Classical galactosemia

A cloud with a silver lining

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Lindsey Welling

CLASSICAL GALACTOSEMIA

A cloud
with a silver lining

Classical Galactosemia

A cloud with a silver lining

Lindsey Welling

NUTRICIA
Advanced Medical Nutrition

Sorgente
Specialist in medische voeding

Classical Galactosemia

A cloud with a silver lining

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op donderdag 5 oktober 2017, te 14:00 uur

door Lindsey Welling
geboren te Hoorn

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Chapter 1

General introduction
and outline of the thesis

Chapter 1 General introduction and outline of the thesis

The first report to describe a probable case of galactosemia dates back to 1908 [1]. Not until decades later it was demonstrated that classical galactosemia (CG, OMIM 230400) is an autosomal recessive inborn error of metabolism, caused by a severe deficiency of the third enzyme of the Leloir pathway, galactose-1-phosphate urid-yltransferase (GALT, EC 2.7.7.12)[2]. This enzyme deficiency results from variations in the gene encoding *GALT* (NM_000155.3), which is located on chromosome 9p13, in which over 300 variations have been reported so far [3]. The incidence of CG varies extremely worldwide, with an estimated incidence of 1 case per 19.000 to 96.000 individuals in various countries in Europe and the United States [4–8], to 1 case per 400.000 to 800.000 individuals in Asian countries, including Taiwan and Japan [9,10].

Diagnosis and enzymatic defect
The gold standard for the diagnosis of CG is demonstration of a deficiency of GALT activity in erythrocytes and/or two known pathogenic variations in the *GALT* gene. There is currently no exact biochemical or clinical definition of CG, however, patients with the typical classical form of galactosemia usually have absent or barely detectable residual GALT enzyme activities in erythrocytes. In the Netherlands, all individuals with an erythrocyte GALT enzyme activity <15% of healthy controls are considered to have CG and are treated accordingly.

β-D-galactose is metabolized in four steps by four different enzymes, collectively designated as the Leloir pathway (Figure 1): galactose mutarotase, galactokinase (GALK), GALT and UDP-galactose-4-epimerase (GALE). In the first step, galactose is converted to galactose-1-phosphate (Gal-1-P) by GALK. After this step, Gal-1-P and uridine diphosphate-glucose (UDP-glucose) are converted into glucose-1-phosphate and UDP-galactose by GALT. In the final step of the pathway, GALE catalyzes the conversion of UDP-galactose to UDP-glucose, as well as their N-acetylated forms: UDP-N-acetylglucosamine (UDP-GlcNAc) to UDP-N-acetylgalactosamine (UDP-GalNAc). Glucose-1-phosphate is then converted to glucose-6-phosphate and is oxidized to CO_2 by the glycolytic pathway and the tricarboxylic acid cycle [11]. As a consequence of GALT deficiency, galactose and Gal-1-P accumulate in the body. Galactose is partially shunted to galactitol (by the enzyme aldose reductase), and galactonate (by the enzyme galactose dehydrogenase).

Galactose

GALK   ATP

ADP

UDP
+
Gluc-1-P

Gal-1-P   UDP-glucose   UDP-galNAc

GALT   GALE

Gluc-1-P   UDP-galactose   UDP-glucNAc

Phosphoglucomutase

Gluc-6-P
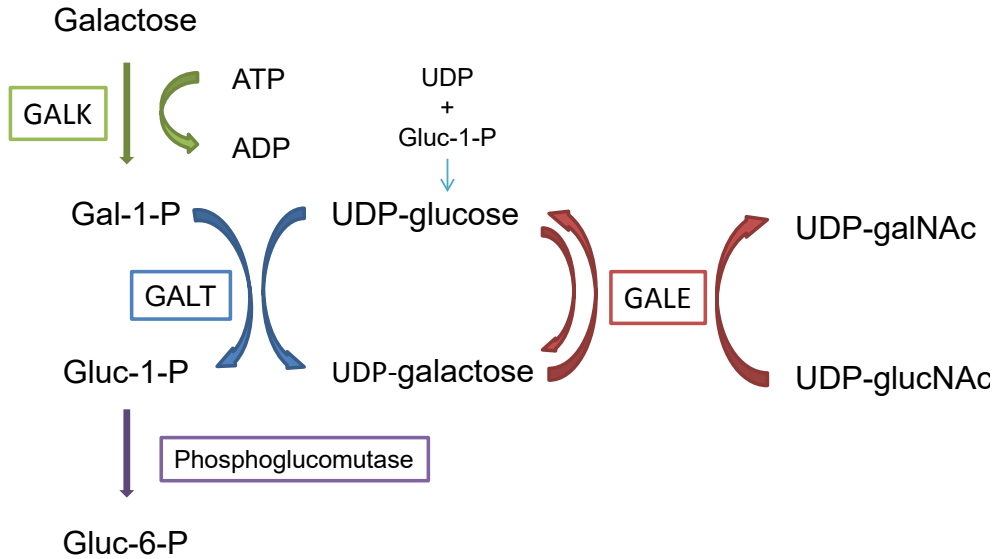
Figure 1. Leloir pathway

Early signs and symptoms
After ingesting galactose from breast milk or infant formula, affected newborn infants generally develop a life-threatening illness in the first week of life. They may present with hypotonia, feeding problems, vomiting, sepsis-like symptoms, *E. Coli* sepsis, liver disease (hepatomegaly, jaundice, bleeding diathesis), renal tubular dysfunction and bilateral cataract. If CG is not recognized early and left untreated, the mortality rate in this period is high. Early diagnosis and timely start of a galactose-restricted diet is life-saving. Several countries, including the Netherlands, have therefore introduced CG into the newborn screening (NBS) program, aiming to prevent critical illness and death in the newborn period [12–14]. There are, however, risks of NBS, such as false-positive cases, and because of uncertainties about the balance between these risks and the aforementioned benefits, several countries have decided not to include CG in their NBS programs [15].

Treatment
A lactose-free and galactose-restricted diet is currently the only available treatment for CG and this diet is the advised treatment for life. Products with a high galactose content are eliminated from the diet, including all animal milks and other dairy products [16,17]. However, there is a remarkably wide variation in the extent to which less obvious sources of galactose (fruits and vegetables, other foods containing trace amounts of lactose) are restricted between countries and between treatment centers within countries.

Pathophysiological processes
Despite early initiation of the diet, and good compliance with this strict diet, patients are at risk for late complications including neurological complications (including cognitive impairment), speech and language problems, psychosocial problems, decreased bone mass density and primary ovarian failure in female patients. Only patients who are not compliant with the diet seem to be at risk for developing cataract after the newborn period. For all of these complications, except for cataract which is caused by accumulation of galactitol in the crystalline lens [18], the exact pathogenesis including the timing of onset of the underlying processes have not been sufficiently unravelled. Most likely multiple pathophysiological processes contribute to the different long-term complications and may already occur in the prenatal and early postnatal intoxication phase.

Gal-1-P is thought to be the most toxic substrate accumulating in CG, as patients with severe GALK deficiency, in whom only galactose (and subsequently galactitol and galactonate) accumulate (Figure 1), likely do not suffer from severe long-term complications and only develop cataract [19] Next to accumulation of substrates, reduced production of other substrates may play a role in the pathophysiology of CG. In the normal situation, GALT converts Gal-1-P and UDP-glucose to UDP-galactose (and glucose-1-phosphate), and three more UDP sugars result from the conversion of UDP-galactose by GALE. UDP sugars are essential precursors for glycosylation processes, driving the biosynthesis of glycoproteins and glycolipids [20], which heavily populate the cell surfaces and extracellular spaces. Ongoing glycosylation defects have indeed been described in some but not all patients with CG, with defects in both N- and O-linked glycans [21].

Long-term complications
Despite the fact that long-term complications are common in CG, the type of complications and the extent varies between patients, even between patients with the same genetic variations in the *GALT* gene. Currently, it is not possible to predict which patients will develop complications of CG and to what extent. It has not been assessed if these complications cause limitations in body functions (such as mental functions and motor functions) and subsequent need for (ongoing) additional care, or necessitate adaptations in daily life.

Neurological complications
The most prominent neurological complication is cognitive impairment. Though there is wide inter-individual variation, with some patients also attaining normal to above average scores, most studies demonstrate mean intelligence quotient (IQ) scores in

the low to low normal range [4,22–36]. A large percentage (54% to 72%) of patients are reported to attain IQ scores below one standard deviation (85) of the general mean of 100 [25,29,31,37]. Other complications include mild to severe ataxia, tremor, dystonia and dysarthria, but these problems occur less frequent [4,7,24–27,36,38–40]. Various types of speech disorders are frequently described in CG (varying from 24% to 88% in different reports), including childhood apraxia of speech (a disorder in which the precision and consistency of movements underlying speech are impaired), dysarthria, deficits in motor planning or programming, decreased respiratory-phonatory support for speech, and disturbance of vocal quality, which are thought to have a neurological origin [4,7,24–27,36,38–43].

On magnetic resonance (MR) scans, white matter abnormalities and myelination abnormalities have been observed in a substantial part of patients [27,38,44,45]. With detailed MR techniques (neurite orientation dispersion and density imaging; voxel-based morphometry), extensive white matter and grey matter abnormalities have been demonstrated in adolescents and young adults [46,47]. The areas of these abnormalities were in agreement with cognitive, language and motor impairments occurring in CG. The myelination abnormalities may be explained by an inability to make sufficient or normally structured galactocerebroside, as a consequence of glycosylation abnormalities, causing abnormalities in the structure of myelin [48]. In a GALT-deficient Drosophila disease model, synaptopmatrix glycosylation losses, altered trans-synaptic signaling pathway components, defective synaptogenesis and impaired coordinated movement have been demonstrated [49]. The (ongoing) glycosylation abnormalities that have been demonstrated in CG, may thus contribute to neurological complications [50].

Social functioning
Problems in psychosocial development and social functioning seem to occur more frequently in patients with CG, but systematic studies are scarce. Studies addressing this topic evaluated Health Related Quality of Life or Course of Life and demonstrated lower scores in the domain of social functioning in adult patients and that less adult patients were married or lived in stable partnership [7,51–55].

Primary ovarian insufficiency
With over 80% of all females suffering from primary ovarian insufficiency (POI), this is the most frequently occurring complication in CG [4,24,56–60]. There is a broad spectrum of severity of POI, but most females suffer from subfertility. It is hypothesized that direct toxicity of galactose or its metabolites to the ovaries, abnormal glycosylation of for example hormones, or wrongful activation of follicular apoptosis, may be underlying mechanism causing POI in women with CG [60–63].
Bone health

Bone health has been evaluated in multiple studies, because patients have various risk factors that may contribute to a decreased bone mineral density (BMD), such as the galactose-restricted diet, and POI in females. Almost all studies demonstrated decreased BMD in both children and adults [4,7,64–69]. Different techniques to measure BMD, and different measurement sites have been used in these studies. Also, outcome measurements differed and sample sizes were small. Furthermore, exploration of bone turnover markers yielded inconsistent results [65,66,70,71]. It therefore remains difficult to determine the extent of bone health impairment in CG and it's clinical relevance.

Outline of the thesis

In **Chapter 2** the primary aim was to assess effectiveness of newborn screening for CG in the Netherlands. Newborn screening was introduced in 2007 and several screening methods and cut-off values for screening markers have been used since. All screening methods in the period 2007 to 2015 were evaluated. The second aim was to identify and study individuals found by newborn screening, with previously unreported clinical and biochemical phenotypes and genotypes (atypical CG), as individualized treatment and prognostication may be warranted.

In **Chapter 3** the aim was to develop a method to provide more insight into whole-cell galactose metabolism, which allows quantitative assessment of residual galactose metabolism in galactosemia patients. Radioisotope labeled galactose oxidation measurements in fibroblasts of classical patients and patients with a variant presentation of galactosemia (in Chapter 2 referred to as atypical patients) were performed. Also, we developed a method for galactose metabolite profiling (GMP) in fibroblasts using [U-^{13}C]-labeled galactose which was performed in the same patients and controls.

In **Chapter 4** the aim was to develop an international clinical guideline for the management of CG. Following consensus procedures, 21 experts from Europe and the United States formulated recommendations addressing diagnosis, treatment and follow-up of CG. Recommendations were based on evidence if available.

In **Chapter 5** the primary aim was to make a more precise estimate of prevalence of cognitive impairment in early-treated patients with CG. A systematic review and meta-analysis, pooling full-scale intelligence quotient scores of individual patients, were performed.

In **Chapter 6** the aim was to further assess the extent of bone health impairment in patients with CG. A systematic review was performed and aggregate and individual patient data of bone mass density Z-scores were pooled in a meta-analysis.

In **Chapter 7** the aim was to assess current capacities and impairments in body functions of children and adults with CG and subsequent need for additional care or adaptations in their daily life. The Capacity Profile, which is a method to classify additional care needs in permanent conditions, was used to determine impairments in the five domains of body functions (physical health, motor functions, sensory functions, mental functions and speech and voice functions) and the subsequent need for additional care.

In **Chapter 8** the aim was to objectify difficulties in social responsiveness in children and adults with CG, using the Social Responsiveness Scale (SRS) questionnaire.

In **Chapters 9 and 10** the content of this thesis is summarized and discussed in both English and Dutch and future perspectives for research are presented.

References

1. Von Reuss A. Zuckeratisseheidung in Sdiuglingsalter. Wiener Medizinische Wochenschrift 1908;58:799–803.
2. Kalckar HM, Anderson EP, Isselbacher KJ. Galactosemia, a congenital defect in a nucleotide transferase. Biochim Biophys Acta 1956;20:262–8.
3. Calderon FRO, Nelson L, Dobrowolski P, Sinitsyna I, Phansalkar A, Longo N, et al. Combination of enzyme analysis, allele-specific PCR and sequencing to detect mutations in the GALT gene. J Inherit Metab Dis 2007;30:818.
4. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis 2013;36:21–7.
5. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis 2006;29:516–25.
6. Ounap K, Joost K, Temberg T, Krabbi K, Tõnisson N. Classical galactosemia in Estonia: selective neonatal screening, incidence, and genotype/phenotype data of diagnosed patients. J Inherit Metab Dis 2010;33:175–6.
7. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis 2012;35:279–86.
8. Hansen TW, Lie SO. Galactosemia--to screen or not to screen? Pediatrics 1988;81:327–8.
9. Cheung KL, Tang NL, Hsiao KJ, Law LK, Wong W, Ng PC, et al. Classical galactosaemia in Chinese: A case report and review of disease incidence. J Paediatr Child Health 1999;35:399–400.
10. Tada K, Tateda H, Arashima S, Sakai K, Kitagawa T, Aoki K, et al. Follow-up study of a nation-wide neonatal metabolic screening program in Japan. A collaborative study group of neonatal screening for inborn errors of metabolism in Japan. Eur J Pediatr 1984;142:204–7.
11. Holden HM, Rayment I, Thoden JB. Structure and function of enzymes of the Leloir pathway for galactose metabolism. J Biol Chem 2003;278:43885–8.
12. Schweitzer-Krantz S. Early diagnosis of inherited metabolic disorders towards improving outcome: the controversial issue of galactosaemia. Eur J Pediatr 2003;162 Suppl:S50-3.
13. Ohlsson A, Guthenberg C, von Döbeln U. Galactosemia screening with low false-positive recall rate: the Swedish experience. JIMD Rep 2012;2:113–7.
14. Viggiano E, Marabotti A, Burlina AP, Cazzorla C, D'Apice MR, Giordano L, et al. Clinical and molecular spectra in galactosemic patients from neonatal screening in northeastern Italy: structural and functional characterization of new variations in the galactose-1-phosphate uridyltransferase (GALT) gene. Gene 2015;559:112–8.
15. Varela-Lema L, Paz-Valinas L, Atienza-Merino G, Zubizarreta-Alberdi R, Villares RV, López-García M. Appropriateness of newborn screening for classic galactosaemia: a systematic review. J Inherit Metab Dis 2016;1–17.

16. Jumbo-Lucioni PP, Garber K, Kiel J, Baric I, Berry GT, Bosch A, et al. Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes. J Inherit Metab Dis 2012;35:1037–49.

17. Adam S, Akroyd R, Bernabei S, Bollhalder S, Boocock S, Burlina A, et al. How strict is galactose restriction in adults with galactosaemia? International practice. Mol Genet Metab 2015;115:23–6.

18. Widger J, O'Toole J, Geoghegan O, O'Keefe M, Manning R. Diet and visually significant cataracts in galactosaemia: is regular follow up necessary? J Inherit Metab Dis 2010;33:129–32.

19. Bosch AM, Bakker HD, van Gennip AH, van Kempen J V, Wanders RJ, Wijburg FA. Clinical features of galactokinase deficiency: a review of the literature. J Inherit Metab Dis 2002;25:629–34.

20. Prydz K, Dalen KT. Synthesis and sorting of proteoglycans. J Cell Sci 2000;113 Pt 2:193–205.

21. Liu Y, Xia B, Gleason TJ, Castaneda U, He M, Berry GT, et al. N- and O-linked glycosylation of total plasma glycoproteins in galactosemia. Mol Genet Metab 2012;106:442–54.

22. Donnell GN, Collado M, Koch R. Growth and development of children with galactosemia. J Pediatr 1961;58:845–51.

23. Komrower GM, Lee DH. Long-term follow-up of galactosaemia. Arch Dis Child 1970;45:367–73.

24. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis 1990;13:802–18.

25. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr 1993;152:36–43.

26. Kaufman FR, Reichardt JK, Ng WG, Xu YK, Manis FR, McBride-Chang C, et al. Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridyltransferase gene. J Pediatr 1994;125:225–7.

27. Kaufman FR, McBride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. Eur J Pediatr 1995;154:S2–5.

28. Hansen TW, Henrichsen B, Rasmussen RK, Carling A, Andressen AB, Skjeldal O. Neuropsychological and linguistic follow-up studies of children with galactosaemia from an unscreened population. Acta Paediatr 1996;85:1197–201.

29. Rasmussen RK, Andreassen AB, Stromme P, Hansen TW. Learning disabilities and language pathology in patients with galactosemia. Logoped Phoniatr Vocol 1996;21:157–62.

30. Badawi N, Cahalane SF, McDonald M, Mulhair P, Begi B, O'Donohue A, et al. Galactosaemia--a controversial disorder. Screening & outcome. Ireland 1972-1992. Ir Med J 1996;89:16–7.

31. Shield JP, Wadsworth EJ, MacDonald A, Stephenson A, Tyfield L, Holton JB, et al. The relationship of genotype to cognitive outcome in galactosaemia. Arch Dis Child 2000;83:248–50.

32. Widhalm K, Miranda-Da-Cruz B, De Sonneville LMJ. Information processing characteristics and uridine treatment in children with classical galactosemia. Nutr Res 2002;22:257–70.

33. Antshel KM, Epstein IO, Waisbren SE. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology 2004;18:658–64.

34. Doyle CM, Channon S, Orlowska D, Lee PJ. The neuropsychological profile of galactosaemia. J Inherit Metab Dis 2010;33:603–9.

35. Schadewaldt P, Hoffmann B, Hammen HW, Kamp G, Schweitzer-Krantz S, Wendel U. Longitudinal assessment of intellectual achievement in patients with classical galactosemia. Pediatrics 2010;125:e374-81.

36. Rubio-Agusti I, Carecchio M, Bhatia KP, Kojovic M, Parees I, Chandrashekar HS, et al. Movement disorders in adult patients with classical galactosemia. Mov Disord 2013;28:804–10.

37. Hoffmann B, Wendel U, Schweitzer-Krantz S. Cross-sectional analysis of speech and cognitive performance in 32 patients with classic galactosemia. J Inherit Metab Dis 2011;34:421–7.

38. Nelson MD, Wolff JA, Cross CA, Donnell GN, Kaufman FR. Galactosemia: evaluation with MR imaging. Radiology 1992;184:255–61.

39. Dubroff JG, Ficicioglu C, Segal S, Wintering NA, Alavi A, Newberg AB. FDG-PET findings in patients with galactosaemia. J Inherit Metab Dis 2008;31:533–9.

40. Krabbi K, Uudelepp M-L, Joost K, Zordania R, Õunap K. Long-term complications in Estonian galactosemia patients with a less strict lactose-free diet and metabolic control. Mol Genet Metab 2011;103:249–53.

41. Potter NL. Voice disorders in children with classic galactosemia. J Inherit Metab Dis 2011;34:377–85.

42. Potter NL, Nievergelt Y, Shriberg LD. Motor and speech disorders in classic galactosemia. JIMD Rep 2013;11:31–41.

43. Shriberg LD, Potter NL, Strand EA. Prevalence and phenotype of childhood apraxia of speech in youth with galactosemia. J Speech Lang Hear Res 2011;54:487–519.

44. Wang ZJ, Berry GT, Dreha SF, Zhao H, Segal S, Zimmerman RA. Proton magnetic resonance spectroscopy of brain metabolites in galactosemia. Ann Neurol 2001;50:266–9.

45. Hughes J, Ryan S, Lambert D, Geoghegan O, Clark A, Rogers Y, et al. Outcomes of siblings with classical galactosemia. J Pediatr 2009;154:721–6.

46. Timmers I, Zhang H, Bastiani M, Jansma BM, Roebroeck A, Rubio-Gozalbo ME. White matter microstructure pathology in classic galactosemia revealed by neurite orientation dispersion and density imaging. J Inherit Metab Dis 2015;38:295–304.

47. Timmers I, Van Der Korput L, Jansma BM, Rubio-Gazalbo ME. Grey matter density abnormalities in patients with classic galactosemia: A voxel based morphometry study. J. Inherit. Metab. Dis.2015;38:S174.

48. Haberland C, Perou M, Brunngraber EG, Hof H. The neuropathology of galactosemia. A histopathological and biochemical study. J Neuropathol Exp Neurol 1971;30:431–47.

49. Jumbo-Lucioni P, Parkinson W, Broadie K. Overelaborated synaptic architecture and reduced synaptomatrix glycosylation in a Drosophila classic galactosemia disease model. Dis Model Mech 2014;7:1365–78.

50. Coss KP, Hawkes CP, Adamczyk B, Stöckmann H, Crushell E, Saldova R, et al. N-glycan abnormalities in children with galactosemia. J Proteome Res 2014;13:385–94.

51. Gubbels CS, Maurice-Stam H, Berry GT, Bosch AM, Waisbren S, Rubio-Gozalbo ME, et al. Psychosocial developmental milestones in men with classic galactosemia. J Inherit Metab Dis 2011;34:415–9.

52. Bhat M, Haase C, Lee PJ. Social outcome in treated individuals with inherited metabolic disorders: UK study. J Inherit Metab Dis 2005;28:825–30.

53. Bosch AM, Maurice-Stam H, Wijburg FA, Grootenhuis MA. Remarkable differences: the course of life of young adults with galactosaemia and PKU. J Inherit Metab Dis 2009;32:706–12.

54. Hoffmann B, Dragano N, Schweitzer-Krantz S. Living situation, occupation and health-related quality of life in adult patients with classic galactosemia. J Inherit Metab Dis 2012;35:1051–8.

