only 30 diseases screened by MS/MS in a sample size of 42 264 newborns,[2] while our comparative results showed an incidence of 1 in 2500. Other countries, such as the United States, have published an incidence rate as high as 1 in 500,[1] as compared to the rate of 1 in 295 in this study; over 50 IEM, endocrinopathies, hemoglobinopathies, and other disorders were included in that study as well. However, when specifically examining

Table 3. Positive Results Obtained From the First Sample of 10 000 Newborns Screened.

IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders Detection	First Tier		Second Tier		First Sample Detection
	Positive or PP	Carriers	Positive or PP	Carriers	
G6PD	26[a]	1[a]	NA		27
CF	11	—	1[a]	4	11
Hemoglobinopathies					
Bart trait	—	7[a]	NA		7
S, C, D, E variants	—	57	—	57[a]	57
BIOT	7	—	NA		7
CH	15	—	NA		15
Acylcarnitine profile	30	—	0	—	30
Amino acid profile	30	—	1	—	30
CAH	4	—	4	—	4
				Total	188

Abbreviations: BIOT, biotinidase deficiency; CAH, congenital adrenal hyperplasia; CH, congenital hypothyroidism; CF, cystic fibrosis; G6PD, glucose-6-phosphate dehydrogenase deficiency; IEM, inborn errors of metabolism; NA, not applicable (no second-tier protocol available for these diseases); PP, presumptive positive.
[a]Newborns confirmed by the identification of a specific mutation, or the exempting of a diagnostic test.

Table 4. Positive Results Obtained From the Second Sample of 10 000 Newborns Screened.

IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders Detection	Second Sample Analyzed	First Tier		Second Tier		Second Sample Detection
		Positive or PP	Carriers	Positive or PP	Carriers	
Acylcarnitine profile	27	2	—	NA		2
Amino acid profile	29	3	—	1	—	3
CF	5	2	—	NA		2
BIOT	7	1	—	1	—	1
CAH	2	0	—	NA		0
CH	14	0	—	NA		0
	84				Total	8

Abbreviations: CAH, congenital adrenal hyperplasia; CH, congenital hypothyroidism; CF, cystic fibrosis; IEM, inborn errors of metabolism; NA, not applicable (no second-tier protocol available for these diseases); PP, presumptive positive.

G6PD, the incidence obtained was nearly 1 in 400, less than that found by Lin et al who reported an incidence rate of 1 in 90.[19]

The prevalence of IEM, endocrinopathies, hemoglobinopathies, and other disorders may vary depending on the number of diseases screened and the technology used for their detection. The higher detection rate reported in this study could be attributed to the inclusion of DNA testing at the first- and second-tier protocol as well as to the combination of technologies used for the screening process. Other variables that should be considered are the following: genotypic differences among populations and races, consanguinity, cutoff value differences among laboratories, and different sample sizes.

A Mexican group of researchers reported vast heterogeneity between medical institutions when exploring the diseases that were screened and the methodologies used.[12] A comparison of the results is difficult, and establishing the actual disease incidence is merely a matter of speculation. The ENS carried out at the Ginequito Hospital used MS/MS as well as biochemical and molecular methods to detect more than 50 diseases related to IEM, endocrinopathies, hemoglobinopathies, and other disorders during first-tier testing, while additional mutation detection was performed during the second-tier testing for CF, BIOT, as well as amino acid and acylcarnitine profiles. This protocol enables the detection of more diseases than other newborn screening programs, offering a closer estimation of the real incidence of these diseases in the Mexican hospital.

The G6PD was the most frequently detected disease, which is in alignment with previously established worldwide incidence reports.[19] Furthermore, this study detected carriers, mainly for hemoglobinopathies,[20,21] and this is beneficial for the families as they received genetic counseling. Moreover, the important detection of TNT was also discovered, accounting for 14 additional cases. Although a benign condition in the short-term, there is evidence that in the long-term it may cause specific learning disabilities.[22] Once again, the importance of early detection and the correct gathering of all data related to the newborn's condition is highlighted, as they enable one to precisely diagnose patients to provide adequate care for these infants.

