Molecular Genetics and Metabolism xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Nine years of newborn screening for classical galactosemia in the Netherlands: Effectiveness of screening methods, and identification of patients with previously unreported phenotypes

Lindsey Welling [a], Anita Boelen [b], Terry G.J. Derks [c], Peter C.J.I. Schielen [d], Maaike de Vries [e], Monique Williams [f], Frits A. Wijburg [a], Annet M. Bosch [a,*]

[a] Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Clinical Chemistry, Laboratory of Endocrinology, Academic Medical Center, University of Amsterdam, The Netherlands
[c] Section of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[d] Centre for Infectious Diseases Research, Diagnostics and Screening (IDS), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[e] Department of Pediatrics, University Medical Center St Radboud Nijmegen, Nijmegen, The Netherlands
[f] Department of Pediatrics, Erasmus MC-Sophia children's Hospital, Erasmus University Rotterdam, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 27 December 2016
Accepted 27 December 2016
Available online xxxx

Keywords:
Classical galactosemia
Newborn screening
Incidence
Prevalence
Galactose oxidation
Variants

ABSTRACT

Introduction: Newborn screening (NBS) for classical galactosemia (CG) was introduced in the Netherlands in 2007. Multiple screening methods have been used since, and currently a two-tier system is used, with residual enzyme activity of galactose-1-phosphate-uridyltransferase (GALT) and total galactose concentration in dried blood spots as the primary and secondary markers. As it is essential to monitor effectiveness of NBS programs, we assessed the effectiveness of different screening methods used over time (primary aim), and aimed to identify and investigate patients identified through NBS with previously unreported clinical and biochemical phenotypes (secondary aim).
Methods: The effectiveness of different screening methods and their cut-off values (COVs), as used from 2007 through 2015, was determined, and the clinical and biochemical data of all identified patients were retrospectively collected.
Results: All screening methods and COVs resulted in relatively high false-positive rates and low positive predictive values. Total galactose levels in dried blood spots were far above the COV for NBS in all true positive cases. A total of 31 galactosemia patients were identified, and when corrected for a family with three affected siblings, 14% had a previously unreported phenotype and genotype. These individuals did not demonstrate any symptoms at the time of diagnosis while still being exposed to galactose, had galactose-1-phosphate values below detection limit within months after the start of diet, and had previously unreported genotypes.
Conclusion: Optimization of NBS for CG in the Netherlands is warranted because of the high false-positive rate, which may result in significant harm. Furthermore, a surprising 14% of newborns identified with CG by screening had previously unreported clinical and biochemical phenotypes and genotypes. For them, individualized prognostication and treatment are warranted, in order to avoid unnecessary stringent galactose restriction.

1. Introduction

Classical galactosemia (CG, OMIM 230400) is an inborn error of galactose metabolism, caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT, EC 2.7.7.12), which converts galactose-1-phosphate (Gal-1-P) and uridine diphosphate galactose (UDP)-glucose to UDP-galactose and glucose-1-phosphate. After ingestion of galactose from breast milk or infant formula, newborn infants develop a life-threatening illness with feeding difficulties, liver failure, renal tubular dysfunction, sepsis and cataract [1]. All acute symptoms resolve quickly after initiation of a lactose-free and galactose-restricted diet. Unfortunately, in spite of a timely diagnosis and start of treatment in the first weeks of life, many patients suffer from long-term complications such as impaired cognitive ability, speech and language defects, neurological complications, decreased bone mass density in some and hypergonadotropic hypogonadism in females [2,16]. CG is defined by a profound impairment of GALT enzyme activity (absent or barely detectable) and/or the presence of two null or severe missense variations. Because there is a large intra-assay variation for GALT enzyme

* Corresponding author at: Department of Pediatrics, room H7-270, Academic Medical Center, PO BOX 22660, 1100 DD Amsterdam, The Netherlands.
E-mail addresses: lindsey.welling@amc.uva.nl (L. Welling), a.boelen@amc.uva.nl (A. Boelen), t.g.j.derks@umcg.nl (T.G.J. Derks), peter.schielen@rivm.nl (P.C.J.I. Schielen), Maaike.deVries@radboudumc.nl (M. de Vries), m.williams@erasmusmc.nl (M. Williams), f.a.wijburg@amc.uva.nl (F.A. Wijburg), a.m.bosch@amc.uva.nl (A.M. Bosch).

http://dx.doi.org/10.1016/j.ymgme.2016.12.012
1096-7192/

measurement, especially in the lower range, it is not possible to define CG with an exact percentage, also because it is yet unknown at which percentage patients will have a clinical presentation and outcome fitting the diagnosis of CG. The recent international guideline for CG states that patients with a red blood cell GALT enzyme activity below 10% and/or pathologic variations on both alleles of the *GALT* gene, should be treated with a galactose-restricted diet, and that there is not enough evidence to conclude whether patients with 10–15% red blood cell residual GALT activity should or should not be treated [17].

A well-known variant of galactosemia is Duarte galactosemia, which is associated with residual enzyme activity of 14–25% [4]. According to the same guideline, there is no need to treat and follow up individuals with the Duarte variant, as these variants are not considered pathogenic. In the Netherlands, it was decided to treat and follow-up all patients with a residual GALT enzyme activity below 15%.

CG is part of the Dutch newborn screening (NBS) panel since 2007, with the aim to prevent critical illness and death in the neonatal period ([13]; [12]; [15]). Due to uncertainties about the risks and benefits of NBS for CG, it is included in only a minority of European NBS programs [7]. Several factors contribute to the potential risks of NBS for CG. First, as applies for all disorders included in NBS programs, there is the risk of identification of false positive (FP) cases, which may cause anxiety and/or depression in parents, parent-child dysfunction and alterations in perception of their child's health, even when the repeat test is normal [5]. Second, screening often results in the detection of individuals with previously unreported phenotypes and genotypes, for whom the need for treatment and potential outcomes are unclear [14]. Third, screening for CG does not seem to prevent long-term complications [13] and finally cost-effectiveness has not been studied sufficiently.