55. Bosch AM, Grootenhuis MA, Bakker HD, Heijmans HSA, Wijburg FA, Last BF. Living with classical galactosemia: health-related quality of life consequences. Pediatrics 2004;113:e423-8.

56. Kaufman FR, Kogut MD, Donnell GN, Goebelsmann U, March C, Koch R. Hypergonadotropic hypogonadism in female patients with galactosemia. N Engl J Med 1981;304:994–8.

57. Kaufman FR, Xu YK, Ng WG, Donnell GN. Correlation of ovarian function with galactose-1-phosphate uridyl transferase levels in galactosemia. J Pediatr 1988;112:754–6.

58. Sanders RD, Spencer JB, Epstein MP, Pollak S V, Vardhana PA, Lustbader JW, et al. Biomarkers of ovarian function in girls and women with classic galactosemia. Fertil Steril 2009;92:344–51.

59. Rubio-Gozalbo ME, Gubbels CS, Bakker JA, Menheere PP, Wodzig WK, Land JA. Gonadal function in male and female patients with classic galactosemia. Hum. Reprod. Update2010;16:177–88.

60. Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo ME. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357–66.

61. Liu G, Hale GE, Hughes CL. Galactose metabolism and ovarian toxicity. Reprod Toxicol 2000;14:377–84.

62. Forges T, Monnier-Barbarino P, Leheup B, Jouvet P. Pathophysiology of impaired ovarian function in galactosaemia. Hum Reprod Updat 2006;12:573–84.

63. FSH isoform pattern in classic galactosemia. J. Inherit. Metab. Dis.2011;34:387–90.

64. Batey LA, Welt CK, Rohr F, Wessel A, Anastasoaie V, Feldman HA, et al. Skeletal health in adult patients with classic galactosemia. Osteoporos Int 2013;24:501–9.

65. Rubio-Gozalbo ME, Hamming S, van Kroonenburgh MJ, Bakker JA, Vermeer C, Forget PP. Bone mineral density in patients with classic galactosaemia. Arch Dis Child 2002;87:57–60.

66. Panis B, Forget PP, Van Kroonenburgh MJPG, Vermeer C, Menheere PP, Nieman FH, et al. Bone metabolism in galactosemia. Bone 2004;35:982–7.

67. Doulgeraki A, Monopolis I, Deligianni D, Kalogerakou M, Schulpis KH. Body composition in young patients with galactose metabolic disorders: a preliminary report. J Pediatr Endocrinol Metab 2014;27:81–6.

68. Kaufman FR, Loro ML, Azen C, Wenz E, Gilsanz V. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. J Pediatr 1993;123:365–70.

69. Karadag N, Zenciroglu A, Eminoglu FT, Dilli D, Karagol BS, Kundak A, et al. Literature review and outcome of classic galactosemia diagnosed in the neonatal period. Clin Lab 2013;59:1139–46.

70. Gajewska J, Ambroszkiewicz J, Radomyska B, Laskowska-Klita T. Bone turnover markers in prepubertal children with classical galactosemia. Indian J Gastroenterol 2006;25:221–2.

71. Gajewska J, Ambroszkiewicz J, Radomyska B, Chelchowska M, Oltarzewski M, Laskowska-Klita T, et al. Serum markers of bone turnover in children and adolescents with classic galactosemia. Adv Med Sci 2008;53:214–20.

PART 1

Newborn screening and
patients with a variant
presentation of galactosemia

2

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

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Frits A. Wijburg and Annet M. Bosch

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 (COV's), 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 COV's 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.

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 [2,3]. 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 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 [4].

A well-known variant of galactosemia is Duarte galactosemia, which is associated with residual enzyme activity of 14-25% [5]. 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 [6–8]. Due to uncertainties about the risks and benefits of NBS for CG, it is included in only a minority of European NBS programs [9]. 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 [10]. 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 [11]. Third, screening for CG

does not seem to prevent long-term complications [6] 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 (COV's)[12]. Effectiveness of different CG screening tests has been reported for only five programs [7,13–16], which all used different screening markers and different methods with varying COV's. 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.

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.

Methods

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.

Screening methods
We refer to Table 1 for an overview of all screening methods and COV's. In the Netherlands, dried blood spots (DBS) on filter paper are ideally collected between 72 and 168 hours 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 blood and TGAL≥900 µmol/l blood [12]. The same methods and COV have been used by all five laboratories since July 1st 2014. From July 1st 2015 the COV's were changed to GALT≤2.0 U/dl blood and TGAL≥1100 µmol/l blood, to further reduce the number of FP cases.

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

	Method 1	Method 2	Method 3	Method 4	Method 5	Total
Screened patients	44174	952191	345685	173656	122027	1637733
Individuals with positive screening result	217	322	87	96	30	752
Individuals with classical galactosemia	1	18	6	3	0	28 (+ 3 patients#*)
Individuals with false-positive result	216	304	81	93	30	724
Individuals with false-negative result	0	0	0#	0*	0	0
Individuals with true negative result	43957	951869	345598	173560	121997	1636981
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 ≥ 1100 µmol/l blood) as a second tier marker.
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.
* 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.

Confirmation of diagnosis
Patients with absent or barely detectable residual red blood cell (RBC) GALT enzyme activity (compared to healthy controls) and/or 2 known pathogenic variations in the GALT gene, plus typical symptoms of CG in the newborn period, and persistently elevated Gal-1-P values in spite of dietary treatment, are considered to be 'typical' CG patients. Newborn screening detects patients with low but not profoundly deficient GALT enzyme activities, and in the Netherlands, all patients with a residual GALT enzyme activity of <15% (compared to healthy controls) and/or two known pathogenic variations in the GALT gene, are treated as if they are classical patients and considered to be true positive (TP).

Outcome of patients identified through NBS
Newborn infants with a positive screening result for CG in the Netherlands are referred to one of the seven Dutch centers for inherited metabolic disorders. We retrospectively retrieved data from medical charts of the TP cases, identified with NBS in the Netherlands from 2007 through 2015, concerning biochemical parameters and clinical outcome.

Sample size/data analysis
All TP cases identified through NBS from 2007 through 2015 were included in this study. We present results of effectiveness of the different methods and COV's of the NBS program for CG, as well as clinical outcome of patients, in a descriptive manner. Except for age at collection of DBS, age data were not normally distributed, and are presented as median with a range. We used SPSS version 22 to calculate these descriptive statistics.

Ethics approval
The Ethical Committee of the Academic Medical Center, Amsterdam, the Netherlands, confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this research and that an official approval of this study by the Ethical Committee was not required.

Results

Effectiveness of screening

Number of screened infants, number of referred infants, percentage of FP referrals, number and percentage of confirmed patients, sensitivity, specificity and positive predictive value (PPV) are reported in Table 1. A total of 1.637.733 newborn children were screened, with a mean age at collection of the DBS for screening ranging from 113 to 118 h after birth over these years. The overall participation rate for the NBS in the Netherlands was >99% for each of the years 2007 through 2015. In total, 752 of these newborn infants had an abnormal screening result for CG. Since 2007, there are no reports of patients diagnosed with CG after a negative newborn screen. Therefore, the sensitivity of NBS was 100% for all of the used screening methods. The highest specificity (99.98%) and PPV (6.9%) were reached with method 3: primary marker residual GALT activity (COV ≤15%), and TGAL (COV ≥ 700 µmol/l blood) as a second tier. We were unable to determine the PPV of the NBS for CG with the latest screening method (July 2015 up to December 2015, total number of infants screened 122.027), because no children with CG were diagnosed in these months.

Diagnosed patients

Newborn screening

Between 2007 and 2015, a total of 28 individuals (out of 1.637.733 screened newborn infants) were TP cases identified by NBS, of whom three were already on a galactose-restricted diet because of a previously diagnosed sibling with CG. Three other patients, also on a diet because of a sibling with CG, were not detected by NBS due to TGAL values below the screening COV. These latter patients are not regarded as false-negative (FN). When taking into account all 31 patients identified with CG, the incidence of CG in the Netherlands is estimated to be 1:52.800. From this total cohort of 31, 25 are clinically considered to have the typical form of CG, based on the criteria defined in the Methods (section 2.3), while six children (three from one family, four families in total) demonstrated a previously unreported biochemical and clinical phenotype and genotype. Patient characteristics are reported in Table 2. These children are referred to as atypical CG patients, and are described in detail in the Results (section 3.3). No FN screens have been reported since the start in 2007 in the DDRMD or in the Dutch newborn screening advisory board. Excluding the six patients with atypical CG (with a previously unreported phenotype/genotype), the incidence of typical CG is estimated at 1:65.500. The median age at referral for a positive screening result was six days (range 3 to 10 days). The 25 infants on a galactose-containing diet at time of screening, demonstrated TGAL values of ≥2413 µmol/l blood. The six infants who were on a galactose-restricted

diet from birth demonstrated TGAL values ranging from 35 to 1464 µmol/l blood. GALT activity measured in DBS ranged from 0% to 14% in the period January 2007 to June 2014, and from July 2014 and onwards was either 0.08 U/dL or ≤0.08 U/dL in all TP cases.

Treatment
Start of dietary treatment was at a median age of six days (range zero to ten days) for the 25 infants who were exposed to galactose after birth. In these children, dietary treatment was started immediately upon NBS referral in the symptomatic children, and after confirmation of the diagnosis in all children without symptoms.

Patient outcomes and detection of previously unreported phenotypes
In the cohort of 31 individuals treated as having CG, six individuals had a previously unreported clinical and biochemical phenotype and genotype. These six individuals are from four families. One family first had twins diagnosed with CG after referral from NBS, and had another child with the same phenotype/genotype a few years later. This child was treated from birth. Because these three children demonstrated the same genotype, as well as the same biochemical phenotype, they will be assessed as one case for our analyses. Therefore, four separate cases, now in a cohort of 29 patients, all initially diagnosed as, and subsequently treated as having typical CG, were identified to have a previously unreported clinical and biochemical phenotype and genotype (14%), and are now labelled as atypical CG for the following reasons.
At time of diagnosis none of these four cases demonstrated CG related illness, in spite of the fact that they were on a galactose-containing diet until confirmation of the diagnosis (day six to ten). In contrast, of the 20 patients considered to have typical CG (and who were on a galactose-containing diet at time of referral from NBS), all but one patient demonstrated CG related illness in the neonatal period (feeding difficulties, weight loss, sepsis or sepsis like symptoms, decreased alertness and liver disease). This one non-symptomatic patient was demonstrated to be heterozygous for a p.S135L mutation, known to cause a milder phenotype. In all but one of them hospitalization was required. One of the 19 symptomatic typical CG patients was diagnosed with *E. coli* sepsis (positive blood culture). The mortality rate was 0%.
Furthermore, TGAL levels at NBS, and Gal-1-P levels in the first weeks of life (measured in 3/4 cases) were significantly raised in the children with atypical CG, comparable to typical CG patients. However, during follow-up, a very rapid decrease of Gal-1-P values was detected in all after initiation of treatment,

Table 2. Biochemical and genetic characteristics of all patients identified with newborn screening

Patient	Start diet (day)	TGAL screening*	GALT activity screening	Symptoms of CG at diagnosis?	GALT activity RBC (confirmation)	GALT variation 1	GALT variation 2	GALT variation 3
1#	6	>3000	8%	No	7,8%	c.563A>G/p.Gln188Arg	c.656T>A/p.Met219Lys	
2	5	>3000	6%	Yes	2,7%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
3#	8	2413	13%	No	9%	c.382G>A/p.Val128Ile	c.382G>A/p.Val128Ile	
4#	8	>3000	12%	No	9%	c.382G>A/p.Val128Ile	c.382G>A/p.Val128Ile	
5#	10	>3000	12%	No	3,6%	c.563A>G/p.Gln188Arg	c.1-96T>G	
6	0	1464	5%	No	<1.2%	c.443G>A/R148Q	c.947G>A/W316X	
7	7	>3000	N A	Yes	0,6%	c.563A>G/p.Gln188Arg	c.855G>T/K285N	
8	0	959	2%	No	0%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
9	5	>3000	<0,8 U/dL	Yes	<3,3%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
10#	8	>3000	0,8 U/dL	No	3,6%	c.563A>G/p.Gln188Arg	c.590A>G/p.Asp197Gly	
11	7	>3000	1%	Yes	0%	N/A	N/A	
12	6	>3000	9%	Yes	0%	c.563A>G/p.Gln188Arg	c.584T>C/p.L195P	
13	9	>3000	6%	Yes	N/A	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
14	5	>3000	5%	Yes	0,8%	N/A	N/A	
15	8	>3000	6%	Yes	0,2%	N/A	N/A	
16	5	>3000	2%	Yes	0,2%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
17	6	2700	6%	Yes	0%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
18	6	>3000	5%	Yes	4,8%	N/A	N/A	
19[3]	4	>3000	4%	Yes	1,4% and 3.9%	c.652C>T/p.Leu218Leu	c.940A>G/p.Asn314Asp	c.1018G>A/p.Glu340Lys
20	6	>3000	10%	Yes	N/A	c.563A>G/p.Gln188Arg	c.1140A>C/p.X380C	
21	5	>3000	0,8 U/dL	Yes	0,7%	c.996-997del	c.1140A>C/p.X380C	

Chapter 2

Patient	Start diet (day)	TGAL screening*	GALT activity screening	Symptoms of CG at diagnosis?	GALT activity RBC (confirmation)	GALT variation 1	GALT variation 2	GALT variation 3
22	4	>3000	9%	Yes	1,2%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
23[1]	6	>3000	10%	Yes	0,5%	c.563A>G/p.Gln188Arg	c.443G>A/p.R148Q	
24[1]	0	705	11%	No	7,5%	N/A	N/A	
25	6	>3000	8%	Yes	1,9%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
26[2]	7	>3000	14%	No	0%	c.404C>T/p.Ser135Leu	c.1138T>C/p.X380ArgextX50	
27	0	522	6%	No	0,7%	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
28	7	>3000	7%	Yes	2,8%	N/A	N/A	
29	7	>3000	0%	Yes	N/A	c.563A>G/p.Gln188Arg	c.563A>G/p.Gln188Arg	
30[2]	0	328	0,08 U/dL	No	N/A	c.404C>T/p.Ser135Leu	c.1138T>C/p.X380ArgextX50	
31[#]	0	35	<0,08 U/dL	No	9,3%	c.382G>A/p.Val128Ile	c.382G>A/p.Val128Ile	

Gal-1-P: galactose-1-phosphate, GALT: galactose-1-phosphate uridyltransferase, NBS: newborn screening, RBC: red blood cell, TGAL: total galactose

N/A: not available
*: μmol/l blood
#: patient with previously unreported genotype and/or clinical and biochemical phenotype
[1]: Patients 23 and 24 are siblings
[2]: Patients 26 and 30 are siblings
[3]: Patient 19: this patient has two variations in the *GALT* gene known as the Los Angeles variant (c.652C>T/p.Leu218Leu plus c.940A>G/p.Asn314Asp), which is associated with superactivity of galactose-1-phosphate uridyltransferase. One known pathogenic variation could be demonstrated (c.1018G>A/p.Glu340Lys), but a second pathogenic variation was not found on repeat testing. Because a very low residual GALT enzyme activity was demonstrated upon repeat testing (1,4% and 3,9% of healthy controls respectively), the patient could be included in our study.

reaching values below the detection limit (defined as <0.05 µmol gram/Hb) before the age of 9 months, comparable to healthy individuals. In contrast, Gal-1-P levels in typical CG patients remained significantly elevated in spite of treatment. Finally, none of the four children with atypical CG demonstrated cataract in the neonatal period. In contrast, six of the 16 typical CG patients (exposed to galactose until diagnosis) who were evaluated, demonstrated cataract. Cataracts were minimal in all cases, not affecting vision, and completely regressed in all within 3 to 14 months after start of diet. The RBC enzyme activities in the six patients with atypical CG were 3.6%, 3.6%, 7.8%, and, in the three siblings 9%, 9%, and 9.3%. Most CG patients demonstrate absent or barely detectable GALT enzyme activities. The three siblings with atypical CG were homozygous for the c.382G>A/p.Val128Ile variation, which has not been reported in the literature before. The other patients were heterozygous for c.563A>G/p.Gln188Arg plus a previously unreported variation (c.656T>A/p.Met219Lys, c.590A>G/p.Asp197Gly and c.1-96T>G respectively).

Discussion

We evaluated the effectiveness of NBS in the Netherlands, also reporting on the identification of patients with atypical presentation and follow-up, designated as atypical CG.

Our study demonstrates that screening for CG, with every screening method reported in this study, and when performed within 72 to 168 h after birth, has the benefit of preventing critical illness in the majority of patients. Most patients in our study demonstrated symptoms of CG at time of referral from screening, but only one patient suffered from severe illness with *E. coli* sepsis. The mortality rate over the period of nine years was 0%. One of the risks of any NBS program is the occurrence of FP cases, which can have significant psychosocial impact. All NBS methods used in the Netherlands resulted in a high FP rate, which has also been reported by others [11]. Therefore, there is a need to further improve the effectiveness of the screening method. Indeed, multiple changes to the methods and COV's were introduced in the Dutch NBS program for CG in order to improve effectiveness. The current screening method consists of a two-tier system with GALT as the primary marker (COV ≤2.0 U/dl blood) and TGAL (COV to ≥1100 µmol/l). As no TP cases have been identified when using this method, the true effectiveness of this screening method cannot be determined. Over the past years, three to four TP's per year were detected. Applying these numbers, the estimated PPV of the current screening method is 3.2% to 6.3%. In five studies providing data on effectiveness of screening tests, the percentage of FP cases ranged from 0.0005% to 0.25% (average 0.05%), and the PPV from 0.9% to 64.3% (average 8.1)[11]. The highest effectiveness was achieved in Sweden (PPV 64.3%), using a two-tier system comparable to the Netherlands with GALT as the primary marker (COV ≤15%) and TGAL as the secondary marker, but with separate COV's for galactose and Gal-1-P [7]. Further improvement of effectiveness of NBS for CG in the Netherlands may be reached by increasing the COV for TGAL, as all diagnosed CG patients (on a galactose-containing diet) had TGAL values ≥2413 µmol/l, which is much higher than the current COV of ≥1100 µmol/l blood.

FP cases from the current screening method most likely are explained by a relatively low residual GALT enzyme activity due to the Duarte variant or heterozygosity for CG, or abnormal results originating from exogenous factors affecting the enzymatic measurements in DBS. According to current guidelines, there is no need to treat and follow up individuals with the Duarte variant, as these variants are not considered pathogenic [4]. Raising the COV for TGAL may help to reduce the number of this type of FP cases. No FN cases have been identified in the Netherlands since the introduction of NBS. However, children with CG who are on parenteral nutrition,

or a soy based formula, in the period before screening, may be missed due to the absence of exogenous galactose intake. Indeed, three of the six CG patients in our cohort, who were already on a galactose restricted diet because of a sibling with CG, were found negative in NBS (patients 27, 30 and 31 in Table 2).

While a number of clinical and genetic variants are well-known in galactosemia (Duarte variant, p.S135L variation), it was remarkable that NBS resulted in the detection of a relatively high number of individuals with a previously unreported biochemical and clinical phenotype (14% of total cohort). These children did not demonstrate clinical symptoms at the time of referral, had undetectable Gal-1-P levels when on dietary treatment, had higher residual GALT enzyme activities, and some demonstrated previously unreported *GALT* variations. These patients with atypical CG may have a higher residual galactose-oxidation capacity compared to the typical CG patients. These children may benefit from a more relaxed diet, as galactose over-restriction may be as harmful in some as a high galactose intake [17]. Current enzymatic assays are not designed to accurately assess residual GALT activity in the low range. Therefore, there is an urgent need for new methods for individualized prognostication and individualized treatment.
A limitation of our study is the relatively short follow-up period, and the fact that clinical data were collected in retrospect. Prospective studies, evaluating long-term clinical outcome in patients with CG identified through NBS, are of major importance [11].

Conclusions

Critical illness and death in patients with CG were prevented with all the used screening methods and COVs, when screening was performed within 72 to 168 hours after birth. The positive predictive value of NBS for CG in the Netherlands can be further improved by increasing the COV for TGAL, as all true positive cases had TGAL values much higher than the current COV. A substantial cohort of patients (14%) detected through NBS demonstrated previously unreported biochemical and clinical phenotypes. This results in a need for individualized prognostication and individualized treatment, as galactose over-restriction may be as harmful in some as a high galactose intake. Prospective studies, evaluating long-term clinical outcome of patients identified with NBS, are warranted.

References

1. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis 2006;29:516–25.

2. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis 2012;35:279–86.

3. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis 2013;36:21–7.

4. Welling L, Bernstein LE, Berry GT, Burlina AB, Eyskens F, Gautschi M, et al. International clinical guideline for the management of classical galactosemia: diagnosis, treatment, and follow-up. J Inherit Metab Dis 2017;40:171–6.

5. Fridovich-Keil JL, Gambello M, Singh R, Sharer J. Duarte Variant Galactosemia. Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K, Ed GeneReviews® [Internet] Seattle Univ Washington, Seattle; 1993-2015 2014;

6. Schweitzer-Krantz S. Early diagnosis of inherited metabolic disorders towards improving outcome: the controversial issue of galactosaemia. Eur J Pediatr 2003;162 Suppl:S50-3.

7. Ohlsson A, Guthenberg C, von Döbeln U. Galactosemia screening with low false-positive recall rate: the Swedish experience. JIMD Rep 2012;2:113–7.

8. Viggiano E, Marabotti A, Burlina AP, Cazzorla C, D'Apice MR, Giordano L, et al. Clinical and molecular spectra in galactosemic patients from neonatal screening in northeastern Italy: structural and functional characterization of new variations in the galactose-1-phosphate uridyltransferase (GALT) gene. Gene 2015;559:112–8.

9. Jumbo-Lucioni PP, Garber K, Kiel J, Baric I, Berry GT, Bosch A, et al. Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes. J Inherit Metab Dis 2012;35:1037–49.

10. Hewlett J, Waisbren SE. A review of the psychosocial effects of false-positive results on parents and current communication practices in newborn screening. J Inherit Metab Dis 2006;29:677–82.

11. Varela-Lema L, Paz-Valinas L, Atienza-Merino G, Zubizarreta-Alberdi R, Villares RV, López-García M. Appropriateness of newborn screening for classic galactosaemia: a systematic review. J Inherit Metab Dis 2016;1–17.

12. Kemper EA, Boelen A, Bosch AM, van Veen-Sijne M, van Rijswijk CN, Bouva MJ, et al. Establishing New Cut-Off Limits for Galactose 1-Phosphate-Uridyltransferase Deficiency for the Dutch Newborn Screening Programme. JIMD Rep 2016;

13. Nennstiel-Ratzel U, Luders A, Blankenstein O, Ceglarek U, Ensenauer R, Fusch C, et al. National screening report Germany 2012. Dtsch Gesellschaft fur Neugeborenenscreeening e V 2012;

14. Item C, Hagerty BP, Muhl A, Greber-Platzer S, Stockler-Ipsiroglu S, Strobl W. Mutations at the galactose-1-p-uridyltransferase gene in infants with a positive galactosemia newborn screening test. Pediatr Res 2002;51:511–6.