Table 5. Final Results of the IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders.[a]

IEM, Endocrinopathies, Hemoglobinopathies, and Other Disorders	Diagnosed	Carriers	False Positive[b]
G6PD	26	1	—
Hemoglobinopathies	—	64	—
CF	2	3	2
CH	—	—	1
Acylcarnitine profile			
3-MCC	1	—	—
Methylmalonic acidemia	1	—	—
CAH	1	—	—
Amino acid profile			
TYR I	1	—	—
MSUD	1		—
BIOT	1	—	
Total	48	68	4

Abbreviations: BIOT, biotinidase deficiency; CAH, congenital adrenal hyperplasia; CH, congenital hypothyroidism; CF, cystic fibrosis; G6PD, glucose-6-phosphate dehydrogenase deficiency; IEM, inborn errors of metabolism; 3-MCC, 3-methylcrotonyl-CoA carboxylase deficiency; MSUD, maple syrup urine disease; TYR I, tyrosinemia type I.

[a]Alterations detected (i.e. disease or carrier state) of the 10 000 samples screened at Ginequito as well as the false positives encountered after diagnostic testing was carried out.

[b]Ruled out after a diagnostic test was done.

There are still inaccuracies in the sample collection that need immediate attention and improvement, such as its technique, shipping, and storage as well as the correct reporting of an infant's age, diet, drugs, transfusions, ethnicity, and so on. However, it is well known that the purpose of screening is to significantly reduce the false-negative rate, leading to a rise in false-positive results.

Concerning follow-up, once a positive result is detected, parent education becomes a priority; so an adequate diet and treatment options can be initiated to avoid additional stress and to prevent permanent damage or even death. More ENS programs should be undertaken in countries where the incidence of IEM, endocrinopathies, hemoglobinopathies, and other disorders is not yet clear in order to establish the frequency of these diseases. This will also allow affected patients to be treated promptly. In the cases presented, preventive measures were undertaken in a timely manner for all G6PD cases. In the same way, treatment was initiated for patients diagnosed with MSUD, tyrosinemia type 1, CAH, 3-MCC, and methylmalonic acidemia. Genetic counseling was provided to all families with diagnosed newborns.

The ENS Program carried out at Ginequito bolsters our comprehension of the real frequency of IEM, endocrinopathies, hemoglobinopathies, and other disorders in the northern Mexican hospital, which can be used to further our understanding of these diseases. Developing the ENS program for all newborns born at this hospital is an ongoing task; however, it is still critical to raise awareness of the importance of a unified nationwide ENS program to obtain the real incidence rates of diseases screened in all of Mexico.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Green NS, Dolan SM, Murray TH. Newborn screening: complexities in universal genetic testing. *Am J Public Health*. 2006; 96(11):1955-1959. doi:10.2105/AJPH.2005.070300.
2. Torres-Sepúlveda Mdel R, Martínez-de Villarreal LE, Esmer C, et al. Tamiz metabólico neonatal por espectrometría de masas en tándem: dos años de experiencia en Nuevo León, México [in Spanish]. *Salud Publica Mex*. 2008;50(3):200-206.
3. Sweetman L, Millington DS, Therrell BL, et al. Naming and counting disorders (conditions) included in newborn screening panels. *Pediatrics*. 2006;117(2):S308-S314. doi:10.1542/peds.2006-1567.
4. Campos Hernández D. Tamizaje neonatal por espectrometría de masas en tándem: actualización. *Rev Panam Salud Pública*. 2010; 27(4):309-318. doi:10.1590/S1020-49892010000400010.
5. Borrajo GJC. Newborn screening in Latin America at the beginning of the 21st century. *J Inherit Metab Dis*. 2007;30(4): 466-481. doi:10.1007/s10545-007-0669-9.
6. Velázquez A, Loera-Luna A, Aguirre BE, Gamboa S, Vargas H, Robles C. Tamiz neonatal para hipotiroidismo congenito y fenilcetonuria [in Spanish]. *Salud Publica Mex*. 1994;36(3): 249-256.
7. Norma Técnica No. 321. Para la prevención del retraso mental producido por hipotiroidismo congénito. *Diario Oficial de la Federación*. Published September 22, 1988. Accessed August 24, 2015.
8. Fernhoff PM. Newborn screening for genetic disorders. *Pediatr Clin North Am*. 2009;56(3):505-513. doi:10.1016/j.pcl.2009.03.002.
9. Norma Oficial Mexicana NOM-034-SSA2-2002. Para la prevención y control de los defectos al nacimiento. *Diario Oficial de la Federación*. Published October 27, 2003. Accessed August 24, 2015.
10. Proyecto de Norma Oficial Mexicana PROY-NOM-034-SSA2-2010. Para la prevención y control de los defectos al nacimiento. *Diario Oficial de la Federación*. Published October 18, 2012. Accessed August 24, 2015.
11. Norma Oficial Mexicana NOM-034-SSA2-2013. Para la prevención y control de los defectos al nacimiento. *Diario Oficial de la Federación*. Published June 24, 2014. Accessed August 24, 2015.
12. Vela-Amieva M, Belmont-Martínez L, Ibarra-González I, Fernández-Lainez C. Variabilidad interinstitucional del tamiz neonatal en México. *Bol Med Hosp Infant Mex*. 2009;66(5): 431-439.