At the start of NBS for CG in the Netherlands, there was limited insight into effectiveness of potential screening methods. As a consequence, to reduce the number of FP results, over the years there have been adaptations in the type of screening method used, such as the number of screening markers and cut-off values (COVs) [8]. Effectiveness of different CG screening tests has been reported for only five programs [3,6,10–12], which all used different screening markers and different methods with varying COVs. In order to assess the benefits and risks of NBS for CG, data on the effectiveness of the different screening methods are needed, as well as detailed knowledge of the biochemical parameters and health status of individuals identified by NBS with previously unreported phenotypes and genotypes.

1.1. Objectives

The primary objective of this study is to evaluate the effectiveness of the NBS program for CG in the Netherlands between 2007 and 2015, by evaluating the different screening methods used during this period.

The secondary objective is to retrospectively evaluate the clinical and biochemical outcome of patients identified through NBS, with a special focus on individuals with a residual GALT enzyme activity <15% and previously unreported phenotypes and genotypes.

2. Methods

2.1. Effectiveness of the newborn screening program

Data relevant for assessing the effectiveness of the used screening methods were provided by the National Institute for Public Health and Environment (RIVM, Ministry of Health, Welfare and Sport). Referral data of the RIVM were cross-checked with data from The Dutch Diagnosis Registration Metabolic Diseases (DDRMD), a registry of patients with a confirmed diagnosis of an inborn error of metabolism and of newborns with a newborn screening result indicative for a metabolic disease (https://www.ddrmd.nl/). Data were also cross-checked in the Dutch newborn screening advisory board.

2.2. Screening methods

We refer to Table 1 for an overview of all screening methods and COVs. In the Netherlands, dried blood spots (DBS) on filter paper are ideally collected between 72 and 168 h after birth, and are sent to one of the five regional newborn screenings laboratories (authority responsible for the entire screening process: RIVM, Centre for population screening, by assignment of the Minister of Health, Welfare and Sport). In the first three months after initiation of the NBS program for CG, total galactose (TGAL: Gal-1-P plus galactose) was the primary and only marker, with a COV of 700 μmol/l blood. At that time, all the laboratories used the Bio-Rad Quantase Neonatal Total Galactose screening assay (Bio-Rad Laboratories Inc., California, USA). Because of a very high number of FP cases, the screening method was changed after three months with GALT activity (COV ≤20%) as a primary marker using the Bio-Rad CODA Neonatal GALT essay, and TGAL as a second tier when GALT was ≤20% (TGAL COV ≥700 μmol/l blood, from April 1st 2007). Patients were referred when both GALT and TGAL were abnormal. After five years of experience with this screening method, the COV for GALT was changed to ≤15% in July 2012, in an attempt to further reduce the high number of FP screening results. In 2012 and 2013, three screenings laboratories switched to the automated GALT assay (3303-0010-assay, PerkinElmer, Turku, Finland) using the Genetic Screening Processor (GSP analyzer, PerkinElmer). Two screenings laboratories had to switch to the same assay without using the GSP, named the manual GSP assay, because of problems with the Biorad Neonatal GALT assay. A good correlation was found between the manual and automated GSP assay, and the COV for both methods was set at GALT ≤2.7 U/dl

Table 1
Effectiveness of different screening methods and cut-off values.

	Method 1	Method 2	Method 3	Method 4	Method 5	Total
Screened patients	44,174	952,191	345,685	173,656	122,027	1,637,733
Individuals with positive screening result	217	322	87	96	30	752
Individuals with classical galactosemia	1	18	6	3	0	28 (+3 patients[ab])
Individuals with false-positive result	216	304	81	93	30	724
Individuals with false-negative result	0	0	0[a]	0[b]	0	0
Individuals with true negative result	43,957	951,869	345,598	173,560	121,997	1,636,981
Sensitivity	100%	100%	100%	100%	Unknown	100%
Specificity	99,51%	99,97%	99,98%	99,95%	0%	99,56%
Positive predictive value	0,46%	5,6%	6,9%	3,1%	Unknown	3,6%

Method 1, January 2015 to 15 April 2015 : TGAL (COV ≥ 700 μmol/l blood) was used as the only marker for NBS.
Method 2, 16 April 2007 to June 2012 : Residual GALT activity (COV ≤20%) as the primary marker, with TGAL (COV ≥ 700 μmol/l blood) as a second tier marker.
Method 3, July 2012 to June 2014 : Residual GALT activity (COV ≤15%) as the primary marker, with TGAL (COV ≥ 700 μmol/l blood) as a second tier marker.
Method 4, July 2014 to June 2015 : Residual GALT activity (≤2.7 U/dl blood) as the primary marker, with TGAL (COV ≥ 900 μmol/l blood) as a second tier marker.
Method 5, July 2015 to December 2015 : Residual GALT activity (≤2.0 U/dl blood) as the primary marker, with TGAL (COV ≥ 1100μmol/l blood) as a second tier marker.
[a] One patient was diagnosed prior to birth and started a galactose restricted diet on the first day of life. Due to immediate start of treatment the TGAL value was in the normal range.
[b] Two patients diagnosed prior to birth and started a galactose restricted diet on the first day of life. Due to immediate start of treatment the TGAL value was in the normal range.

Please cite this article as: L. Welling, et al., Nine years of newborn screening for classical galactosemia in the Netherlands: Effectiveness of screening methods, and identificat..., Mol. Genet. Metab. (2016), http://dx.doi.org/10.1016/j.ymgme.2016.12.012