15. Lund AM, Hougaard DM, Simonsen H, Andresen BS, Christensen M, Dunø M, et al. Biochemical screening of 504,049 newborns in Denmark, the Faroe Islands and Greenland--experience

and development of a routine program for expanded newborn screening. Mol Genet Metab 2012;107:281–93.

16. Couce ML, Castiñeiras DE, Bóveda MD, Baña A, Cocho JA, Iglesias AJ, et al. Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme. Mol Genet Metab 2011;104:470–5.

17. Knerr I, Coss KP, Kratzsch J, Crushell E, Clark A, Doran P, et al. Effects of temporary low-dose galactose supplements in children aged 5-12 y with classical galactosemia: a pilot study. Pediatr Res 2015;78:272–9.

3

Profiling of intracellular metabolites produced from galactose and its potential for galactosemia research

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Abstract

Introduction Clinical outcome of patients with a classical presentation of galactosemia (classical patients) varies substantially, even between patients with the same genotype. With current biomarkers, it is not possible to predict clinical outcome early in life. The aim of this study was to develop a method to provide more insight into whole-cell galactose metabolism, which allows quantitative assessment of residual galactose metabolism in galactosemia patients. We therefore performed: (1) radioisotope labeled galactose oxidation measurements in fibroblasts of classical patients and patients with a variant presentation of galactosemia (variant patients), and (2) developed a method for galactose metabolite profiling (GMP) in fibroblasts using [U-^{13}C]-labeled galactose.

Methods Galactose oxidation measurements and GMP were performed in fibroblasts of three classical patients, three variant patients and three healthy controls. The following metabolites were analyzed: radioactive $^{14}CO_2$ release for the oxidation measurements, and [U13C]-galactose, [U13C]-galactose-1-phosphate (Gal-1-P) and [$^{13}C_6$]-UDP-galactose for GMP. The ratio of [U^{13}C]-Gal-1-P/ [$^{13}C_6$]-UDP-galactose was defined as the galactose index (GI).

Results Galactose oxidation in fibroblasts of classical patients was impaired, but could not discriminate variant patients from classical patients. With GMP, variant patients had lower levels of [U^{13}C]-galactose and [U^{13}C]-Gal-1-P (though substantially higher than healthy controls) and higher levels of [$^{13}C_6$]-UDP-galactose (though substantially lower than healthy controls). Classical patients had a higher GI than variant patients and classical patients had a higher GI than healthy controls.

Conclusion Both methods were able to discriminate classical patients from variant patients, but with GMP, variant patients could also be discriminated from healthy controls. GMP may therefore be a useful method for early prognostication after further validation in a larger cohort of patients representing the full phenotypic spectrum of galactosemia.

Introduction

Deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12) causes galactosemia (OMIM # 230400), an autosomal recessive inborn error of galactose metabolism. Despite early start of and good compliance with the galactose-restricted diet, which is the only available treatment, a substantial percentage of galactosemia patients suffers from burdensome complications, including decreased cognitive abilities, other neurological complications and primary ovarian insufficiency in females. The mechanisms of disease are not fully understood yet. In the normal situation, GALT facilitates the conversion of galactose-1-phosphate (Gal-1-P) and UDP-glucose to glucose-1-phosphate and UDP-galactose (Figure 1). Highly elevated levels of Gal-1-P in the fetus and newborn infant with galactosemia and persistently elevated Gal-1-P levels in patients even on dietary treatment (due to significant endogenous galactose production by the human body), are thought to play an important role in the pathophysiology. Elevated Gal-1-P levels have been demonstrated to competitively inhibit many metabolic pathways including glycosylation of proteins and lipids [1–3]. As UDP-sugars are essential in the biosynthesis of glycoproteins and glycolipids [4], a disturbed balance in UDP-sugars may also contribute to the glycosylation defects demonstrated in galactosemia [5]. The clinical outcome spectrum in galactosemia is highly variable, even in siblings with identical mutations and erythrocyte enzyme activities and ranges from fully normal to severely impaired development, which is poorly understood [1,6–8]. At this time, it is impossible to predict clinical outcome at the time of diagnosis based on the available biochemical, enzymatic and genetic data. Prognostic uncertainty is a major burden on parents and patients, especially since unnecessary treatment may occur and galactose over-restriction may even be harmful in some patients [5]. This is especially relevant since the extended newborn screening program (NBS) in the Netherlands has resulted in identifying individuals with remarkable differences in biochemical and clinical phenotypes compared to patients with a classical presentation of galactosemia (from here called `classical patients')[12]. These children did not demonstrate any symptoms of galactosemia at the time of referral and demonstrated an unusually rapid decrease of their highly-elevated levels of Gal-1-P after initiation of a galactose-restricted diet. Some but not all patients had a higher residual erythrocyte GALT enzyme activity (up to 9% of healthy controls) than patients diagnosed before NBS and some had previously unreported genotypes. These patients are referred to as having a variant (clinical and biochemical) presentation of galactosemia and are hypothesized to have a higher residual capacity to metabolize galactose (from here on called 'variant patients')(Welling et al 2017). Furthermore, even in classical patients differences in galactose tolerance have been reported and in some

patients improved glycosylation with a somewhat increased galactose intake (using the IgG galactosylation marker) was observed [9]. Thus, individualized prognostication is highly relevant and should ideally be followed by individualized treatment which may improve outcome.

While at diagnosis the severity of GALT deficiency is determined by measurement of GALT activity in erythrocytes, the available enzyme assays have as limitation that they do not provide information on the effect of the enzyme deficiency on overall metabolism of galactose. In this respect, it is important to mention that the *in* vitro activity of GALT is determined under highly unphysiological conditions including saturated concentrations of the two substrates Gal-1-P and UDP-glucose. The highly variable clinical outcome spectrum may be a consequence of differences in the residual capacity to metabolize galactose in other tissues than the erythrocyte. In another inborn error of metabolism, very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, fatty acid oxidation flux in fibroblasts was demonstrated to correlate much better with clinical severity than the enzyme activity in lymphocytes [10]. Galactose oxidation measurements and galactose metabolites profiling (GMP) in fibroblasts may provide new insights into whole-cell galactose metabolism, and maybe predictive of clinical outcome and dietary galactose tolerance in galactosemia.

In this study, we developed a method which provides information on whole-cell galactose metabolism, by quantifying the intracellular levels of the metabolites generated from galactose including Gal-1-P and UDP-galactose. In fibroblasts of classical patients and of patients with a variant presentation we performed galactose oxidation measurements using radioactive labeled [$U^{14}C$]-Gal-1-P and we developed a method for GMP using stable labeled [$U^{13}C$]-galactose.
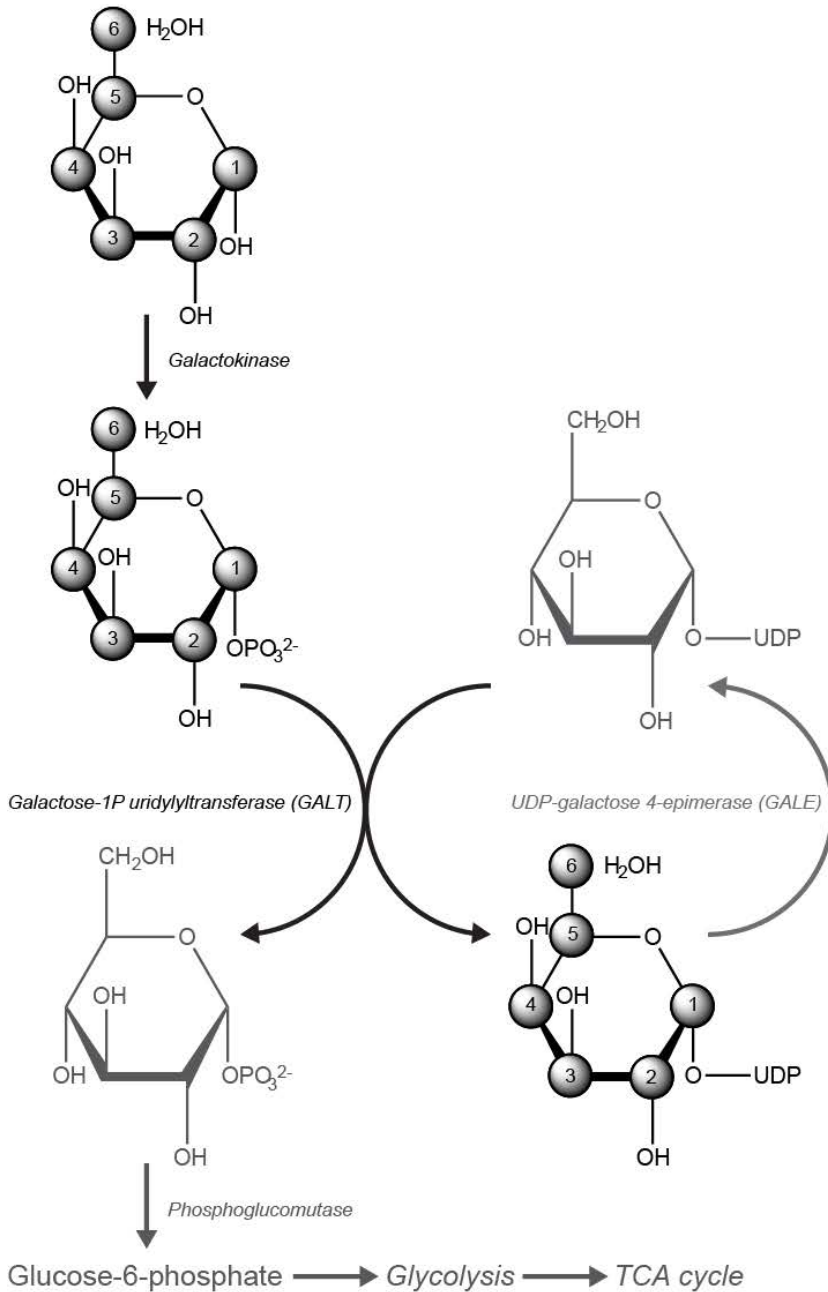
Figure 1. Schematic overview of galactose metabolism
Galactose (1) is converted by galactokinase (GALK) to Gal-1-P (2) which is subsequently converted to UDP-galactose (3) by galactose-1-phosphate uridyltransferase (GALT) using UDP-glucose (4) as donor for the UDP and receptor for the phosphate to produce glucose-1-phosphate (5). Produced glucose-1-phosphate can enter glycolysis via phosphoglucomutase to glucose-6-phosphate and subsequently enter the TCA cycle. UDP-galactose (3) can be converted by UDP-galactose-4-epimerase (GALE) to UDP-glucose (4). The numbered carbons are used in the galactose index (GI) measurements.

Methods and materials

Patients
Galactose oxidation measurements and GMP were performed in fibroblasts of: three classical patients who were diagnosed based on clinical presentation, three variant patients not demonstrating symptoms at the time of diagnosis by NBS, and three healthy controls. All fibroblasts had been taken previously for reasons of patient care and all patients (and/or parents) had provided informed consent for the use of these fibroblasts in further studies. This study was submitted to the Medical Ethical Review Board of the Academic Medical Center in Amsterdam, who declared that ethical approval was not necessary because the Medical Research Involving Human Subjects Act (WMO) is not applicable for this study.

GALT enzyme activity and genetic analysis
Measurement of residual erythrocyte GALT enzyme activity and genetic analysis had been performed in all patients as part of the diagnostic work-up. Measurement of residual GALT enzyme activity in fibroblasts, essentially performed as described by Shin-Buehring [11], was performed as part of the diagnostic work-up in variant patients and for research purposes in the included classical patients.

Whole cell galactose metabolism and outcome measures
In fibroblasts, galactose is converted by galactokinase (GALK) to Gal-1-P which is subsequently converted by GALT to UDP-galactose, using UDP-glucose as donor for the UDP group and as acceptor for the phosphate group. UDP-galactose can be further converted via UDP-galactose-4-epimerase (GALE) to UDP-glucose which subsequently is converted via GALT to glucose-1-phosphate. Phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate which enters glycolysis and eventually the tricarboxylic acid (TCA) cycle (Figure 1). For the radioactive ^{14}C-labeled galactose oxidation measurements the $^{14}CO_2$ produced by the TCA cycle was used as output for galactose oxidation. For the stable ^{13}C-labeled GMP measurements, the intermediate metabolites of galactose metabolism, including galactose, Gal-1-P and UDP-galactose were measured as output for GMP.

Cell culture procedure
Human fibroblasts were cultured at 37°C under 5% CO_2 in HAM F10 supplemented with 10% FCS and 100 µg/mL of penicillin/streptomycin. For the experiment, 50-100 µg of cells were plated to 6 wells plates. After 24h the medium was removed and cells were starved for 16h using Dulbecco's PBS without any extra additions. After starvation, the cells were used for ^{14}C-labeled galactose oxidation or ^{13}C-labeled GMP analysis.

Radioactive [^{14}C]-labeled galactose oxidation measurements

For the galactose oxidation measurements, [U^{14}C]-Gal-1-P (Perkin Elmer, NEC 579) was used as substrate. For [U^{14}C]-Gal-1-P to enter the cell it had to be converted to [U^{14}C]-galactose first using alkaline phosphatase. Therefore, alkaline phosphatase was added to the incubation mixture. Specifically, after 16h of starvation, cells of healthy controls, classical patients and variant patients were incubated for 8h with 12 μl of [U^{14}C]-Gal-1-P (276 mCi/mmol; ~2 μM), 0.5 μl alkaline phosphatase (~42 U/ml) and 488 μl Dulbecco's PBS at 37°C. Reaction was stopped using 0.1 ml perchloric acid (2.6 M) causing radioactive $^{14}CO_2$ release. The released $^{14}CO_2$ was then captured in 0.5 ml NaOH (2 M) and counted on a scintillation beta counter.

Stable isotope [^{13}C]-labeled galactose metabolites profiling (GMP)

We developed a GMP analysis using [U^{13}C]-galactose as substrate in combination with mass spectrometry, allowing determination of the endogenous levels of glucose, galactose and their downstream metabolites from the [U^{13}C]-galactose by mass. In fibroblasts, [U^{13}C]-galactose is converted by galactokinase (GALK) to [U^{13}C]-Gal-1-P which is subsequently converted by GALT to [$^{13}C_6$]-UDP-galactose using UDP-glucose as donor for the UDP group, and as acceptor for the phosphate group (Figure 1). After 16h of starvation, cells of healthy controls, classical patients and variant patients were incubated with 1 mM of U^{13}C-galactose for 1h, 2h, 4h or 7h (Figure 3a). Cells were washed three times with ice-cold saline solution (0.9%; w/v). Metabolism was quenched by adding 1 mL ice-cold methanol followed by 1 mL ice-cold water. For the extraction of metabolites, the 6 well plates were placed in a sonication bath and sonicated for 15 minutes. The homogenate was transferred to a 2 mL tube and after addition of 1 mL of chloroform, the homogenate was vortexed and centrifuged for 5 minutes at 14.000 rpm at 4°C. The "polar" top layer was transferred to a new 1.5 mL tube and dried in a vacuum concentrator. Dried samples were dissolved in 100 μL methanol/water (6/4; v/v). The following metabolites were determined: [U^{13}C]-galactose, [U^{13}C]-Gal-1-P and [$^{13}C_6$]-UDP-galactose. The galactose index (GI) was defined as the ratio of [U^{13}C]-Gal-1-P/[$^{13}C_6$]-UDP-galactose. For the analysis, we used a Thermo Scientific ultra-high-pressure liquid chromatography system (Waltman, MA, USA) coupled to a Thermo Q Exactive (Plus) Orbitrap mass spectrometer (Waltman, MA, USA). The autosampler was held at 10°C during the runs and 5 μL of sample was injected on the analytical column. The chromatographic separation was established using a SeQuant ZIC-cHILIC column (PEEK 100 x 2.1mm, 3.0μm particle size, Merck, Darmstadt, Germany) and kept at 15°C. The flow rate was 0.250 mL/min. The mobile phase was composed of (A) 9/1 acetonitrile/water with 5 mM ammonium acetate; pH 6.8 and (B) 1/9 acetonitrile/water with 5 mM ammonium acetate; pH 6.8, respectively. The LC gradient program was: beginning with 100% (A) hold 0-3 min; ramping 3-20 min to 36% (A); ramping from

20-24 min to 20% (A); hold from 24-27 min at 20% (A); ramping from 27-28 min to 100% (A); and re-equilibrate from 28-35 min with 100% (A). The MS data were acquired at full scan range, 140.000 resolution and in negative ionization mode. Interpretation of the data was performed in the Xcalibur software (Thermo scientific, Waltman, MA, USA).

Statistical analysis
Statistical analysis was performed with Prism 7.02 (GraphPad, San Diego, CA, USA). Data are expressed as the means ± SD. Differences were evaluated with the one-way ANOVA multiple-comparisons test. When significant, the post hoc Bonferroni multiple-comparisons test was used to test differences between groups for significance. Statistical significance is indicated as detailed in the figure legends; p-values of ≤ 0.05 were considered significant. Isotope labeling correction was calculated using Mathworks Matlab (Natick, US).

Results

Patients

The three classical patients were homozygous for the c.563A>G (p.Gln188Arg) mutation. Residual GALT enzyme activities in erythrocytes were below the limit of quantification of the enzyme assay for all three patients. The three variant patients had genotypes which were reported in our recent study [12] and had residual GALT enzyme activities in erythrocytes ranging from 4% to 9% of the mean of the reference values (Table 1).

Table 1. Genetic and biochemical characteristics of classical patients and variant patients

Patient	Genotype	Residual GALT enzyme activity in erythrocytes in μmol/(h.gram Hb) (reference value or range)	Residual GALT enzyme activity in fibroblasts in μmol/(h.mg protein) (reference range)
1 Classical	c.563A>G (p.Gln188Arg)/ c.563A>G (p.Gln188Arg)	<0.5 (18-28)	<0.01 (0.15-0.34)
2 Classical	c.563A>G (p.Gln188Arg)/ c.563A>G (p.Gln188Arg)	<0.5 (18-28)	<0.01 (0.15-0.34)
3 Classical	c.563A>G (p.Gln188Arg)/ c.563A>G (p.Gln188Arg)	0.25 (19.4) 0.36 (20.6)	<0.01 (0.15-0.34)
4 Variant	c.563A>G (p.Gln188Arg)/ c.1-96T>G	1.2 (21.8-44.9)	<0.01 (0.15-0.34)
5 Variant	c.563A>G (p.Gln188Arg)/ c.656T>A (p.Met219Lys)	2.4 (21.8-44.9)	0.04 (0.15-0.34)
6 Variant	c.382G>A (p.Val128Ile)/ c.382G>A (p.Val128Ile)	3.1 (21.8-44.9)	0.01 (0.15-0.34)

Radioactive [^{14}C]-labeled galactose oxidation measurements

The radioactive [^{14}C]-labeled galactose oxidation measurement was performed to investigate if there is a difference in overall galactose oxidation between classical patients, variant patients and healthy controls. The galactose oxidation capacity was markedly reduced in classical patients and was significantly different from patients with a variant presentation and healthy controls (Figure 2). The galactose oxidation capacities in fibroblasts of variant patients were not deficient and were in fact higher compared to healthy controls, at least in two of the three variant patients. This were two independent experiments where each circle in the graph represents the mean of two individual measurements for the respective patient's cells. Multiple control cell lines were used for the measurements (Figure 2).

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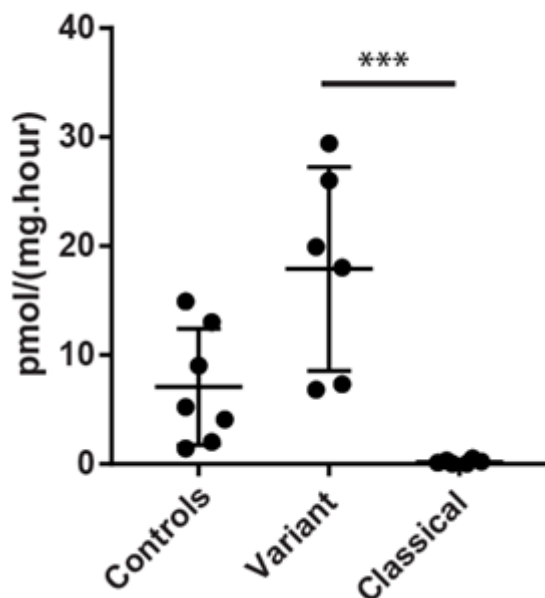
Figure 2. [14C]-galactose oxidation analysis
$^{14}CO_2$ produced from [^{14}C]-galactose prepared from [^{14}C]-Gal-1-P was measured in two independent experiments for classical patients, variant patients and healthy control after 6h. Significance is as follows: The mean of variant patients vs the mean of classical patients ***$p < 0.001$.