13. Vela-Amieva M, Blemont-Martínez L, Fernández-Lainez C, Ramírez-Frías C, Ibarra-González I. Frecuencia de enfermedades metabólicas congénitas susceptibles de ser identificadas por el tamiz neonatal. *Acta Pediátrica México*. 2009;30(3): 156-162.
14. Levy HL. Historical perspectives: newborn metabolic screening. *Neoreviews*. 2005;6(2):e57-e60. doi:10.1542/neo.6-2-e57.
15. Wilcken B, Wiley V, Hammond J, Carpenter K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. *N Engl J Med*. 2003;348(23):2304-2312. doi:10.1056/NEJMoa025225.
16. Lindner M, Gramer G, Haege G, et al. Efficacy and outcome of expanded newborn screening for metabolic diseases—report of 10 years from South-West Germany. *Orphanet J Rare Dis*. 2011; 6(44):1-10. doi:10.1186/1750-1172-6-44.
17. Shah VS, Ohlsson A. Venepuncture versus heel lance for blood sampling in term neonates. *Cochrane Database of Syst Rev*. 2011;(10):CD001452. doi:10.1002/14651858.CD001452.pub4.
18. Velásquez A, Vela-Amieva M, Waylor EW, Chace DH. Resultados del tamiz neonatal ampliado, como nueva estrategia para la prevención de los defectos al nacimiento. *Rev Mex Pediatría*. 2000;67(5):206-213.
19. Lin Z, Fontaine JM, Freer DE, Naylor EW. Alternative DNA-based newborn screening for glucose-6-phosphate dehydrogenase deficiency. *Mol Genet Metab*. 2005;86(1-2):212-219. doi:10.1016/j.ymgme.2005.05.008.
20. Madan N, Sharma S, Sood SK, Colah R, Bhatia LHM. Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. *Indian J Hum Genet*. 2010;16(1):16-25. doi:10.4103/0971-6866.64941.
21. Adorno EV, Couto FD, Moura Neto JP de, et al. Hemoglobinopathies in newborns from Salvador, Bahia, Northeast Brazil. *Cad Saude Publica*. 2005;21(1):292-298. doi:10.1590/S0102-311X2005000100032.
22. Rice DN, Houston IB, Lyon ICT, et al. Transient neonatal tyrosinaemia. *J Inherit Metab Dis*. 1989;12(1):13-22. doi:10.1007/BF01805526.