Stable [^{13}C]-labeled galactose metabolism profiling (GMP)

To obtain more insight in galactose metabolism and to determine whether there is a difference in galactose metabolism between classical patients, variant patients and healthy controls we performed GMP measurements (Figure 3). When comparing the three groups, U^{13}C-galactose and [U^{13}C]-Gal-1-P were increased in fibroblasts of classical patients and variant patients, whereas, [^{13}C$_6$]-UDP-galactose was decreased in these patients. This increase in [U^{13}C]-Gal-1-P and decrease in [^{13}C$_6$]-UDP-galactose was time dependent (Figure 3a). The GI, defined as the ratio of [U^{13}C]-Gal-1-P/ [^{13}C$_6$]-UDP-galactose, was determined for all groups and was increased in all patients compared to the healthy controls but was significantly higher in classical patients ($p < 0.001$) than in variant patients (10 fold change, one-way ANOVA multiple-comparisons test not significant)(Figure 3c and 3d). These results show that the GI can be used as measure of the severity of the GALT deficiency and residual galactose metabolism (Figure 3c and 3d). The difference in GI between the different groups were most prominent for the shorter incubation times (Figure 3a), however, the variations between the different measurements was higher at these time points and therefore 7 hours was chosen as optimal incubation time.
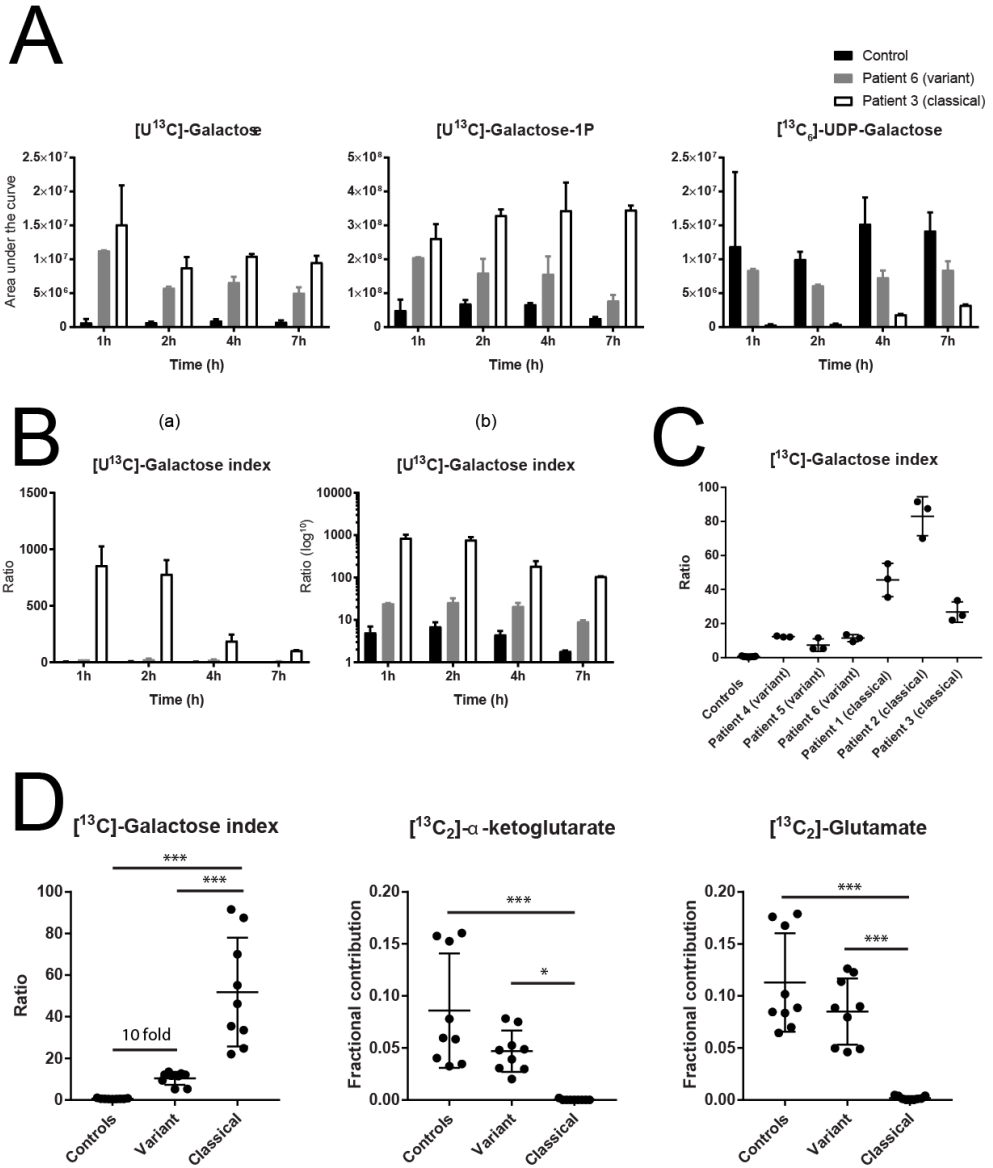
Figure 3. Stable 13C-labeled galactose metabolism profiling (GMP) analysis
(A) Fibroblast metabolites of galactose metabolism incubated on different time points with [U^{13}C]-galactose measured with mass spectrometry. (B) [U^{13}C]-galactose index (GI) with Y-axis linear (a) and log10 (b). (C) The galactose index, measured after 7h of incubation in cells of classical patients, variant patients and healthy controls. (D) Mean of the GI and downstream [^{13}C]-labeled metabolites after 7h incubation with [U^{13}C]-galactose in cells of classical patients, variant patients and healthy controls. Significance is as follows: controls vs the mean of classical patients ***$p<0.001$ and the mean of variant patients vs the mean of classical patients *$p<0.05$, ***$p<0.001$.

To establish whether galactose oxidation in fibroblasts from classical patients is truly fully impaired we also studied the incorporation of [^{13}C]-labeled in other downstream metabolites including ^{13}C-labeled alpha-ketoglutarate and glutamate (Figure 3d). In fibroblasts of classical patients there was no formation of α-keto-glutarate and glutamate from ^{13}C –labeled galactose (p<0.001), suggesting that galactose metabolism is fully blocked in fibroblasts of classical patients. In fibroblasts from variant patients however, α-ketoglutarate and glutamate were formed from [^{13}C]-labeled galactose in comparable amounts to those found in control cells. The latter results are in good agreement with the results of the ^{14}C-galactose oxidation experiments which overall suggests normal flux through the galactose degradation pathway in cells of healthy control and also variant patients.

Discussion

The aim of this study was to develop a method to provide more insight into whole-cell galactose metabolism, which allows quantitative assessment of residual galactose metabolism in galactosemia patients. Radioisotope labeled galactose oxidation measurements (using [U^{14}C]-Gal-1-P as a substrate) were performed and a method for GMP in fibroblasts using [U^{13}C]-galactose as a substrate was developed, followed by metabolite analysis with tandem mass spectrometry. Both methods were studied in patients with a classical presentation and variant presentation of galactosemia.

Our results show that the developed GMP analysis is a sensitive method allowing discrimination of classical patients from variant patients, and the latter from healthy controls. These results indicate that variant patients have a higher residual capacity to metabolize galactose compared to classical patients and that the GI can be used as a measure for the severity of the GALT deficiency and residual galactose metabolism in galactosemia patients. The differences in the GI between the three groups could already be observed after 1h of incubation, though less variability between the different measurements and more significant differences between the groups were seen when measurements were performed at 7h after incubation. For this reason, 7h was chosen as optimal incubation time. Before implementation in the diagnostic workup, the developed GMP analysis in fibroblasts needs further validation in a larger group of galactosemia patients representing the whole clinical outcome spectrum. It is essential to determine if patients with a classical presentation, but with different outcomes, have different profiles of galactose metabolites and if GMP can thus be used as predictor of outcome.

With the current method for GMP, we could not differentiate ^{13}C$_6$-UDP-galactose from ^{13}C$_6$-UDP-glucose and [U^{13}C]-Gal-1-P from [U^{13}C]-glucose-1-phosphate. For the current study this is only a small limitation, as the labeled substrate first has to pass the GALT enzyme before it can be detected either as [^{13}C$_6$]-UDP-galactose, [^{13}C$_6$]-UDP-glucose or [U^{13}C]-glucose-1-phosphate. If the GALT deficiency is more severe, less of the substrate will pass this step and less [^{13}C$_6$]-UDP-galactose and [^{13}C$_6$]-UDP-glucose will be detected, which is clear from the differences between classical patients and variant patients. A next step in future research will be to discriminate [^{13}C$_6$]-UDP-galactose from [^{13}C$_6$]-UDP-glucose and discriminate [U^{13}C]-Gal-1-P from [U^{13}C]-glucose-1-phosphate in this analysis. This may be important because in classical patients, differences in residual capacities to metabolize galactose affecting clinical outcome may be smaller. Furthermore, if [^{13}C$_6$]-UDP-galactose can be separated from [^{13}C$_6$]-UDP-glucose, GALE deficiency can also be studied.

In contrast to GMP, no differences were observed in galactose oxidation measurements between fibroblasts of healthy controls and variant patients, although in

classical patients the galactose oxidation was severely impaired. These findings support the hypothesis that variant patients have a higher residual capacity to metabolize galactose compared to classical patients. The difference in results between the GMP and the galactose oxidation studies can be explained by incorporation of enough label coming from galactose metabolism into the TCA cycle intermediates in variant patients of galactosemia, whereas incorporation of label into the TCA cycle intermediates was completely absent in the classical patients as demonstrated in the GMP analysis (Figure 3d).

In conclusion, GMP in fibroblasts is a sensitive method to determine residual galactose metabolism capacity which can discriminate between patients with a classical presentation of galactosemia, patients with a variant presentation and healthy controls. With galactose oxidation measurement in fibroblasts, classical patients could be discriminated from healthy controls, but the latter not from variant patients. Results of both GMP and galactose oxidation measurements indicate that variant patients have a higher residual capacity to metabolize galactose compared to classical patients. The developed GMP may be good method for early prognostication of individuals with GALT deficiency, though this method should be further validated in a larger group of individuals with several degrees of GALT deficiency representing the full outcome spectrum.

References

1. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis. 2006;29:516–25.

2. Huidekoper HH, Bosch AM, Van Der Crabben SN, Sauerwein HP, Ackermans MT, Wijburg FA. Short-term exogenous galactose supplementation does not influence rate of appearance of galactose in patients with classical galactosemia. Mol Genet Metab. 2005;84:265–72.

3. Berry GT, Nissim I, Lin Z, Mazur AT, Gibson JB, Segal S. Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia. Lancet. 1995;346:1073–4.

4. Prydz K, Dalen KT. Synthesis and sorting of proteoglycans. J Cell Sci. 2000;113 Pt 2:193–205.

5. Knerr I, Coss KP, Kratzsch J, Crushell E, Clark A, Doran P, et al. Effects of temporary low-dose galactose supplements in children aged 5-12 y with classical galactosemia: a pilot study. Pediatr Res. 2015;78:272–9.

6. Hughes J, Ryan S, Lambert D, Geoghegan O, Clark A, Rogers Y, et al. Outcomes of siblings with classical galactosemia. J Pediatr. 2009;154:721–6.

7. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis. 2013;36:21–7.

8. Fridovich-Keil JL, Walter JH. Galactosaemia chapter 72. D. Valle, B. Vogelstein, K.W. Kinzler, S.E. Antonarakis, A. Ballabio (Eds.), et al., The Online Metabolic and Molecular Bases of Inherited Disease, OMMBID, Part 7: Carbohydrates, McGraw Hill, New York (2008)(Chapter 72). 2008;

9. Coss KP, Treacy EP, Cotter EJ, Knerr I, Murray DW, Shin YS, et al. Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism? Mol Genet Metab. 2014;113:177–87.

10. Diekman EF, Ferdinandusse S, van der Pol L, Waterham HR, Ruiter JPN, Ijlst L, et al. Fatty acid oxidation flux predicts the clinical severity of VLCAD deficiency. Genet Med. 2015;17:989–94.

11. Shin-Buehring YS, Stuempfig L, Pouget E, Rahm P, Schaub J. Characterization of galactose-1-phosphate uridyl-transferase and galactokinase in human organs from the fetus and adult. Clin Chim Acta. 1981;112:257–65.

12. Welling L, Boelen A, Derks TGJ, Schielen PCJI, de Vries M, Williams M, et al. Nine years of newborn screening for classical galactosemia in the Netherlands: Effectiveness of screening methods, and identification of patients with previously unreported phenotypes. Mol Genet Metab. 2017;120:223–8.

Chapter 3

PART 2

Management of classical
galactosemia

4

Chapter 4

International clinical guideline for the management of classical galactosemia: diagnosis, treatment and follow-up

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Abstract

Classical galactosemia (CG) is an inborn error of galactose metabolism. Evidence-based guidelines for the treatment and follow-up of CG are currently lacking, and treatment and follow-up have been demonstrated to vary worldwide. To provide patients around the world the same state-of-the-art in care, members of The Galactosemia Network (GalNet) developed an evidence-based and internationally applicable guideline for the diagnosis, treatment and follow-up of CG. The guideline was developed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system. A systematic review of the literature was performed, after key questions were formulated during an initial GalNet meeting. The first author and one of the working group experts conducted data-extraction. All experts were involved in data-extraction. Quality of the body of evidence was evaluated and recommendations were formulated. Whenever possible recommendations were evidence-based, if not they were based on expert opinion. Consensus was reached by multiple conference calls, consensus rounds via e-mail and a final consensus meeting. Recommendations addressing diagnosis, dietary treatment, biochemical monitoring, and follow-up of clinical complications were formulated. For all recommendations but one, full consensus was reached. A 93% consensus was reached on the recommendation addressing age at start of bone density screening. During the development of this guideline, gaps of knowledge were identified in most fields of interest, foremost in the fields of treatment and follow-up.

Introduction

Classical galactosemia (CG, MIM 230400) is an autosomal recessive inborn error of galactose metabolism caused by a profound (absent or barely detectable) deficiency of galactose-1-phosphate-uridyltransferase (GALT; EC 2.7.7.12), which leads to the accumulation of the metabolites galactose-1-phosphate (Gal-1-P), galactitol and galactonate. The human GALT gene maps to chromosome 9p13 (Flanagan 2009). The incidence of CG widely varies worldwide, with an estimated incidence of 1:19,000 to 1:44,000 in Europe (with a higher incidence in the Irish Traveller population) and the USA [1–4]. After the ingestion of galactose from breast milk or infant formulas, affected neonates develop a life-threatening illness with feeding difficulties, liver failure, *E. coli* sepsis and bilateral cataract in the first weeks of life. While the acute symptoms resolve rapidly upon initiation of a lactose-free and galactose-restricted diet, such as a soy-based formula, many patients, irrespective of the severity of the illness in the newborn period [5], suffer from long-term complications such as cognitive deficits, speech and language deficits, neurological abnormalities and hypergonadotropic hypogonadism in females [6–10]. The phenotypic spectrum of the disease is extremely wide, varying from normal development to severe complications affecting independence. It is debated whether these complications are progressive. The disease mechanism is not fully understood. Endogenous production of galactose is significant, causing a persistent elevation of Gal-1-P and galactitol in patients with CG, even on a galactose restricted diet [11–13]. Elevated Gal-1-P levels competitively inhibit several metabolic pathways including those involved in the galactosylation of proteins and lipids [14]. Both Gal-1-P and galactitol levels have a high inter- and intra-personal variability and do not seem to predict outcome, limiting their usefulness for biochemical monitoring [15].

The UK Galactosemia Steering Group established general national recommendations [16], but did so over a decade ago and without a formal assessment of the evidence. No other guidelines, meeting current standards of evidence-based medicine, have been published to date. Treatment and follow-up of CG vary significantly worldwide [17]. To provide patients around the world the same state-of-the-art care, we developed an evidence-based and internationally applicable guideline. This guideline addresses all important topics with regard to diagnosis, treatment and follow-up of CG, and can be used as a reference. The authors have chosen not to address newborn screening (NBS) in this guideline. Additionally, a summary of all recommendations is provided as a supplement for easy use in clinical setting. The target users of this guideline are medical doctors, dieticians, psychologists, speech and language therapists and other multidisciplinary team members involved in care for patients with CG. At this time we propose that this guideline may be applied to all patients with a GALT enzyme activity below 15%. While 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, through newborn screening patients with low but not profoundly deficient GALT enzyme activities up to 15% are detected. Future research is necessary for evidence based advise on treatment in these children.

Methodology

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used to methodologically design and develop this guideline [18].

Guideline participants and key questions
The development of this guideline was initiated by the Galactosemia Network (GalNet). Important topics and problems in the field of diagnosis, treatment, and follow-up of CG were explored. Ten different fields of interest were identified: 1) diagnostics, 2) biochemical follow-up, 3) dietary management, 4) cognitive development, 5) speech and language development, 6) neurological complications, 7) psychosocial development and mental health, 8) endocrinology and fertility, 9) bone health and 10) ophthalmological complications. At the start of the GalNet in 2012, all Society for the Study of Inborn Errors of Metabolism (SSIEM) members were invited to participate in the network. All who expressed their interest in the GalNet were invited to a first meeting in January 2014 in Maastricht (the Netherlands), where key questions in each field of interest were formulated by the experts of the GalNet, in collaboration with representatives of the European Galactosemia Society (patient organization)(Table 1). Experts attending this meeting were invited to participate in guideline development, and a 21-member guideline expert panel was formed. Based on their specialty, experts from this panel participated in working groups focusing on key questions related to their field of interest.

Information sources and search strategy
The first author and an experienced clinical librarian conducted formalized literature searches, using a different search strategy for each set of key questions belonging to a specific field of interest (for example: 'Bone health'). Databases searched included MEDLINE, EMBASE, PsychInfo, Web of Science and Cochrane library, as applicable per set of key questions. No filters were used for the searches. Search strategies are provided in Supplement 1 (via http://www.thesisgalt.nl/).

Eligibility criteria of studies
Study design: Studies with the following design were included: Randomized Controlled Trials (RCT), non-RCT, cohort studies, case-control studies, case series, cross-sectional studies and experimental studies. Case reports and conference abstracts were excluded. Studies in humans and *in vitro* studies with human tissue were included, animal studies were excluded.
Characteristics: Studies published in any year and written in English were included. Studies reported in any other language were excluded. Full-text version of the articles had to be available.

Table 1. Key questions

Field of interest	Key Questions
Diagnostics (recommendations 1, to 3)	• What is the gold standard for the diagnosis of Classical Galactosemia? (enzyme activity and *GALT* gene mutation analysis, is enzyme alone enough, is mutation alone enough?) • Who needs to be treated? (cut-off enzyme activity?)
Diet (recommendations 4 to 7)	• What is the safe amount of dietary galactose (for the different age groups)? • Based on the answer to above question: should fruit/vegetables/mature cheese/offal/legumes be restricted in the diet? • Should the diet be evaluated regularly for deficiencies? Which deficiencies and how frequently?
Biochemical follow-up (recommendations 8 to 11)	• What parameters need to be followed until stabilization in the first year of life and how frequently? • What (if any) parameters need to be followed up after age 1 year? What is the value of the parameters? At what ages, with what frequency?
Developmental follow-up (recommendations 12 to 14)	• Should IQ be tested? If so, how? At what ages? • Should executive functions be tested? If so how? At what ages?
Speech and language (recommendations 15 to 17)	• Should speech and language be evaluated? If so, how? At what ages? • What treatment should be advised in case of speech and language disorders?
Neurology (recommendations 18 to 20)	• Should patients be screened for neurological pathology? (ataxia, tremor) How? What age and frequency? • Should MRI scan be included in the follow-up of patients?
Psychosocial development / Mental health (recommendations 21 to 23)	• Should patients be screened for psychosocial deficits? How? What ages? • Should patients be screened for mental health issues? How? What ages? • Should Quality of Life (QoL) be regularly evaluated?
Endocrinology/Fertility follow-up (recommendations 24 to 33)	• How should girls be screened for endocrine dysfunction, and at what ages? (What markers? Is there a role for ultrasound/MRI?) • When should hormonal supplementation be started? Which supplementation is best? Up to what age? • What should be the endocrine follow-up in females at adult age? • Is there a need for endocrine follow-up in males? • Counselling fertility: what do we say? • Fertility preservation: what do we recommend?
Bone (recommendations 34 to 37)	• Should bone health be assessed? How? From what age? How frequently? • What is the clinical relevance of a decrease of -2SD in bone mass? (later in the process this key question was omitted, because this is a general question not concerning CG) • What is advised treatment for bone mass below -1 SD, bone mass -2SD? • Which bone parameters are relevant for follow-up and treatment assessment?
Ophthalmological complications (recommendations 38 to 40)	• In the newborn period which patients need ophthalmological examination? • Which patients need ophthalmological follow-up? At what age, with what frequency?

Study selection

Titles of the identified articles were screened (by first and last author) and immediately discarded when clearly not on the topic or not meeting the inclusion criteria. Abstracts of the remaining articles were read (by first and last author) and relevant articles meeting the inclusion criteria were included. When necessary the entire article was read (by first author) before deciding to include or exclude the article.

Data-extraction

The first author and one of the working group experts conducted data-extraction (identification of key data elements) per manuscript. All experts were involved in data-extraction for one or multiple key questions. Evidence was summarized per recommendation (see Summary of Evidence Tables, provided in Supplement 2, via http://www.thesisgalt.nl/). Based on this summary, each recommendation was categorized as "supported by evidence" or as "expert opinion". If the recommendation was categorized as 'expert opinion', this was mentioned after the statement.

Critical appraisal and risk of bias assessment

Risk of bias was assessed with the appropriate checklist from SIGN when available. To our knowledge no standardized critical appraisal checklists exist to date for articles with a descriptive study design (case series, cross-sectional studies, experimental studies). Therefore we did not formally assess risk of bias, but did acknowledge the low level of evidence available in these observational, descriptive studies. We recognized in advance that almost all evidence in the field of galactosemia is from descriptive studies. This assumption was confirmed. Thus, the body of evidence in our guideline was uniformly rated as 'low to very low' in terms of the GRADE system. Individual studies were not assigned a level of evidence. Major issues as noted by the investigators were reported in the 'Remarks' section of the Summary of Evidence Table and were taken into account when making recommendations.

Strength of recommendation

The body of evidence for each recommendation was 'low to very low'. Accordingly all recommendations (also the recommendations labeled 'expert opinion') were assigned a 'discretionary' strength of recommendation. Only if highly consistent results were found across multiple studies, and if experts had confidence in these results, was the strength of recommendation upgraded to 'strong'. The strength of recommendation is mentioned after the recommendation: Strong recommendation:++; discretionary recommendation: +. The body of evidence supporting a recommendation is presented in the Summary of Evidence Tables (Supplement 2, via http://www.thesisgalt.nl/). Evidence is summarized per key question or set of

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key questions, and not per recommendation, due to overlap in evidence for multiple key questions. Also, in some cases, multiple recommendations were formulated based on one key question.

Consensus procedures
Experts in speech and language, gynecology, psychology and nutrition participated in separate working groups that developed recommendations and achieved consensus on topics related to their discipline. An 11-person clinical consensus committee comprised of physicians overseeing care of patients with CG (AB, AMB, FE, IK, EPT, EM, GTB, KO, MERG, MG, PL) not only participated in one or more working groups, but also participated in the consensus process of the recommendations of all the other topics. After the recommendations from each working group were completed on specific topics, and the working group members all agreed with the recommendations, the clinical consensus committee reviewed them to identify potential disagreements. The first and last author made minor revisions and incorporated major revisions for review by the specific working group as well as the clinical consensus committee. A third review took place at a final in-person consensus meeting, to which all members of the clinical consensus committee were invited. During this final meeting a mediator guided the sessions. Recommendations that were adapted were sent for approval to all experts of the relevant working groups and to Clinical Consensus Committee members not present during the meeting. All authors endorsed the final manuscript prior to its submission.

External review
This guideline was externally reviewed by two independent experts; a pediatric neurologist and internal medicine specialist for endocrinology and metabolic disorders, both with experience in CG and rare disorders. In addition, representatives of the European Galactosemia Patient Society reviewed the guideline. The goal of this review by independent experts was to improve the quality of the guideline and to assess applicability and feasibility. This external review was undertaken with open-ended questions. The main findings of the reviewers were 1) Lengthy but easy to read manuscript 2) Clear, concise and feasible recommendations 3) Suggestions to improve quality and readability of the text. The suggestions of the reviewers were taken into account by incorporating major revisions to several paragraphs, to shorten the text and improve the quality.

Implementation of this guideline

This guideline is aimed for worldwide adoption and implementation. During the development of the guideline, it was recognized that not all centers would have state-of-the-art facilities or test instruments. Thus, alternatives are provided. All participating experts, the GalNet (www.galactosemianetwork.org/) as well as the European Galactosemia Society (www.galactosaemia.eu/) and the USA Galacto-semia Foundation (www.galactosemia.org/), have agreed to be involved in the implementation of this guideline. A short version of all recommendations, easy to utilize in the clinic, is provided as a supplement (Supplement 3, via http://www.thesisgalt.nl/).

Results

Study selection process
The results of the different search strategies and the results of the study selection processes are presented in Supplement 1 (via http://www.thesisgalt.nl/).

Risk of bias assessment
Only one study was identified for which an appropriate critical appraisal and risk of bias assessment checklist from SIGN was available. This study, a RCT, was scored to be of high quality with low risk of bias [19]. Two studies with a descriptive study design were excluded as evidence [20,21], as determined by the authors, for reasons reported in the Summary of Evidence tables (Supplement 2, via http://www.thesisgalt.nl/).

Consensus procedures
E-mail rounds: A total of 40 recommendations were formulated. After the clinical consensus committee reviewed the recommendations via one or two e-mail rounds, a 100% consensus was reached for all recommendations with regard to dietary management, diagnostics, neurology and speech and language. Less than a full consensus was reached for one recommendation in each of the fields of bone health, developmental follow-up and endocrinology/fertility follow-up, and for two recommendations in each of the fields of biochemical follow-up, cataract and psychosocial development/mental health.

Final consensus meeting: The final consensus meeting took place in October 2015 in Amsterdam, the Netherlands. Nine of eleven clinical consensus committee members attended the meeting. All nine recommendations for which no consensus was reached during the e-mail rounds were discussed, adapted and adopted during the meeting. All members attending the meeting agreed to 8 of these 9 recommendations, and the two members not attending the meeting provided consensus for adoption of all nine recommendations later via e-mail. Experts that were not part of clinical consensus committee, but who were involved in formulating these 9 recommendations, were also contacted afterwards via e-mail and all gave consensus for the recommendations that were adapted during the final consensus meeting (and adopted by the clinical consensus committee members attending this meeting). One member did not provide consensus for one recommendation (#35) with regard to bone health. Therefore a 93% consensus was reached for this particular recommendation.

Recommendations

Diagnosis

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. Untreated patients demonstrate a multi-organ toxicity in the newborn period that is lactose intake- and duration-dependent. Through newborn screening (NBS), patients with low but not profoundly deficient GALT enzyme activities up to 15% are detected who do not demonstrate the p.S135L variation (c.404C>T (p.Ser135Leu)) or Duarte genotypes. Future research is necessary for evidence-based advise on treatment in these children. At this time we propose that this guideline may be applied to all patients with a GALT enzyme activity below 15%.

In some countries, CG patients are identified through NBS, but other countries chose not to include CG in their NBS program due to ongoing uncertainties about the balance between risks and benefits [17]. NBS prevents development of critical illness and death, but it probably does not change frequency of long-term complications. Varela-Lema et al. recently concluded that existing evidence remains insufficient to establish the appropriateness of NBS for CG [22]. NBS is not further addressed in this guideline.

The most commonly used methods to diagnose CG, after clinical suspicion or identification through NBS, are measurement of GALT enzyme activity in red blood cells (RBC), and (confirmation by) *GALT* genetic analysis. Usually GALT activity is expressed as the percentage of the activity of healthy non-carrier controls. In the database of Calderon et al., last updated in January 2013 (www.arup.utah.edu/database/GALT/GALT_welcome.php), 336 different variations had been reported [23]. Only one study reports on the diagnostic process in CG with a combination of GALT enzyme activity measurement and genetic analysis of the most common variations in the *GALT* gene [23]. Detection of genetic variations accorded with enzyme activity measurement in 93% of samples, increasing to 99% after samples with discordant results were fully sequenced. For measurement of GALT enzyme activity most laboratories use radioactive assays, which are laborious and/or are incapable of measuring low enzyme activity [24]. Other methods have been developed, including assays using ultra performance liquid chromatography-tandem mass spectrometry, liquid chromatography-tandem mass spectrometry and reversed-phase high performance liquid chromatography with UV detection [24–28]. Measurement of GALT activity in RBC is unreliable after blood transfusion, and genetic analysis analysis or enzyme activity measurement in lymphocytes should be performed. Supportive diagnostic methods (before the final diagnosis is made) include measurements of total blood galactose, RBC Gal-1-P and/or urinary galactitol.

Recommendation #1 (+)
Clinicians should confirm the diagnosis of CG by the measurement of GALT enzyme activity in red blood cells (absent or significantly decreased), and/or *GALT* gene analysis. It is enough to confirm the diagnosis by genetic analysis only, if the detected variations are reported as disease causing in genetic variation databases (Calderon et al. 2007;
http://www.arup.utah.edu/database/galt/galt_welcome.php) and the biological parents each carry one variation.

Treatment
There is worldwide consensus that patients with the classical form of galactosemia should be treated with a galactose-restricted diet [17].

p.S135L variant
A well-known variant with a GALT activity <15%, is the p.S135L variation. Worldwide patients homozygous for this variation, which is most often seen in people of African descent, are treated with a galactose-restricted diet. Homozygous patients have RBC GALT activities with values between 0.2-1.7% of normal activity, with enzyme activities of up to 10% in other tissues such as liver and intestinal mucosa, and may have better long-term clinical outcomes than patients with CG [29,30]. Genotype was not confirmed in the patients in the cited studies.
One study showed a lower galactitol excretion in 4 patients with p.S135L/p.S135L than in patients homozygous for the p.Q188R (c.563A>G (p.Gln188Arg)) variation and p.Q188R/other patients, but the levels were still above the reference range [31]. *In vivo* galactose oxidation capacity in patients with p.S135L/p.S135L is comparable to healthy controls [32–35]. *In vitro* galactose oxidation capacity in lymphoblastic cells lines of 2 patients homozygous for p.S135L was significantly higher than in patients homozygous for p.Q188R, but reduced compared to control cells [36], and after incubation with 1-^{13}C galactose Gal-1-P levels in p.Q188R/p.Q188R and p.S135L/p.S135L lymphoblastic cells were fully comparable to control cells [37]. There is no difference in the UDPgal and UDPglu levels between p.S135/p.S135L and p.Q188R/p.Q188R cells[37]. IgG N-glycans from one pediatric patient with p.S135L/p.S135L (on a galactose intake of 300 mg/day) showed decreased galactosylation in comparison with healthy children, similar to p.Q188R/p.Q188R patients (a galactose intake of <50 mg/day)[38]. This is indicative of ongoing N-glycan processing defects in these patients.

Duarte galactosemia
Patients with Duarte variant galactosemia (DG) have one GALT allele that is severely impaired, and a second GALT allele (Duarte-2, D2) that is partially impaired. At least five sequence changes on D2 alleles have been demonstrated so far: a p.N314D

(c.940A>G (p.Asn314Asp) missense substitution, three intronic base changes, and a 4 bp deletion in the 50 proximal sequence [39,40]. DG is associated with a mean residual enzyme activity of 14-25% [41], in contrast to most patients homozygous for the classical p.Q188R variation, who usually have a severely deficient (<1%) residual GALT enzyme activity [29]. Individuals with DG have a galactose oxidation capacity comparable to healthy controls [32–35]. Children with the DG variant are not known to present with clinical symptoms, but are detected by newborn screening, and since the start of these programs there is debate in the USA about whether or not these children need treatment and/or follow-up. Long-term clinical outcome in untreated affected individuals is assumed to be normal, but data are scarce with a limited amount number of studies with regard to clinical outcome and biochemical follow-up of DG. To our knowledge, currently the most common practice in Europe is not to treat and follow-up individuals with DG, while in the USA some metabolic centers prescribe a galactose-restricted diet in the first year of life [41,42].

Three papers reported on neonatal symptoms in DG variants (genotype confirmed). One paper reported mild unspecified symptoms in DG variants, however, this manuscript was excluded as evidence as the 5 reported DG variant children suffered from multiple pathologies such as cardiac disease and dysmorphic features [21]. Two other papers reported no symptoms and no abnormalities of liver function [42,43]. Reports of long-term outcomes in DG indicate normal IQ scores, language skills, FSH values and ophthalmologic examinations in untreated children aged 1-6 years with DG, as well as in those treated with a galactose-restricted diet in the first year of life [42,44]. Levels of FSH in female children with DG (up to 10.5 years) are comparable to healthy controls [45]. One study reported a higher percentage of children with DG enrolled in special education services, primarily speech and language, compared to the general population, but these results were not significant [46], and detailed information about the nature of the special educational services was not available for all the children with DG. A pilot study assessed developmental outcome in 10 children with DG compared to 5 unaffected siblings from the same group of families (all children aged 6-11 years)[47]. In this small sample, some differences in socio-emotional development, in delayed recall, and in auditory processing speed between children with DG and the unaffected siblings were found.

During the first year of life, children with DG who are untreated have significantly higher levels of RBC Gal-1-P, galactitol and galactonate when compared to those children with DG started on a diet after diagnosis (who have levels of Gal-1-P and galactitol within the reference range at the age of 4 weeks) and also have higher levels than patients with CG on a galactose-restricted diet [42,48]. In children with DG, who are untreated, levels of Gal-1-P and galactitol gradually decrease to a level within the reference range at the age of 1 year without intervention [42]. After children with DG have RBC Gal-1-P values within the reference range, they still demon-

strate increased levels of other galactose metabolites, including RBC galactitol (<10 years) and RBC galactonate (1-6 years), that correlate with galactose intake [44,49].

Recommendation #2 (expert opinion, +)
Clinicians should treat patients with a red blood cell GALT enzyme activity below 10% and/or pathologic variations on both alleles of the *GALT* gene, including p.S135L, with a galactose-restricted diet. There is not enough evidence to conclude whether patients with 10-15% red blood cell residual GALT activity should or should not be treated.

Recommendation #3 (expert opinion, +)
We recommend not to treat patients with the Duarte variant.

Dietary management
Ingestion of galactose derived from lactose in breast milk or whey-based formula causes life-threatening symptoms in the first weeks of life in patients with a severe deficiency of the GALT enzyme activity. These symptoms quickly resolve upon initiation of a galactose-restricted diet. While in some countries CG is part of the newborn screening panel, many patients will have presented with symptoms before referral for abnormal newborn screening. For most infants, the galactose-restricted diet includes discontinuation of breast milk or whey-based infant formulas and initiation of a soy-based formula, but an elemental formula may also be chosen[17]. There is an ongoing debate about the safety of soy-based formulas, due to the mild estrogenicity of soya. However, a recent review and meta-analysis demonstrated no effects on long-term growth, bone health and metabolic, reproductive, endocrine, immune and neurological functions and neurocognitive parameters in non-galactosemic children treated with soy-based formulas [50]. Elemental formulas containing L-amino acids are more expensive than soy-based formulas and, at this time, there is no evidence that consuming an elemental formula provides a clinical benefit for infants with CG. Casein hydrolysate formulas, containing medium-chain fatty acids, may be beneficial for infants with significant liver disease. Casein protein hydrolysate formula (derived from cow's milk) does contain traces of residual lactose (<10mg/100mL), but this is considered safe in CG. In contrast, whey-based hydrolysates contain more residual lactose and are not advocated for infants with CG. Due to the high galactose content of all animal milks and other dairy products (cow's milk contains 2400 mg galactose/100 mL) all clinics eliminate these products from the diet [17,51], but extent of galactose and lactose restriction varies between countries and even from clinic-to-clinic within the same country. Also there is a variation in the extent of restriction of less obvious sources of galactose (e.g. fruits and vegetables that contain free galactose or foods containing trace amounts of

lactose).

Recently, Van Calcar et al. reviewed the available literature on the galactose content of fruits, vegetables, legumes, dairy products, aged cheeses and caseinates [52]. In this section of the guideline we refer to Table 2 summarizing the reported galactose contents of food products, as reviewed by Van Calcar et al 2014 and Portnoi and MacDonald 2009. The free galactose content of most fresh or processed fruits, vegetables and legumes is less than 50 mg/100 gram serving [52], and an adult diet enriched in fruits and vegetables was found to contain only 54 mg of galactose per day [53]. This galactose intake is negligible compared to the endogenous galactose production in humans, which is thought to contribute to development of long-term complications. The endogenous production is strongly age-dependent, with the highest production in newborns (> 24.8 mg/kg/day) decreasing to a minimum of 8.4 mg/kg/day in adults [12,13,33,35,54–56]. Thus, for a 70 kg adult, endogenous galactose production would be more than 580 mg/day. In addition, endogenous production of galactose does not appear to be affected by exogenous intake of galactose [12]. The disparity between dietary intake and endogenous production has prompted many countries to recommend a galactose-restricted diet without restrictions of fruit, vegetables and legumes. There is no evidence to suggest that consumption of these minor sources of galactose has any adverse effects on long-term clinical status [52,57,58]. Importantly, Portnoi et al. 2009 and Van Calcar et al. 2014 demonstrated that the galactose content is low or even negligible in various aged cheeses including Gruyere, mature Parmesan and Emmentaler cheese (alternative spellings are Emmenthaler, Emmental, Emmenthal) produced in both Europe and North America, although the galactose content in the same type of cheese can vary due to variation in maturation and other biological and processing factors. Cheese is an excellent source of calcium, and many clinics allow and encourage including aged cheese in the diet of patients with galactosemia [59]. However, low-lactose milk aimed at the lactose-intolerant population is contra-indicated in patients with CG. In these products lactose has been hydrolyzed to glucose and galactose by addition of lactase, but still contains considerable galactose content. There is a continuing debate as to whether galactose restriction could be relaxed further with increasing age, especially since there is concern that an overly strict galactose restriction might be harmful [60]. In adolescents and adults with CG, intake of oral galactose of up to 200 mg over three weeks, 600 mg over six weeks and 4000 mg galactose over 14 weeks had no effect on RBC Gal-1-P concentrations and these subjects did not develop any clinical manifestations over this short time frame [53,57,60]. Coss et al. further demonstrated that patients with more severe complications have more abnormal IgG glycan patterns and, with exposure up to 2000 mg galactose/day for 16 weeks, the abnormal glycosylation of serum IgG improved in some of the subjects; however, the

improvement in glycosylation was highly individual, especially at higher galactose intakes [60]. There are also two published case reports of adults, both homozygous for the p.Q188R variation, who have been off-diet since 3 years of age. These patients ingested approximately 2500 and 9000 mg of galactose per day, yet their clinical outcome and biochemical parameters were comparable to those seen in treated adult patients [61,62]. However, experience is limited and there is little evidence to support the safety of discontinuation of the galactose-restricted diet.

Recommendation #4 (++)
Clinicians should immediately commence a galactose-restricted diet (e.g. soy-based, casein hydrolysate or elemental formula) if classical galactosemia is suspected in an infant, without waiting for confirmation of the diagnosis.

Recommendation #5 (expert opinion, +)
We recommend treating patients with classical galactosemia with a life-long galactose-restricted diet that only eliminates sources of lactose and galactose from dairy products, but permits galactose from non-milk sources that contribute minimal dietary galactose. Within this definition we accept that small amounts of galactose are present in specific mature cheeses and caseinates. At present there is insufficient evidence to support a specific age-related recommendation for the quantity of galactose allowed in the diet.

Recommendation #6 (+)
We recommend allowing any amount and type of fruits, vegetables, legumes, unfermented soy-based products, mature cheeses (with galactose content <25 mg/100g), and the food additives sodium or calcium caseinate, in the diet for classical galactosemia. Although higher in galactose, all fermented soy-based products can be allowed in the small quantities that are typically used in the diet.

The opinion about whether to restrict offal in the diet is divided; its galactose content is unknown, but there is no direct evidence of harm. It is a theoretical risk only, therefore it has been decided to put offal in the 'in moderation' section in the 'Current diet restriction for classical galactosemia' table.

Deficiencies
With elimination of dairy products from the diet, patients with CG are at risk for calcium and vitamin D deficiency. The majority of studies report serum calcium levels in the reference range in children and adolescent patients with CG [63–66]. Only one study with five patients with CG reports significantly lowered concentrations of calcium compared to healthy controls, but the age of these patients was not

Table 2. Examples of galactose content of food products (adapted from Portnoi et al. 2012 and 2015, Van Calcar et al. 2014[52,59,144])

	United States (Van Calcar 2014)	United Kingdom (Portnoi 2009, 2015)
	Galactose content (mg/100g food)[1] Mean ±SD (range[2])	Lactose[5] content (mg/100g food) Mean (range) (l.o.d.=limit of detection)
Dairy based products		
Cheddar cheese aged traditional	9.5 ± 17.9 (<2.8 to 104.3)	
UK west country Cheddar aged traditional		3.6 (<2.8-11.4)
Gruyere	4.1 ± 1.2 (<2.8 to 5.1)	
UK Gruyere		Not detectable (<3.5 l.o.d.)
Emmentaler/Swiss	3.5 ± 1.2 (<2.8 to 7.4)	
UK Emmentaler		Not detectable (<3.5 l.o.d.)
Jarlsberg	<2.8 (all <2.8)	
UK Jarlsberg		Not detectable (<10 l.o.d.)
American Parmesan, Brick (aged >10 mo) momo)months)	18.3 ± 33.3 (<2.8 to 156)	
American Parmesan, Grated	9.7 ± 12.0 (<2.8 to 23.6)	
UK Italian Parmesan (usually 2 year old) block/grated		Not detectable
Sodium or Calcium Caseinate	35.5 ± 37.7 (<5.1 to 95.5)	
UK Comte Cheese Butter oil/milkfat		Not detectable (0.43 (0.05-1.86)) 3 samples mean 0.9mg/100g Need median 4.32 and range <0.05-1.86
UK Butter oil/milk fat		<0.05 to 2.3
UK Ghee		<0.05 to 2.9
UK Butter		685 to 688
Plant-Based Products[3]		
Various Fruits (Raw or Processed)	9.7 ± 7.9 (1.0 to 44.5)	
Various Vegetables (Raw or Processed)	9.3 ± 11.4 (ND to 77.2)	
Fruit and Vegetable Juices	18.3 ± 14.0 (4.0 to 46.4)	
Legumes		

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	United States (Van Calcar 2014)	United Kingdom (Portnoi 2009, 2015)
	Galactose content (mg/100g food)[1] Mean ±SD (range[2])	Lactose[5] content (mg/100g food) Mean (range) (l.o.d.=limit of detection)
Garbanzo beans (Cooked or Processed)	149.5 ± 197.(24.6 to 443.8) t443.8)443.8443.8)	
Other legumes (Cooked or Processed)[4]	46.2 ± 63.1 (ND to 174.8)	
Soy Products		
Soy Beans, Whole	43.8	
Soy Milk (Made from whole soy beans)	5.1 ± 0.4 (4.8 and 5.3)	
Tofu, Silken	90 (dry wt)	
Fermented Soy Products		
Miso paste	290.7 ± 121.2 (139 to 433)	
Soy sauce[1]	361.7 ± 147.3 (240 to 590)	
Sufu (fermented tofu)	912 (dry wt)	

[1] All values are reported as mg galactose in 100 g of product except for soy sauce values which were reported as mg galactose in 100 mL. All reported values are based on 100 g wet weight; values for dried weight were not considered in the determination of means and ranges. The only exceptions to this are for tofu and sufu since wet weights were not given in the references.

[2] The lower detection limit varied depending on the methodology utilized in each paper and is reported with a "b" sign or ND (not detected). For any sample containing lactose, 53% of total lactose was considered galactose, based on molecular weight of 342 for lactose and 180 for galactose.

[3] For all plant products, only the reported free galactose content was considered in the determination of mean ± SD. Any galactose in a bound form was not considered to contribute to the galactose content of any food.

[4] Other legumes include one or more analyses for kidney beans, pinto beans, black beans, white beans, lentils and pink-mottled cream beans.

[5] The amount (in mg) of galactose is approximately half the amount of lactose.

reported[67]. Vitamin D is required for optimal calcium utilization, and normal concentrations of both 1,25-OH vitamin D and 25-OH-vitamin D have been measured in children and adolescents with CG [63–66]. However, in 80% of adults with CG, 25-OH vitamin D levels were reported to be below the reference range [4]. While some studies report an adequate daily intake of calcium and vitamin D in children and adolescents [63,64], others report deficient intakes of calcium [68,69]. One study reported that 75% of adult patients have an intake of vitamin D below the daily [4].

Recommendation #7 (+)
We recommend an annual dietary assessment of calcium and vitamin D intake with measurement of plasma total 25-OH-vitamin D levels. Both calcium and vitamin D should be supplemented as necessary following the age-specific recommendations for the general population.

Biochemical follow-up
Biochemical monitoring in CG is aimed at the follow-up of abnormal parameters of galactose metabolism and evaluation of adherence to the galactose-restricted diet. Currently, monitoring varies widely between centers, and markers measured most frequently include blood galactose, RBC Gal-1-P and/or urinary galactitol levels [17]. Levels of RBC Gal-1-P and urinary galactitol are raised at birth, decrease rapidly after initiation of a galactose-restricted diet, and then stabilize, but remain elevated compared to healthy controls [13,15,31,56,58,70–72]. There are serious doubts regarding the usefulness of these markers in monitoring the disease and adherence to the diet. There is no clear association/correlation between galactose metabolites and other markers and the development of both acute and long-term complications in patients with CG [5,71–73]. There are no prospective longitudinal studies assessing the predictive value of these markers for the development of long-term complications. Also, several studies have demonstrated no clear increase in RBC Gal-1-P and urinary galactitol levels after short-term oral galactose loading (up to 4000 mg) was given to patients, thus questioning the usefulness of these markers in monitoring (short-term) adherence to the diet [53,57,60]. It also has been reported that blood Gal-1-P and urinary galactitol have a high biological variability with high inter- and intra-individual variation, making single measurements of little value [15]. Gal-1-P is useful in detecting gross dietary deviations and acute intoxication [57]. There is (limited) evidence that monitoring of galactosylation may be an effective parameter in the future [60,74]. At this time Gal-1-P measurement, using each patient as his own reference, appears the best parameter for monitoring patients. There is, however, a strong need for improved monitoring biomarkers.

Table 3. Current diet restriction for classical galactosemia (adapted from Bernstein et al, Children's Hospital Colorado in collaboration with the Galactosemia Foundation Task Force[145])

Allowed foods and ingredients*
Soy-based infant formulas containing soy protein isolate, amino acid-based elemental infant formulas
All fruits, vegetables and their juices, pickled fruit and vegetables
All legumes (e.g. navy beans, kidney beans, garbanzo beans/chick peas, soybeans)
Soy-based products that are not fermented (soy milk, tofu, textured soy protein, hydrolyzed vegetable protein, soy protein concentrate, meat analogs)
Aged cheeses[1]: Jarlsberg, Emmentaler, Swiss, Gruyere, Tilsiter, mature Parmesan, mature Cheddar cheese
Sodium and calcium caseinate
All cacao products except milk chocolate
Eggs
Additional ingredients: natural and artificial flavorings, all gums, including carrageenan
Foods used in moderation *
Soy sauce, soy products that are fermented (e.g. miso, natto, tempeh, sufu)
Meat by-products
Offal
Restricted foods and ingredients*
Breast milk, all milk-based infant formulas
Processed meats using lactose
All milk-based foods and beverages, including low lactose milk, except for caseinates and aged cheeses, listed above
All milk-based ingredients including buttermilk solids, casein, dry milk protein, dry milk solids, hydrolyzed whey protein, hydrolyzed casein protein, lactose, lactalbumin, whey
All cheese and cheese-based products except those listed above
Butter

[1] Galactose content and consequently allowed types of cheese may vary in different countries
* All manufactured foods need to be checked for the presence of milk by reading food ingredient labels

Recommendation #8 (++)
In the first year of life clinicians should measure red blood cell Gal-1-P levels at diagnosis, and after three and nine months of dietary galactose restriction.

Recommendation #9 (expert opinion, +)
We recommend measuring red blood cell Gal-1-P levels yearly after the first year of life until an individual baseline has been established.

Recommendation #10 (expert opinion, +)
We recommend measuring red blood cell Gal-1-P levels in case of increase in galactose intake and concern about intoxication.

Recommendation #11 (expert opinion, +)
The clinical utility of serial blood or urinary galactitol measurement is limited.

Chapter 4

Long-term complications

Cognitive development

Despite initiation of a lactose- and galactose-restricted diet early in life, patients are at risk for decreased intellectual ability. Therefore, intellectual quotient (IQ) tests are frequently performed in the follow-up of patients with CG. IQ scores can also serve as a baseline for interpreting deficits in other areas; For example, poor executive functioning is common in children with low IQ. In addition, periodic evaluation of cognitive abilities can serve to assess the following: adequacy of treatment in preventing cognitive decline, the risk for developmental delay in specific domains (such as verbal abilities, reasoning abilities, memory and processing speed), and the potential need for early intervention and special education services. In CG, most studies report poor cognitive outcomes with mean IQ scores below average or in the low average range, but there is considerable variability between individual patients and scores range from very low to above average [1,7,8,10,43,71,72,75–83]. The reported percentage of patients with an IQ score below 85 varies from 45-72% [71,76,79,84]. A subject of debate in the follow-up of CG is whether the cognitive impairment is progressive. Some studies report a negative correlation between age and performance [8,72,75]. This finding may be an artifact of these studies' cross-sectional design and not measuring scores within the same patients over time. Other cross-sectional studies reported no significant difference in IQ scores between older and younger patients [1,84]. In longitudinal studies, no deterioration of cognitive function over time was reported during childhood/adolescence [72,85,86] and adulthood [72,82]. A number of studies report significantly lower IQ scores in females compared to males [8,72], yet other studies could not confirm this finding [81,84,86]. Mean IQ scores and range of IQ for patients with DG (aged 1-6 years), both on an early-initiated lactose-free diet and on a regular diet, were found to be comparable to the general population [42].

Recommendation #12 (++)

Clinicians should refer patients for testing of developmental quotient (DQ) and intellectual quotient (IQ), to obtain a well-validated measure of development and cognitive abilities. At minimum, testing should be done at:

Age 2-3 years: to assess early speech/language and motor development in time for early intervention, using a standardized test instrument such as the Bayley Scales of Infant and Toddler Development (BSID) or a similar measure.

Age 4-5 years: to assess school readiness and need for occupational therapy and speech-language therapy, using a standardized test instrument such as the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) or a similar measure.

Age 8-10 years: to assess cognitive development, specific areas of strengths and weaknesses and the need for special therapies, using a standardized test instrument such as the Wechsler Intelligence Scale for Children (WISC) or a similar measure.

Age 12-14 years: to assess cognitive development and specific areas of strengths and weaknesses and to assess the need for special therapies, using a standardized test instrument such as the Wechsler Intelligence Scale for Children (WISC) or a similar measure.

Age 15 years and older: according to needs, specific questions.
(consider combining these assessments with speech and language screening, recommendation #15, and psychosocial development screening, recommendation #21)

Recommendation #13 (expert opinion, +)
For obtaining a measure of functioning when formalized testing is not possible or when additional assessments are needed between formalized testing points, we recommend using a validated parent/informant questionnaire, such as the Adaptive Behavior Assessment System (ABAS) or a similar measure.

There is no correlation between IQ and the time of initiation of dietary treatment as long as treatment is started within the first 8 weeks of life [71,82]. Correlation with genotype is unclear as some studies suggest that IQ is not correlated to the p.Q188R variation [7,73,87], while another study reports that patients with homozygosity for p.Q188R have lower IQ scores than patients with less common genotypes[76].

Executive functions
Only a few studies have evaluated the executive function of patients with CG. Mean scores of executive functioning in adult patients with CG are below the average, but there is considerable variability between individual patients [75]. Overall, 15% of adult patients demonstrate deficits in executive functioning as self-reported on the Behavior Rating Inventory of Executive Function (BRIEF)[4]. As evidenced by direct evaluation of children and parent responses on the BRIEF, children with CG exhibit less well-developed executive functioning compared to peers [77]. Sustained attention and information processing may also be impaired [78].

Recommendation #14 (expert opinion, +)
We recommend a clinical assessment of executive function, if feasible in the clinic, with specific attention to processing speed and visual spatial comprehension. In children (8-10 years) as a first screening use the Behavior Rating Inventory of Executive Function (BRIEF), and in adolescents (12-14 years) and in young adults

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(18-20 years) use the Cambridge Neuropsychological Test Automated Battery (CAN-TAB), the Amsterdam Neuropsychological Tasks program (ANT) or a similar measure, with follow-up, as needed.

Speech and language impairment

Various speech and language disorders have been reported in CG. Language is the tool by which we communicate thoughts, feelings, and ideas either spoken or written, and speech is the tool by which we verbally communicate with others. In some reports on speech and language disorders in CG the disorder is well-defined such as childhood apraxia of speech (also called developmental verbal dyspraxia, a motor speech disorder with problems saying sounds, syllables, and words), articulation disorders, dysarthria, and receptive language disorders. In many reports more general speech/language delays or disorders are reported, relating primarily to producing rather than understanding speech and language. Few studies have used standardized and validated test instruments, but overall 24%-88% of children and adults with CG are reported to have a speech and language disorder [1,4,5,71,72,79,80,83,84,88–91]. Speech motor function may be affected as well, specifically reduced tongue strength (73%), decreased breath support for speech (32-64%)[4,92], and disturbed vocal quality to laryngeal insufficiency (33% of children with CG and speech disorders) [93]. Almost 10% of patients with CG are affected by vocal tremors of unknown origin [93]. Children with CG and a history of speech disorders also have a 4- to 6-fold greater relative risk for co-occurring language disorders [88]. Patients with CG show difficulties in language production tasks, both behaviorally (less accurate and slower) and in their brain's signature measured by functional magnetic resonance imaging (fMRI) and by event-related potentials (ERPs), compared to healthy controls. The ERP differences continue throughout consecutive linguistic preparation phases, which indicates an affected lexical access and impaired syntactic planning [94], while the fMRI findings point towards both affected linguistic preparation and motor speech planning [95]. Many individuals with CG (up to 86%) have received speech therapy, with most children receiving direct speech therapy from a speech and language therapist or, less frequently, indirect speech therapy with the speech and language therapist working with the child's family [71,72,88,94]. There is no evidence in the literature addressing therapeutic options or therapeutic efficacy. In CG, language disorders and type of language disorder are associated with, but not fully explained by, cognitive function[72,84,90]. More than half (56%) of patients with CG with average cognition and most (88%) patients with CG with borderline-low cognition have co-occurring speech and language disorders [88]. Nelson et al. (1991) reported that the presence or severity of CAS is not related to age at start of diet, the presence of neonatal symptoms, gender or age at time of speech evaluation. Another study reported that the number of days consuming milk prior to diagnosis is associated with poorer speech

outcomes in males, not females, with CG [92]. Patients with the p.Q188R/p.Q188R genotype are reported to be at greater risk for speech and language impairment than participants with p.Q188R/other genotypes [88,96]. Two-thirds of children with CG and speech disorders have co-occurring coordination disorders and children with CG and CAS or dysarthria have poorer balance and manual dexterity [92].

Recommendation #15 (++)
All children with CG should be screened for speech and language delay at ages 7-12 months, 2 years, 3 years and 5 years (consider combining with screening for cognitive disorders, recommendation #12). If children show low or borderline speech and language development, full assessments should be conducted.

Recommendation #16 (expert opinion, +)
We recommend that an assessment of speech and language includes hearing screening, a brief assessment of pre-linguistic communication (<2 years of age) and expressive, receptive, and pragmatic language use, structure-function examination, motor speech (observation of respiration, resonance, voice, articulation), and speech intelligibility for all children not meeting age appropriate milestones. We recommend a cognitive evaluation, as well if, a disorder is suspected.

Recommendation #17 (expert opinion, +)
For children who are not meeting age appropriate speech or language milestones, we recommend treatment based on guidelines for treatment of speech, language, and voice disorders in the general population. Therapy should begin during the first year of life and include modelling and training of gestural communication to increase infant and toddler language development. Play-based milieu for language development is recommended during the second year of life. Individual speech therapy focused on high repetition of a small number of targets should begin during the second year of life and continue as needed throughout the preschool and elementary school years. Respiration, phonation, and resonance deficits should also be addressed.

Neurological complications
Patients with CG are at risk for central nervous system dysfunction, not only manifesting itself as neurological symptoms and motor problems, but also as cognitive impairment, speech and language problems and psychiatric symptoms. The latter three are discussed in a separate part of this guideline.
Affected newborns may develop encephalopathy and signs of increased intracranial pressure with cerebral edema after ingestion of galactose [97–99]. In the long-term it

is known that patients may develop neurological complications, with an overall frequency of motor dysfunction of up to 66% [5,21,83,100]. The studies addressing specific neurologic symptoms all report on different symptoms and have very heterogeneous populations with large variation in sample sizes, making it difficult to reliably estimate the percentage of patients that suffer from each of these symptoms. Frequently noted signs are mild to severe ataxia, tremor, dystonia, dysarthria and dysmetria [1,4,7,58,71,72,81,83,101,102]. Epilepsy is reported in a minority of patients [58,71,83,103]. One study, that followed 22 patients with CG diagnosed early, did not find any cases with ataxia or dysmetria in young patients aged 0-6 years [104]. One study reported eye movement abnormalities and pyramidal signs in some patients (including brisk tendon reflexes and clonus, extensor plantar response, spastic paraparesis and pseudobulbar signs)[83].

Recommendation #18 (++)
Clinicians should screen patients with CG for neurological involvement by clinical examination from the age of 2-3 years. Such screening should include examination for ataxia, tremor, dysmetria and dystonia. If a specific neurological deficit is noted, monitoring of progression with a designated scale is advised. It is suggested to screen adult patients annually and to record progression, if any. Pediatric patients could be screened more frequently (every 6 months) in order to identify potentially modifiable neurological problems.

Recommendation #19 (+)
We recommend asking patients or caregivers about onset of seizure and seizure-like activity since previous examination and perform an EEG, if indicated.

Cerebral imaging
An abnormal white matter signal is present in the majority of MRI scans (>75%) of patients with CG, indicative of abnormal myelination [5,58,81,101,105,106]. Additional reported MRI findings are focal white matter lesions, white matter volume loss, (mild) cerebral atrophy, enlargement of the fourth ventricle and cerebellar sulci (suggesting cerebellar atrophy), and enlargement of lateral ventricles [5,58,81,83,101,105–107]. One study followed patients over time with MRI scans [101]. All eight patients younger than one year of age had normal white matter signaling. All patients over the age of one year either had an abnormal peripheral myelin pattern, or developed abnormalities within 1-2 years without apparent progression in time. 22/63 patients from this study had mild lateral ventricular enlargement at the initial MRI. This enlargement was unchanged in four patients that had follow-up MRIs 1-2 years later. Of the 20/63 patients without lateral ventricular enlargement that had follow-up examinations, none developed lateral ventricular enlargement. In some

studies only patients with neurological symptoms had MRI examination, and in other studies clinical status was not reported. It is unclear if the abnormalities on MRI-scans are representative for the whole population of patients with CG and if the findings are correlated with clinical symptoms. One study also performed magnetic resonance spectroscopy (MRS) in addition to brain MRI to assess *in vivo* brain metabolism [106]. MRS showed a normal spectrum of metabolites, with no indications of elevated Gal-1-P levels or an impairment of energy supply in the brain. No relationship between MRS and clinical data was found. A study on white matter microstructure pathology using neurite orientation dispersion and density imaging (NODDI) showed extensive white matter abnormalities with a lower neurite density index and increased orientation dispersion index in the regions involved with higher order cognitive functions and language and motor functions [107].

Recommendation #20 (expert opinion, +)
We do not recommend routine brain and spinal cord imaging in the follow-up of patients with CG. In those patients with significant or progressive neurological symptoms and signs, imaging may be warranted to (1) determine if a second condition is present or (2) further define the development and progression of neuroradiology findings in individual patients.

Psychosocial development
Due to the chronicity of the disease, the need to adhere to a life-long diet and the impact of the long-term complications, patients with CG are at risk for problems in psychosocial development, including personality, social relationships and emotional well-being.

Psychosexual and social development, marital status
Specific testing of social and psychosocial milestones in adult patients showed that patients achieved fewer developmental milestones in the psychosexual and social domain (having friends and engaging in social activities)[108,109]. Multiple studies assessed marital status in adult patients with CG, with the percentage of patients married or living in stable partnership varying between 13% and 57% [4,109–111]. Bosch et al. 2009 reported that the percentage of patients married (14.3%) was significantly lower compared to the reference population (39.1%). Hoffmann et al. 2012 found a difference in marital status between sexes with more married females (57%) compared to males (11%), with the percentage of married males much lower than in the reference population (47.2%). In one study assessing males only, 5% of patients were married, compared to 30.9% in the reference group of males that did not differ in age, though this difference was not statistically significant [108]. Few patients with CG had children (0-5%), while a desire to have children was reported by nearly half of patients [110,111].

The percentage of patients actually trying to conceive a child or the percentage successful was not reported. While primary ovarian insufficiency (present in the majority of female patients) might be an important contributing factor to the minority of females having children, there is no evidence of decreased fertility in males (see Endocrinology and Fertility part of this guideline)[108,112]. The percentage of patients trying to conceive might also be low, due to the fact that fertility counselling in the recent past was often focused on the expected low chances of achieving pregnancy.

Educational attainment and employment
Bosch et al. reported that 44% of children with CG, aged 6-11 years, attended special schools as opposed to 3% of the general population [113]. Educational attainment was significantly lower than the general population with 61.5% completing basic school and low vocational training only, compared with 27.2% of the general population [113]. Lower education attainment was confirmed in another study which reported that fewer individuals with CG (10.8%) had a school leaving certificate, compared to 3.5% in the general population, and a minority achieved a university entrance diploma (8.1%) compared to the general population (28.6%). However, a higher percentage of individuals with CG (81%) earned a secondary school degree compared to the general population (59.5%)[111]. In adulthood, up to 30% of individuals with CG were unemployed, with no differences between males and females [4,109,111]. A recent survey of 60 adult patients with galactosemia in the UK revealed that 58% were in paid employment, compared with 74% of the general population (Unpublished data, Charles Dent Metabolic Unit, UK, 2015).

Psychiatric symptoms and emotional problems
Patients with CG have been reported to suffer more frequently from psychiatric symptoms and emotional disturbance, including depression, anxiety, obsessive-compulsive disorder and autism spectrum disorder [83,87]. Parents of children with CG report their children exhibit more internalizing symptoms (e.g. depression and anxiety) without elevated levels of dysphoric mood compared to controls [77]. Depression (as detected with the Beck Depression Inventory), was present in 12% of adult patients, and anxiety (as measured with the Beck Anxiety Inventory) was present in 52% [4].

Coping with CG
Coping with CG by patients was assessed in two studies, both using condition specific but non-validated questionnaires. It was demonstrated that over 75% of the patients rated their coping with CG as 'very good' or 'good'. The remainder had difficulties in coping with their condition. CG was seen as a burden by 39% of patients [111,113]. Many patients (42%) had a problem maintaining the diet, and patients reported that

'diet/nutrition' was the primary aspect of life influenced by galactosemia, followed by 'school/work' and 'friends/leisure'. Many parents of patients with CG (60%) considered it a burden to take care of a child with CG, and many believed that CG influenced their relationship with their child. More than half of parents of girls frequently worried about possible infertility. However, a high percentage of parents (86%) believed that one could live a good life with this disorder [113] and only 7.7% of adults with CG reported that galactosemia had a negative effect on family life [111].

Screening for psychosocial deficits
Currently no universal and validated checklist is available to screen for psychosocial deficits.

Recommendation #21 (expert opinion, +)
We recommend screening children for psychosocial deficits, including autism spectrum disorders, sensory integration problems, depression and anxiety, using standardized questionnaires such as the Behavior Assessment System for Children, Second Edition (BASC-2) in English or a similar tool in other language. We recommend performing this screening at age 2 years in combination with screening for speech and language delays (see recommendation #15) and to combine this screening with developmental testing at ages 4-5 years, 8-10 years and 12-14 years (see recommendation #12).

Recommendation #22 (+)
We recommend screening adults for mental health issues with validated questionnaires that include brief scales for Anxiety and Depression, such as the NIH PROMIS Questionnaires, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI) or similar measures. With adults, we recommend discussing living situations, work and/or educational situations, satisfaction with social relationships, and sexual intimacy during outpatient clinic visits and to refer for professional consultation, if necessary.

Health-Related Quality of Life (HRQoL)
Three studies report on HRQoL in patients with CG. One study demonstrated that having CG negatively affects HRQoL of both children and adults [113]. Children aged 6-15 years had a lower HRQoL in the domains of cognitive function and of motor function. Patients >16 years of age reported significantly lower scores in the domains of cognitive function and social function. Hoffmann et al. showed that adult patients with CG scored significantly lower on the domains 'positive mood' and 'social well-being' when compared to the general population and compared to PKU patients [111]. Finally, a study evaluating HRQoL in patients >6 years of age report-

ed that patients with CG did not differ from their peers in their physical activities, mobilization, overall health and their self-esteem, but that they did have difficulties in their relationships with others. Despite feeling 'not as good as most people', all patients had been happy at some point in the 4 weeks preceding the interview [114]. The HRQoL of parents of children with CG did not differ from the HRQoL of parents of healthy children [115].

Recommendation #23 (expert opinion, +)
We do not recommend routine Health-Related Quality of Life (HRQoL) evaluations.

Fertility
Over 80% of females with CG develop primary ovarian insufficiency (POI)[1,6,72,83,116–119]. The clinical spectrum of POI in women with CG varies from primary amenorrhea with or without lack of development of secondary sexual characteristics, to normal pubertal development followed by irregular menses, oligomenorrhea or secondary amenorrhea. Most patients experience subfertility, and often show a diminished ovarian reserve. Many females need puberty induction and/or hormone replacement therapy to prevent sequelae of POI [120]. The mechanisms underlying POI and the timing of the ovarian damage in CG are not understood to date. Possible underlying pathophysiological mechanisms of POI in CG include direct toxicity of accumulated galactose or one of its metabolites, abnormal glycosylation of glycoproteins or glycolipids (including hormones such as follicle stimulating hormone (FSH)), and wrongful activation of follicular apoptosis [118,119,121,122]. One study reported that female patients with CG have additional FSH isoforms besides normal acidic FSH isoform, [123], but another study did not confirm these findings [124]. FSH-inactivity seems not to be a probable cause of POI, as most female patients with CG do not respond significantly to stimulation with exogenous FSH and/or luteinizing hormone (LH)[125,126].

Biomarkers for POI
Corresponding to the incidence of POI, most (>80%) female patients with CG demonstrate raised levels of FSH [72,127], while estradiol levels are decreased in adolescent girls and women [112]. Levels of anti-Müllerian hormone (AMH), a marker for ovarian reserve which is produced by granulosa cells of (healthy) pre-antral and small antral follicles of the ovary [128], are reported to be abnormally low among girls and women with CG across all age groups, even in patients <1 years of age [117,125,129]. However there is no significant difference in AMH levels between girls with CG with spontaneous menarche compared to hormone replacement therapy assisted menarche [129]. Female patients with CG demonstrated normal gonadotropin levels that increase as POI becomes apparent, normal levels of prolactin, total cholesterol, tri-

glycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, thyroid-stimulating hormone (TSH), thyroid-binding globulin (TBG), free thyroxine (FT4), basal testosterone, free testosterone, andostenedione, and dehydroepiandrosterone sulfate(DHEAS)[6,112,126]. Also, no ovarian antibodies were found [6].

Imaging
Ovaries of girls and women with CG measured on MRI were significantly smaller when compared to age-matched controls, but did not differ significantly from post-menopausal controls [125]. In more than half of patients with CG ovaries could not be visualized on ultrasound, compared to 10% in healthy controls. On ultrasound most patients had antral follicle counts below the control range [129].

Recommendation #24 (++)
Girls with CG should be screened for hypergonadotropic hypogonadism if they reach the age of 12 years with insufficient secondary sex characteristics or if they reach the age of 14 years with no regular menses. Screening should include follicle-stimulating hormone and 17-beta-estradiol.

Recommendation #25 (expert opinion, +)
We recommend considering follicle stimulating hormone level, growth, and psycho-social maturity of the individual girl, for determination of age at start of treatment. For puberty inducement, a low dose estrogen in a step-wise escalating dose is used, then later combined with cyclic progesterone for regular withdrawal bleeds. We recommend considering referral to a pediatric endocrinologist.

Recommendation #26 (expert opinion, +)
We recommend not using anti-Müllerian hormone and ovarian imaging routinely for follow-up as these have not been shown to accurately predict pubertal development or fertility outcome.

Recommendation #27 (+)
We do not recommend endocrine follow-up for Duarte Galactosemia, as there is no evidence that the ovaries are affected.

Recommendation #28 (expert opinion, +)
We recommend that girls and women with CG, who have gone through puberty and established regular menstrual periods, should be monitored annually for menstrual abnormalities, secondary amenorrhea and symptoms of primary ovarian insufficiency (POI). Changes in menses or POI symptoms should be evaluated with a

serum follicle-stimulating hormone level. Anti-Müllerian hormone measurement is not helpful in determining which women will undergo POI, but may be helpful in identifying women at risk for imminent POI when it is undetectable. Imaging by pelvic ultrasound or MRI is not recommended unless otherwise clinically indicated.

Recommendation #29 (expert opinion, +)
We recommend that women with hypergonadotropic hypogonadism, or primary ovarian insufficiency should be provided counseling and support about their reproductive options and management of irregular or absent menses. Hormone replacement therapy should be initiated with the onset of secondary amenorrhea to reduce the risk of osteoporosis and other complications of primary ovarian insufficiency.

Recommendation #30 (++)
We recommend considering a referral to a reproductive endocrinologist for women who desire pregnancy and have been unable to conceive naturally, or for women who desire additional counseling about fertility treatment options including oocyte donation.

Recommendation #31 (expert opinion, +)
We recommend providing counseling about adequate birth control methods for women who do not desire pregnancy. While combined oral or transdermal contraceptives may provide cycle control, bone protection and attenuate hot flashes, they may fail to provide adequate birth control in women with very elevated follicle-stimulating hormone levels. An intrauterine device may provide the lowest failure rate.

Risk factors for POI
The risk of POI in CG is positively associated with higher mean Gal-1-P levels after 1 year of dietary treatment, reduced galactose oxidation capacity, and homozygosity for the p.Q188R variation (16-fold increased risk) compared to heterozygosity for this variation or two different variations [127]. Homozygosity for the p.Q188R variation, however, is not predictive for the development of primary amenorrhea versus secondary amenorrhea [7]. Because of the high frequency of the mild p.S135L variation in African American patients, there is an association between ethnicity and the outcome of POI, with a high proportion of the Caucasian females but no African American females diagnosed with POI [127]. Residual GALT activity might be a modifier of ovarian function as well, as female patients with >0.4% predicted wild-type GALT activity are more likely to show AMH levels of >0.1 ng/ml when compared to girls with <0.4% GALT activity [129]. There is no association between POI and the age at ini-

tiation of dietary treatment, degree of dietary control, highest erythrocyte Gal-1-P level [127], and urinary galactitol levels [6].

Pregnancy, counselling and fertility preservation
Chances of pregnancy are reduced in POI, but pregnancies in women with CG have been reported, and appear not to be as rare as is generally assumed (reviewed by Gubbels et al. 2008). As recommendations on fertility preservation were lacking, Van Erven et al. issued recommendations for physicians based on current knowledge concerning galactosemia and fertility preservation [130]. Oocyte donation may be used to establish pregnancy in women with CG and POI, but has some psychological disadvantages [131]. Three fertility preservation techniques are currently offered to patients in need of fertility preservation: ovarian tissue, mature oocyte and/or embryo cryopreservation. The application of fertility preservation in these patients is complicated however, because the underlying mechanisms and onset of POI in CG are not fully understood. Also the experience with fertility preservation is mainly derived from cancer patients with previously unaffected ovaries, and some procedures like cryopreservation of ovarian tissue are still experimental [130].

Recommendation #32 (expert opinion, +)
Fertility preservation may not be successful. Currently, fertility preservation techniques are not yet readily used in everyday practice. We recommend fertility preservation should only be offered with appropriate institutional research ethics review board approval to girls with classical galactosemia at a young pre-pubertal age.

Males
In contrast to female patients, fertility does not seem to be affected in males, but there is a paucity of data about reproductive function and male patients fathering children, possibly due to psychosocial rather than biological reasons. One study reports a confirmed delayed onset of puberty in 1 of 18 males over 12 years of age [71]. A higher rate of cryptorchidism [112,132], lower semen volumes as a group and sperm concentrations in individuals are reported [132]. Males demonstrated normal levels of gonadotropins, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, prolactin, TSH, TBG, FT4, basal testosterone, free testosterone, androstenedione and DHEAS [112,132].

Recommendation #33 (+)
We do not recommend routine endocrinology follow-up in males.

Bone health
Patients with CG might be at risk for impaired bone health due to various reasons, such as restrictions in dietary intake, decreased physical activity in some patients,

POI in females, and currently unknown pathophysiologic factors intrinsic to the disease. The preferred and most frequently used method to measure BMD in children is dual-energy X-ray absorptiometry (DXA) [133]. Other types of bone densitometry measurements, such as quantitative computed tomography (CT) and quantitative ultrasound, are also available. BMD can be measured at different sites and is frequently reported as a T- or Z-score, which are both units of standard deviation. In children the preferred sites to measure BMD are the lumbar spine and the total body less head (TBLH); In adults the lumbar spine, total hip and/or femoral neck [134]. Two-dimensional cross-sectional area measurements (areal BMD (aBMD)) or estimated volumetric BMD (vBMD) measurements can be performed. The diagnosis of osteoporosis in children, premenopausal women and males under 50 years of age should be based on densitometric criteria combined with fracture history. The definition of osteoporosis for these groups is a BMD Z-score <-2 ('BMD below the expected range for age' or 'low BMD for chronological age') combined with a significant fracture history [133].

Bone health in CG

Three studies in adult patients report vBMD Z-scores of -1.9 and -1.4 in males. [135]; aBMD Z-scores -1.19 in females (lumbar spine) and -1.25 (total hip) in females and -0.80 (lumbar spine) and -0.81 (total hip) in males [136]; and BMD Z-scores greater than 2 standard deviations below the normative mean in 8 of 33 patients [4].

Four studies reported on BMD Z-scores in children and adolescents, three using DXA scanning BMD [63,64,137] and one using quantitative CT scanning [135]. Two studies demonstrated decreased lumbar spine aBMD Z-scores (-0.65 and -0.6)[63,137], and two other studies found decreased total body aBMD Z-scores (-0.3 and -0.99)[64,137]. Panis et al 2004 and Rubio-Gozalbo et al. 2002 found decreased femoral neck vBMD Z-scores as well (-0.28 and -1.76). It should be noted that recent insight is that Z-scores should not be used for volumetric measurements. Kaufman et al. found a significantly decreased mean BMD of the lumbar spine (assessed with quantitative CT) when compared to age-matched healthy controls, in both children and adult patients [135]. Karadag et al. demonstrated normal BMD in all patients aged 0-6 years, tested with DXA and quantitative ultrasound, but decreased BMD was not defined [104]. Coss and colleagues demonstrated osteopenia or osteoporosis (in children >10 years old and adults) in 14.7% of the Traveller population and in 39.6% of the non-Traveller population, using Z-scores generated from DXA scans, but it is unclear how the authors defined osteopenia and osteoporosis [1]. A randomized controlled trial of two years in children with CG evaluated the effect of calcium and vitamin K1 and D3 supplementation versus placebo on bone mineral content (BMC), and showed a significant increase in BMC of lumbar spine in the treatment group compared to placebo group, but only in prepubertal children [19]. Self-report of fractures in adult patients with CG showed that 63% of women and 31% of men

sustained at least one lifetime fracture [136], which seems to be comparable to the general population [138]. However, further studies will need to validate these results.

Recommendation #34 (++)
Clinicians should assess bone mineral density (BMD) by age appropriate dual-energy X-ray absorptiometry (DXA) scan.

Recommendation #35 (expert opinion, +)(consensus: 93%)
We recommend BMD screening from age 8-10 years. With evidence of reduced bone density (Z-score ≤-2.0), follow-up according to current pediatric bone health guidelines is advised. Without evidence of reduced bone density, we recommend performing a repeat dual-energy X-ray absorptiometry scan when puberty is complete. We recommend performing follow-up thereafter every five years and treatment instituted according to WHO FRAX recommendations.

Recommendation #36 (+)
We recommend comprehensive dietary evaluation, optimization of calcium intake if needed, monitoring and if necessary supplementation of vitamin D, hormonal status evaluation and hormone replacement therapy consideration, as well as a regular exercise and assessment of skeletal problems and clinically significant fractures in all patients with CG. Supplementation of vitamin K might be beneficial when combined with an adequate intake of calcium and vitamin D, but currently there is not enough evidence to recommend the routine use of vitamin K.

Bone metabolism
Measurement of bone metabolism parameters, such as minerals, vitamins, hormones, bone formation markers and bone resorption markers, might be helpful in understanding underlying mechanisms of a decreased BMD and evaluation of treatment (e.g. supplementation of calcium and vitamin D). Studies measuring these parameters in patients with CG have only been performed in children and adolescents. Levels of carboxylated osteocalcin, N-terminal telopeptide, C-terminal telopeptide, and IGF-1 Z-scores were found to be significantly decreased in patients with CG, while values of bone alkaline phosphatase, osteocalcin and C-terminal telopeptide were found to be higher than in controls [63–66]. All other tested parameters in these four studies were in the reference range or comparable to healthy controls.

Recommendation #37 (expert opinion, +)
At present there is not enough evidence to justify routine determination of bone turnover markers in patients with CG.

Cataracts

Cataracts are a frequently encountered complication of CG, mainly in the newborn period. Cataracts always occur bilaterally, and the enhancement of nuclear or peri-nuclear refractive power leads to the appearance of a refractile ring or a drop of oil in the crystalline center, which is an early sign. Later stages appear as nuclear or zonular opacities, and in all stages vacuoles might be present. The cause of cataract is accumulation of galactitol in the crystalline lens, the result of activation of the aldose reductase shunt [139]. Cataracts are typically already present in the first weeks of life, with a prevalence which varies from 6-25% across several studies [43,140–142]. In a retrospective case series, 14/100 patients with CG were diagnosed with cataracts, with the average age at cataract diagnosis of 6.3 years [139]. However, this article did not report on age of diagnosis, previous ophthalmologic examinations or dietary adherence. A study on long-term complications in adulthood reported the presence of cataracts in 21% of adult patients with CG, as noted in medical records or reported during the medical history [4]. Overall prevalence of cataracts, regardless of age at start of diet, is extremely variable across different studies, with the larger retrospective case series reporting 7.7% [1] and 14% [139]. A smaller study reported cataract in 17/22 patients, including 13/18 patients who were diagnosed before 17 days, and in 4/4 patients who were diagnosed after 17 days. One prospective case series with a mean follow-up period of 8.5 years reported an overall prevalence of cataracts of 36% in 33 patients [143], but the time of detection of the cataracts was not reported, except for two patients in this cohort who developed reversible cataract at the age of 2.5 and 3.7 years respectively, both after a three-month period off diet [141–143].

Severity of the cataracts reported in patients with CG varies, but in the vast majority of cases visual acuity is not affected, and lens opacities frequently resolve spontaneously over time in patients on a galactose-restricted diet [43,71,72,140–143]. Reports of cataract necessitating surgery are rare: one study reports on a 33 year old male suffering from blindness due to cataract, but age of diagnosis and dietary adherence were not reported [71]. Karadag et al. reported four patients diagnosed after age 17 days required cataract surgery [104]. Waggoner et al. reported 8 cases (out of 314 patients) requiring surgery, including one patient that had been treated from birth but with unknown adherence [72].

Widger et al. could not demonstrate a direct relationship between dietary adherence and cataract formation, however in this study dietary nonadherence was defined by the relatively low galactose intake of >50 mg/day [139]. Levels of Gal-1-P have not been demonstrated to correlate with cataract formation [143].

The current available literature is inconclusive regarding which patients will develop cataracts and at what age, and if adherence to the diet plays a direct role in the development of cataracts. There are however strong suggestions that cataracts in the neonatal period usually do not affect visual acuity and, with dietary adherence, will often resolve spontaneously. Furthermore it seems that, patients with a good dietary adherence do not develop cataracts later in life or if they do develop cataracts, visual acuity is not affected.

Recommendation #38 (++)
Clinicians should refer all patients to an ophthalmologist for evaluation of cataract at the time of diagnosis.

Recommendation #39 (+)
We recommend performing ophthalmological follow-up in patients with a cataract at diagnosis until it has fully resolved.

Recommendation #40 (+)
We recommend performing ophthalmological screening in all patients who are non-compliant with diet.

Chapter 4

Closing remarks

The presented guideline is the first international and evidence-based guideline for the diagnosis, treatment and follow-up of CG, and aimed to be applicable world-wide. This guideline should serve as a guide for clinicians and other experts caring for patients with CG. Though great effort was undertaken to formulate evidence-based recommendations, this was frequently hampered by limited evidence resulting in numerous recommendations based on expert opinion (18/40 recommendations, 45%). The literature concerning CG available to date mostly consists of studies with an observational study-design. In the current era of evidence-based medicine these studies are labeled as having a low to very low level of evidence. Therefore strength of recommendation is 'discretionary' for a majority of recommendations in the guidelines, (32/40 recommendations, 80%) including the recommendations labeled expert opinion. However, as other study designs (such as RCTs or cohort studies) are usually not feasible or may not provide the best design to study characteristics of rare diseases, the strength of the recommendation was upgraded to 'strong' when results were consistent across multiple studies, and experts had confidence in the validity of these results (9/40 recommendations, 23%).

Future perspectives
Following this conclusion, it is not unexpected that gaps of knowledge were iden-tified in most discussed fields of interest, foremost in the fields of treatment and follow-up. Topics of major importance for future research include: further assess-ment of which patients should be treated (cut-off enzyme activity), exploration for possible further relaxation of the diet for patients after childhood, exploration of new biomarkers for biochemical follow-up as well as reproductive function, assess-ment of executive functions in children and adults, and further exploration of bone turnover markers in relation to BMD.

Guideline update
Revision of this guideline is important as it only represents evidence in predefined areas up to October 2015. Since research in the field of CG is flourishing, it is ex-pected that new information will be gained in the next decade. This guideline is scheduled to be updated in the next ten years by representatives of the GalNet.

References

1. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis 2013;36:21–7.

2. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis 2006;29:516–25.

3. Ounap K, Joost K, Temberg T, Krabbi K, Tõnisson N. Classical galactosemia in Estonia: selective neonatal screening, incidence, and genotype/phenotype data of diagnosed patients. J Inherit Metab Dis 2010;33:175–6.

4. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis 2012;35:279–86.

5. Hughes J, Ryan S, Lambert D, Geoghegan O, Clark A, Rogers Y, et al. Outcomes of siblings with classical galactosemia. J Pediatr 2009;154:721–6.

6. Kaufman FR, Kogut MD, Donnell GN, Goebelsmann U, March C, Koch R. Hypergonadotropic hypogonadism in female patients with galactosemia. N Engl J Med 1981;304:994–8.

7. Kaufman FR, Reichardt JK, Ng WG, Xu YK, Manis FR, McBride-Chang C, et al. Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridyltransferase gene. J Pediatr 1994;125:225–7.

8. Komrower GM, Lee DH. Long-term follow-up of galactosemia. Arch Dis Child 1970;45:367–73.

9. Waisbren SE, Norman TR, Schnell RR, Levy HL. Speech and language deficits in early-treated children with galactosemia. J Pediatr 1983;102:75–7.

10. Donnell GN, Collado M, Koch R. Growth and development of children with galactosemia. J Pediatr 1961;58:845–51.

11. Ning C, Reynolds R, Chen J, Yager C, Berry GT, Leslie N, et al. Galactose metabolism in mice with galactose-1-phosphate uridyltransferase deficiency: sucklings and 7-week-old animals fed a high-galactose diet. Mol Genet Metab 2001;72:306–15.

12. Huidekoper HH, Bosch AM, Van Der Crabben SN, Sauerwein HP, Ackermans MT, Wijburg FA. Short-term exogenous galactose supplementation does not influence rate of appearance of galactose in patients with classical galactosemia. Mol Genet Metab 2005;84:265–72.

13. Schadewaldt P, Kamalanathan L, Hammen HW, Wendel U. Age dependence of endogenous galactose formation in Q188R homozygous galactosemic patients. Mol Genet Metab 2004;81:31–44.

14. Fridovich-Keil JL, Walter JH. Galactosaemia chapter 72. D. Valle, B. Vogelstein, K.W. Kinzler, S.E. Antonarakis, A. Ballabio (Eds.), et al., The Online Metabolic and Molecular Bases of Inherited Disease, OMMBID, Part 7: Carbohydrates, McGraw Hill, New York (2008)(Chapter 72). 2008;

15. Hutchesson AC, Murdoch-Davis C, Green A, Preece MA, Allen J, Holton JB, et al. Biochemical monitoring of treatment for galactosaemia: biological variability in metabolite concentrations. J Inherit Metab Dis 1999;22:139–48.

16. Walter JH, Collins JE, Leonard J V. Recommendations for the management of galactosaemia. UK Galactosaemia Steering Group. Arch Dis Child 1999;80:93–6.

17. Jumbo-Lucioni PP, Garber K, Kiel J, Baric I, Berry GT, Bosch A, et al. Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes. J Inherit Metab Dis 2012;35:1037–49.

18. The GRADE Working Group. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2 [updated March 2009]. 2009.

19. Panis B, Vermeer C, van Kroonenburgh MJPG, Nieman FHM, Menheere PPCA, Spaapen LJ, et al. Effect of calcium, vitamins K1 and D3 on bone in galactosemia. Bone 2006;39:1123–9.

20. Pesce MA, Bodourian SH. Clinical significance of plasma galactose and erythrocyte galactose-1-phosphate measurements in transferase-deficient galactosemia and in individuals with below-normal transferase activity. Clin Chem 1982;28:301–5.

21. Milánkovics I, Schuler A, Kámory E, Csókay B, Fodor F, Somogyi C, et al. Molecular and clinical analysis of patients with classic and Duarte galactosemia in western Hungary. Wien Klin Wochenschr 2010;122:95–102.

22. Varela-Lema L, Paz-Valinas L, Atienza-Merino G, Zubizarreta-Alberdi R, Villares RV, López-García M. Appropriateness of newborn screening for classic galactosaemia: a systematic review. J Inherit Metab Dis 2016;1–17.

23. Calderon FRO, Nelson L, Dobrowolski P, Sinitsyna I, Phansalkar A, Longo N, et al. Combination of enzyme analysis, allele-specific PCR and sequencing to detect mutations in the GALT gene. J Inherit Metab Dis 2007;30:818.

24. Li Y, Ptolemy AS, Harmonay L, Kellogg M, Berry GT. Ultra fast and sensitive liquid chromatography tandem mass spectrometry based assay for galactose-1-phosphate uridylyltransferase and galactokinase deficiencies. Mol Genet Metab 2011;102:33–40.

25. Ko D-H, Jun S-H, Park H-D, Song SH, Park KU, Kim JQ, et al. Multiplex enzyme assay for galactosemia using ultraperformance liquid chromatography-tandem mass spectrometry. Clin Chem 2010;56:764–71.

26. Li Y, Ptolemy AS, Harmonay L, Kellogg M, Berry GT. Quantification of galactose-1-phosphate uridyltransferase enzyme activity by liquid chromatography-tandem mass spectrometry. Clin Chem 2010;56:772–80.

27. Lindhout M, Rubio-Gozalbo ME, Bakker JA, Bierau J. Direct non-radioactive assay of galactose-1-phosphate:uridyltransferase activity using high performance liquid chromatography. Clin Chim Acta 2010;411:980–3.

28. Xu YK, Kaufman FR, Donnell GN, Ng WG. Radiochemical assay of minute quantities of galactose-1-phosphate uridyltransferase activity in erythrocytes and leukocytes of galactosemia patients. Clin Chim Acta 1995;235:125–36.

29. Wang BB, Xu YK, Ng WG, Wong LJ. Molecular and biochemical basis of galactosemia. Mol Genet Metab 1998;63:263–9.

30. Elsas LJ, Dembure PP, Langley S, Paulk EM, Hjelm LN, Fridovich-Keil J. A common mutation associated with the Duarte galactosemia allele. Am J Hum Genet 1994;54:1030–6.

31. Palmieri M, Mazur A, Berry GT, Ning C, Wehrli S, Yager C, et al. Urine and plasma galactitol in patients with galactose-1-phosphate uridyltransferase deficiency galactosemia. Metabolism 1999;48:1294–302.

32. Berry GT, Singh RH, Mazur AT, Guerrero N, Kennedy MJ, Chen J, et al. Galactose breath testing distinguishes variant and severe galactose-1-phosphate uridyltransferase genotypes. Pediatr Res 2000;48:323–8.

33. Berry GT, Nissim I, Gibson JB, Mazur AT, Lin Z, Elsas LJ, et al. Quantitative assessment of whole body galactose metabolism in galactosemic patients. Eur J Pediatr 1997;156 Suppl:S43-9.

34. Lai K, Langley SD, Singh RH, Dembure PP, Hjelm LN, Elsas LJ. A prevalent mutation for galactosemia among black Americans. J Pediatr 1996;128:89–95.

35. Berry GT, Nissim I, Lin Z, Mazur AT, Gibson JB, Segal S. Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia. Lancet 1995;346:1073–4.

36. Yager C, Gibson J, States B, Elsas LJ, Segal S. Oxidation of galactose by galactose-1-phosphate uridyltransferase-deficient lymphoblasts. J Inherit Metab Dis 2001;24:465–76.

37. Wehrli SL, Reynolds R, Chen J, Yager C, Segal S. Metabolism of 13C galactose by lymphoblasts from patients with galactosemia determined by NMR spectroscopy. Mol Genet Metab 2002;77:296–303.

38. Coss KP, Hawkes CP, Adamczyk B, Stöckmann H, Crushell E, Saldova R, et al. N-glycan abnormalities in children with galactosemia. J Proteome Res 2014;13:385–94.

39. Kozak L, Francova H, Pijackova A, Macku J, Stastna S, Peskovova K, et al. Presence of a deletion in the 5' upstream region of the GALT gene in Duarte (D2) alleles. J Med Genet 1999;36:576–8.

40. Carney AE, Sanders RD, Garza KR, McGaha LA, Bean LJH, Coffee BW, et al. Origins, distribution and expression of the Duarte-2 (D2) allele of galactose-1-phosphate uridylyltransferase. Hum Mol Genet 2009;18:1624–32.

41. Fridovich-Keil JL, Gambello M, Singh R, Sharer J. Duarte Variant Galactosemia. Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K, Ed GeneReviews® [Internet] Seattle Univ Washington, Seattle; 1993-2015 2014;

42. Ficicioglu C, Thomas N, Yager C, Gallagher PR, Hussa C, Mattie A, et al. Duarte (DG) galactosemia: a pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening. Mol Genet Metab 2008;95:206–12.

43. Badawi N, Cahalane SF, McDonald M, Mulhair P, Begi B, O'Donohue A, et al. Galactosaemia--a controversial disorder. Screening & outcome. Ireland 1972-1992. Ir Med J 1996;89:16–7.

44. Ficicioglu C, Hussa C, Gallagher PR, Thomas N, Yager C. Monitoring of biochemical status in children with Duarte galactosemia: utility of galactose, galactitol, galactonate, and galactose 1-phosphate. Clin Chem 2010;56:1177–82.

45. Badik JR, Castaneda U, Gleason TJ, Spencer JB, Epstein MP, Ficicioglu C, et al. Ovarian function in Duarte galactosemia. FertilSteril 2011;96:469–73.

46. Powell KK, Van Naarden Braun K, Singh RH, Shapira SK, Olney RS, Yeargin-Allsopp M. Long-term speech and language developmental issues among children with Duarte galactosemia. Genet Med 2009;11:874–9.

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47. Lynch ME, Potter NL, Coles CD, Fridovich-Keil JL. Developmental Outcomes of School-Age Children with Duarte Galactosemia: A Pilot Study. JIMD Rep 2015;19:75–84.

48. Ficicioglu C, Yager C, Segal S. Galactitol and galactonate in red blood cells of children with the Duarte/galactosemia genotype. Mol Genet Metab 2005;84:152–9.

49. Schwarz HP, Schaefer T, Bachmann C. Galactose and galactitol in the urine of children with compound heterozygosity for Duarte variant and classical galactosemia (GtD/gt) after an oral galactose load. Clin Chem 1985;31:420–2.

50. Vandenplas Y, Castrellon PG, Rivas R, Gutiérrez CJ, Garcia LD, Jimenez JE, et al. Safety of soya-based infant formulas in children. Br J Nutr 2014;111:1340–60.

51. Adam S, Akroyd R, Bernabei S, Bollhalder S, Boocock S, Burlina A, et al. How strict is galactose restriction in adults with galactosaemia? International practice. Mol Genet Metab 2015;115:23–6.

52. Van Calcar SC, Bernstein LE, Rohr FJ, Scaman CH, Yannicelli S, Berry GT. A re-evaluation of life-long severe galactose restriction for the nutrition management of classic galactosemia. Mol Genet Metab 2014;112:191–7.

53. Berry GT, Palmieri M, Gross KC, Acosta PB, Henstenburg JA, Mazur A, et al. The effect of dietary fruits and vegetables on urinary galactitol excretion in galactose-1-phosphate uridyltransferase deficiency. J Inherit Metab Dis 1993;16:91–100.

54. Berry GT, Moate PJ, Reynolds RA, Yager CT, Ning C, Boston RC, et al. The rate of de novo galactose synthesis in patients with galactose-1-phosphate uridyltransferase deficiency. Mol Genet Metab 2004;81:22–30.

55. Schadewaldt P, Kamalanathan L, Hammen HW, Kotzka J, Wendel U. Endogenous galactose formation in galactose-1-phosphate uridyltransferase. Arch Physiol Biochem 2014;Dec;120:228–39.

56. Ning C, Fenn PT, Blair IA, Berry GT, Segal S. Apparent galactose appearance rate in human galactosemia based on plasma [(13)C]galactose isotopic enrichment. Mol Genet Metab 2000;70:261–71.

57. Bosch AM, Bakker HD, Wenniger-Prick LJM de B, Wanders RJA, Wijburg FA. High tolerance for oral galactose in classical galactosaemia: dietary implications. Arch Dis Child 2004;89:1034–6.

58. Krabbi K, Uudelepp M-L, Joost K, Zordania R, Õunap K. Long-term complications in Estonian galactosemia patients with a less strict lactose-free diet and metabolic control. Mol Genet Metab 2011;103:249–53.

59. Portnoi PA, MacDonald A. Determination of the lactose and galactose content of cheese for use in the galactosaemia diet. J Hum Nutr Diet 2009;22:400–8.

60. Coss KP, Byrne JC, Coman DJ, Adamczyk B, Abrahams JL, Saldova R, et al. IgG N-glycans as potential biomarkers for determining galactose tolerance in Classical Galactosaemia. Mol Genet Metab 2012;105:212–20.

61. Lee PJ, Lilburn M, Wendel U, Schadewaldt P. A woman with untreated galactosaemia. Lancet 2003;362:446.

62. Panis B, Bakker JA, Sels JPJE, Spaapen LJM, van Loon LJC, Rubio-Gozalbo ME. Untreated classical galactosemia patient with mild phenotype. Mol Genet Metab 2006;89:277–9.

63. Panis B, Forget PP, Van Kroonenburgh MJPG, Vermeer C, Menheere PP, Nieman FH, et al. Bone metabolism in galactosemia. Bone 2004;35:982–7.

64. Rubio-Gozalbo ME, Hamming S, van Kroonenburgh MJ, Bakker JA, Vermeer C, Forget PP. Bone mineral density in patients with classic galactosaemia. Arch Dis Child 2002;87:57–60.

65. Gajewska J, Ambroszkiewicz J, Radomyska B, Chelchowska M, Oltarzewski M, Laskowska-Klita T, et al. Serum markers of bone turnover in children and adolescents with classic galactosemia. Adv Med Sci 2008;53:214–20.

66. Gajewska J, Ambroszkiewicz J, Radomyska B, Laskowska-Klita T. Bone turnover markers in prepubertal children with classical galactosemia. Indian J Gastroenterol 2006;25:221–2.

67. El-Bassyouni HT, Ashour AM, Ezzat A, Bassiouni R, Fateen EM. The effect of diet on antioxidant status in patients with galactosemia. J Med Sci 2006;6:452–7.

68. Rutherford PJ, Davidson DC, Matthai SM. Dietary calcium in galactosaemia. J Hum Nutr Diet 2002;15:39–42.

69. Wiesmann UN, Rosé-Beutler B, Schlüchter R. Leguminosae in the diet: the raffinose-stachyose question. Eur J Pediatr 1995;154:93–6.

70. Yager CT, Chen J, Reynolds R, Segal S. Galactitol and galactonate in red blood cells of galactosemic patients. Mol Genet Metab 2003;80:283–9.

71. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr 1993;152:36–43.

72. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis 1990;13:802–18.

73. Cleary MA, Heptinstall LE, Wraith JE, Walter JH. Galactosaemia: relationship of IQ to biochemical control and genotype. J Inherit Metab Dis 1995;18:151–2.

74. Knerr I, Coss KP, Kratzsch J, Crushell E, Clark A, Doran P, et al. Effects of temporary low-dose galactose supplements in children aged 5-12 y with classical galactosemia: a pilot study. Pediatr Res 2015;78:272–9.

75. Doyle CM, Channon S, Orlowska D, Lee PJ. The neuropsychological profile of galactosaemia. J Inherit Metab Dis 2010;33:603–9.

76. Shield JP, Wadsworth EJ, MacDonald A, Stephenson A, Tyfield L, Holton JB, et al. The relationship of genotype to cognitive outcome in galactosaemia. Arch Dis Child 2000;83:248–50.

77. Antshel KM, Epstein IO, Waisbren SE. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology 2004;18:658–64.

78. Widhalm K, Miranda-Da-Cruz B, De Sonneville LMJ. Information processing characteristics and uridine treatment in children with classical galactosemia. Nutr Res 2002;22:257–70.

79. Rasmussen RK, Andreassen AB, Stromme P, Hansen TW. Learning disabilities and language pathology in patients with galactosemia. Logoped Phoniatr Vocol 1996;21:157–62.

Chapter 4

80. Hansen TW, Henrichsen B, Rasmussen RK, Carling A, Andressen AB, Skjeldal O. Neuropsychological and linguistic follow-up studies of children with galactosaemia from an unscreened population. Acta Paediatr 1996;85:1197–201.

81. Kaufman FR, McBride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. Eur J Pediatr 1995;154:S2–5.

82. Schadewaldt P, Hoffmann B, Hammen HW, Kamp G, Schweitzer-Krantz S, Wendel U. Longitudinal assessment of intellectual achievement in patients with classical galactosemia. Pediatrics 2010;125:e374-81.

83. Rubio-Agusti I, Carecchio M, Bhatia KP, Kojovic M, Parees I, Chandrashekar HS, et al. Movement disorders in adult patients with classical galactosemia. Mov Disord 2013;28:804–10.

84. Hoffmann B, Wendel U, Schweitzer-Krantz S. Cross-sectional analysis of speech and cognitive performance in 32 patients with classic galactosemia. J Inherit Metab Dis 2011;34:421–7.

85. Manis FR, Cohn LB, McBride-Chang C, Wolff JA, Kaufman FR. A longitudinal study of cognitive functioning in patients with classical galactosaemia, including a cohort treated with oral uridine. J Inherit Metab Dis 1997;20:549–55.

86. Fishler K, Koch R, Donnell G, Graliker B V. Psychological correlates in galactosemia. Am J Ment Defic 1966;71:116–25.

87. Lee DH. Psychological aspects of galactosaemia. J Ment Defic Res 1972;16:173–91.

88. Potter NL, Lazarus JA, Johnson JM, Steiner RD, Shriberg LD. Correlates of language impairment in children with galactosaemia. J Inherit Metab Dis 2008;31:524–32.

89. Webb AL, Singh RH, Kennedy MJ, Elsas LJ. Verbal dyspraxia and galactosemia. Pediatr Res 2003;53:396–402.

90. Nelson CD, Waggoner DD, Donnell GN, Tuerck JM, Buist NR. Verbal dyspraxia in treated galactosemia. Pediatrics 1991;88:346–50.

91. Shriberg LD, Potter NL, Strand EA. Prevalence and phenotype of childhood apraxia of speech in youth with galactosemia. J Speech Lang Hear Res 2011;54:487–519.

92. Potter NL, Nievergelt Y, Shriberg LD. Motor and speech disorders in classic galactosemia. JIMD Rep 2013;11:31–41.

93. Potter NL. Voice disorders in children with classic galactosemia. J Inherit Metab Dis 2011;34:377–85.

94. Timmers I, Jansma BM, Rubio-Gozalbo ME. From mind to mouth: event related potentials of sentence production in classic galactosemia. PLoS ONE [Electronic Resour 2012;7:e52826.

95. Timmers I, van den Hurk J, Hofman PA, Zimmermann LJ, Uludag K, Jansma BM, et al. Affected functional networks associated with sentence production in classic galactosemia. Brain Res 2015;1616:166–76.

96. Robertson A, Singh RH, Guerrero N V, Hundley M, Elsas LJ. Outcomes analysis of verbal dyspraxia in classic galactosemia. Genet Med 2000;2:142–8.

97. Najafi M, Khodadad A, Khatami GR. Cerebral edema: A rare complication in galactosemia. Med J Islam Repub Iran 2005;18:371–3.

98. Belman AL, Moshe SL, Zimmerman RD. Computed tomographic demonstration of cerebral edema in a child with galactosemia. Pediatrics 1986;78:606–9.

99. Huttenlocher PR, Hillman RE, Hsia YE. Pseudotumor cerebri in galactosemia. J Pediatr 1970;76:902–5.

100. Viggiano E, Marabotti A, Burlina AP, Cazzorla C, D'Apice MR, Giordano L, et al. Clinical and molecular spectra in galactosemic patients from neonatal screening in northeastern Italy: structural and functional characterization of new variations in the galactose-1-phosphate uridyltransferase (GALT) gene. Gene 2015;559:112–8.

101. Nelson MD, Wolff JA, Cross CA, Donnell GN, Kaufman FR. Galactosemia: evaluation with MR imaging. Radiology 1992;184:255–61.

102. Dubroff JG, Ficicioglu C, Segal S, Wintering NA, Alavi A, Newberg AB. FDG-PET findings in patients with galactosaemia. J Inherit Metab Dis 2008;31:533–9.

103. Aydin-Özemir Z, Tekturk P, Uyguner ZO, Baykan B. Galactosemia and phantom absence seizures. J Pediatr Neurosci 2014;9:253–6.

104. Karadag N, Zenciroglu A, Eminoglu FT, Dilli D, Karagol BS, Kundak A, et al. Literature review and outcome of classic galactosemia diagnosed in the neonatal period. Clin Lab 2013;59:1139–46.

105. Wang ZJ, Berry GT, Dreha SF, Zhao H, Segal S, Zimmerman RA. Proton magnetic resonance spectroscopy of brain metabolites in galactosemia. Ann Neurol 2001;50:266–9.

106. Moller HE, Ullrich K, Vermathen P, Schuierer G, Koch HG. In vivo study of brain metabolism in galactosemia by 1H and 31P magnetic resonance spectroscopy. Eur J Pediatr 1995;154:S8-13.

107. Timmers I, Zhang H, Bastiani M, Jansma BM, Roebroeck A, Rubio-Gozalbo ME. White matter microstructure pathology in classic galactosemia revealed by neurite orientation dispersion and density imaging. J Inherit Metab Dis 2015;38:295–304.

108. Gubbels CS, Maurice-Stam H, Berry GT, Bosch AM, Waisbren S, Rubio-Gozalbo ME, et al. Psychosocial developmental milestones in men with classic galactosemia. J Inherit Metab Dis 2011;34:415–9.

109. Bosch AM, Maurice-Stam H, Wijburg FA, Grootenhuis MA. Remarkable differences: the course of life of young adults with galactosaemia and PKU. J Inherit Metab Dis 2009;32:706–12.

110. Bhat M, Haase C, Lee PJ. Social outcome in treated individuals with inherited metabolic disorders: UK study. J Inherit Metab Dis 2005;28:825–30.

111. Hoffmann B, Dragano N, Schweitzer-Krantz S. Living situation, occupation and health-related quality of life in adult patients with classic galactosemia. J Inherit Metab Dis 2012;35:1051–8.

112. Rubio-Gozalbo ME, Panis B, Zimmermann LJ, Spaapen LJ, Menheere PPCA. The endocrine system in treated patients with classical galactosemia. Mol Genet Metab 2006;89:316–22.

113. Bosch AM, Grootenhuis MA, Bakker HD, Heijmans HSA, Wijburg FA, Last BF. Living with classical galactosemia: health-related quality of life consequences. Pediatrics 2004;113:e423-8.

114. Lambert C, Boneh A. The impact of galactosaemia on quality of life--a pilot study. J Inherit Metab Dis 2004;27:601–8.

Chapter 4

115. ten Hoedt AE, Maurice-Stam H, Boelen CCA, Rubio-Gozalbo ME, van Spronsen FJ, Wijburg FA, et al. Parenting a child with phenylketonuria or galactosemia: implications for health-related quality of life. J Inherit Metab Dis 2011;34:391–8.

116. Kaufman FR, Xu YK, Ng WG, Donnell GN. Correlation of ovarian function with galactose-1-phosphate uridyl transferase levels in galactosemia. J Pediatr 1988;112:754–6.

117. Sanders RD, Spencer JB, Epstein MP, Pollak S V, Vardhana PA, Lustbader JW, et al. Biomarkers of ovarian function in girls and women with classic galactosemia. Fertil Steril 2009;92:344–51.

118. Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo ME. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357–66.

119. Rubio-Gozalbo ME, Gubbels CS, Bakker JA, Menheere PP, Wodzig WK, Land JA. Gonadal function in male and female patients with classic galactosemia. Hum. Reprod. Update 2010;16:177–88.

120. Gubbels CS, Land JA, Rubio-Gozalbo ME. Fertility and impact of pregnancies on the mother and child in classic galactosemia. Obstet Gynecol Surv 2008;63:334–43.

121. Forges T, Monnier-Barbarino P, Leheup B, Jouvet P. Pathophysiology of impaired ovarian function in galactosaemia. Hum Reprod Updat 2006;12:573–84.

122. Liu G, Hale GE, Hughes CL. Galactose metabolism and ovarian toxicity. Reprod Toxicol 2000;14:377–84.

123. Prestoz LL, Couto AS, Shin YS, Petry KG. Altered follicle stimulating hormone isoforms in female galactosaemia patients. Eur J Pediatr 1997;156:116–20.

124. Gubbels CS, Thomas CMG, Wodzig WKWH, Olthaar AJ, Jaeken J, Sweep FCGJ, et al. FSH isoform pattern in classic galactosemia. J Inherit Metab Dis 2011;34:387–90.

125. Gubbels CS, Land JA, Evers JLH, Bierau J, Menheere PPCA, Robben SGF, et al. Primary ovarian insufficiency in classic galactosemia: role of FSH dysfunction and timing of the lesion. J Inherit Metab Dis 2013;36:29–34.

126. O'Herlihy C, Danks D. Endocrine evidence of prenatal follicular destruction in girls with galactosaemia. Pediatr Adolesc Gynecol 1985;3:1–6.

127. Guerrero N V, Singh RH, Manatunga A, Berry GT, Steiner RD, Elsas LJ. Risk factors for premature ovarian failure in females with galactosemia. J Pediatr 2000;137:833–41.

128. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;

129. Spencer JB, Badik JR, Ryan EL, Gleason TJ, Broadaway KA, Epstein MP, et al. Modifiers of ovarian function in girls and women with classic galactosemia. J Clin Endocrinol Metab 2013;98:E1257-65.

130. Van Erven B, Gubbels CS, van Golde RJ, Dunselman GA, Derhaag JG, De WG, et al. Fertility preservation in female classic galactosemia patients. Orphanet J Rare Dis 2013;8:107.

131. Sauer M V, Kaufman FR, Paulson RJ, Lobo RA. Pregnancy after oocyte donation to a woman with ovarian failure and classical galactosemia. Fertil Steril 1991;55:1197–9.

132. Gubbels CS, Welt CK, Dumoulin JC, Robben SGF, Gordon CM, Dunselman GA, et al. The male reproductive system in classic galactosemia: Cryptorchidism and low semen volume. J Inherit Metab Dis 2013;36:779–86.

133. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, El-Hajj Fuleihan G, Kecskemethy HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom 2014;17:225–42.

134. ISCD. http://www.iscd.org/official-positions/6th-iscd-position-development-conference-adult/. Date last accessed 2015-11-292015;

135. Kaufman FR, Loro ML, Azen C, Wenz E, Gilsanz V. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. J Pediatr 1993;123:365–70.

136. Batey LA, Welt CK, Rohr F, Wessel A, Anastasoaie V, Feldman HA, et al. Skeletal health in adult patients with classic galactosemia. Osteoporos Int 2013;24:501–9.

137. Doulgeraki A, Monopolis I, Deligianni D, Kalogerakou M, Schulpis KH. Body composition in young patients with galactose metabolic disorders: a preliminary report. J Pediatr Endocrinol Metab 2014;27:81–6.

138. Jones IE, Williams SM, Dow N, Goulding A. How many children remain fracture-free during growth? a longitudinal study of children and adolescents participating in the Dunedin Multidisciplinary Health and Development Study. Osteoporos Int 2002;13:990–5.

139. Widger J, O'Toole J, Geoghegan O, O'Keefe M, Manning R. Diet and visually significant cataracts in galactosaemia: is regular follow up necessary? J Inherit Metab Dis 2010;33:129–32.

140. Kossowicz H, Zbieg Sendecka E. Cataracts in galactosemia. Metab Ophthalmol 1977;1:115–6.

141. Burke JP, O'Keefe M, Bowell R, Naughten ER. Ophthalmic findings in classical galactosemia--a screened population. J Pediatr Ophthalmol Strabismus 1989;26:165–8.

142. Burke JP, O'Keefe M, Bowell R, Naughten ER. Cataracts in children with classical galactosaemia and in their parents. J Inherit Metab Dis 1988;11 Suppl 2:246–8.

143. Beigi B, O'Keefe M, Bowell R, Naughten E, Badawi N, Lanigan B. Ophthalmic findings in classical galactosaemia--prospective study. Br J Ophthalmol 1993;77:162–4.

144. Portnoi PA, MacDonald A. The Lactose and Galactose Content of Cheese Suitable for Galactosaemia: New Analysis. JIMD Rep 2015;

145. Bernstein LE, Van Calcar SC, Rohr F, Yanicelli S, Cusimano C. Galactosemia: The Diet. Children's Hospital Colorado in collaboration with the Galactosemia Foundation Task Force. 2014.

Chapter 4

PART 3

Long-term complications studied in more depth

5

Chapter 5

Systematic review and meta-analysis of intelligence quotient in early-treated individuals with classical galactosemia

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Abstract

Introduction Cognitive impairment is a well-known complication of classical galactosemia (CG). Differences in patient characteristics and test methods have hampered final conclusions regarding the extent of intellectual disabilities in CG. The primary aim of this systematic review was to assess intellectual performance in early-treated (≤4 weeks of life) individuals with confirmed CG (defined by absent or barely detectable GALT enzyme activity and/or the presence of two null or severe missense variations), assessed with comparable test instruments. The full scale IQ (FSIQ) was the variable of interest.

Methods A clinical librarian developed search strategies and two independent investigators performed the study selection, risk of bias assessment, and data-extraction. Individual patient data were pooled for meta-analysis using linear mixed-effect models with a random intercept per study, and including covariates (age or gender) as fixed effects where appropriate.

Results Four articles were included in this meta-analysis. Data of 87 individuals (median age 13 years, range 3 to 38 years) were used to assess mean FSIQ in CG. The FSIQ ranged from 47 to 122, and the mean score was 87 (95% CI; 81 to 94). Forty-five percent of individuals attained scores <85, almost forty percent attained scores of 85-100, and a minority (15%) attained scores above 100. There was no significant correlation between FSIQ and age.

Conclusions Results from this meta-analysis fortify conclusions from previous studies that early-treated individuals with CG are at risk for having impaired cognitive abilities. However, IQ varies considerably between affected individuals.

Introduction

Classical galactosemia (CG, MIM 230400) is an inborn error of galactose metabolism, caused by a severe deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12). The incidence in Europe and the United States has been estimated for several countries and varies from 1 case per 19.000 to 1 case per 44.000 individuals [1–4]. When exposed to galactose from breast milk or infant formula, affected newborn infants develop life-threatening symptoms that quickly resolve once a galactose-restricted diet is initiated. A very strict life-long galactose-restricted diet is the only available treatment since CG was first reported in more detail [5]. Despite good dietary adherence, individuals are at risk for developing long-term complications including speech and language impairment and impaired cognitive abilities [2]. Evaluation of cognitive abilities, including measurement of intelligence, is therefore an important aspect of the follow-up of children and adults. Studies show mean IQ scores below average, however, with large interindividual variability [1,6–20], with many patients (45-72%) scoring in the low to very low range (IQ <85)[9,13,15,21]. Start of treatment after the 8th week of life seems to increase the risk for decreased cognitive abilities [8,9]. A negative correlation between age and IQ in individuals is reported in cross-sectional studies [7,8,18], but was not confirmed by longitudinal studies [8,19,22,23]. Drawing conclusions from the individual studies about the extent of cognitive impairment in CG remains controversial, because many studies did not (1) define the diagnosis of CG, possibly including individuals with a variant form of galactosemia who have a better outcome; (2) define inclusion criteria (patients may have been tested on indication [cognitive impairment]), resulting in sampling bias; and (3) report age at start of dietary treatment. Patient samples were frequently small, results of different instruments could not be compared, and study results were presented in variable manners. Because results may vary from one study to another, validity of a hypothesis cannot be based on the results of single studies. An individual patient data (IPD) meta-analysis systematically assesses the results of previous studies to derive conclusions about that body of research [24]. It pools patient-level data from multiple studies in a statistically responsible manner, including individual patients according to predefined inclusion and exclusion criteria. A meta-analysis can increase power of the results and may include a more precise estimate of the outcome than any individual study contributing to the pooled analysis [25].

The primary aim of this systematic review with meta-analysis was to assess cognitive abilities in a large group of individuals with a confirmed diagnosis of CG, who commenced dietary treatment before the age of 4 weeks and who were not tested on because of (suspected) cognitive impairment. The primary variable of interest was

full scale intelligence quotient (FSIQ), determined by comparable test instruments. Secondary aims were to assess a possible correlation between FSIQ and age and to evaluate performance IQ (PIQ) and verbal IQ (VIQ) scores in individuals with this disorder.

Methods

Eligibility criteria

Inclusion and exclusion criteria
In the meta-analysis, these criteria apply to individual patients.

Patients: Individuals of all ages with a confirmed diagnosis of CG, defined by a profound impairment of GALT enzyme activity (absent or barely detectable) and/or the presence of two null or severe missense variations. Individuals with reported residual GALT enzyme activity >1%, as well as individuals from the Traveller community, were excluded. To avoid any influence of later treatment on cognition, we decided to include only patients with age at diagnosis/start of dietary treatment ≤4 weeks of life.
Outcome: Cognitive testing with any of the following test instruments yielding full/total IQ score: any version of the Wechsler Preschool and Primary Scale of Intelligence (WPSSI), Wechsler Intelligence Scale for Children (WISC), Wechsler Adult Intelligence Scale (WAIS), the McCarthy Scales of Children's Abilities or Stanford-Binet (and any translated, validated versions of these test instruments).
Study design: Randomized controlled trials (RCT), non-RCT, cohort studies, case-control studies; cross-sectional studies and case series were included, case reports and (systematic) reviews were excluded. Only studies in which the majority of patients treated in a specific hospital participated, were included, aiming to avoid bias caused by only testing patients because of (suspected) cognitive impairment. Studies published ≥1980 were included, as it was not considered feasible to contact authors of the articles published before 1980.
Characteristics: Human studies, studies conducted in any year, and articles (including conference abstracts) written in English were included.

It is well known that the Irish Traveller community may have decreased access to full educational opportunities. Traveller pupils are reported to have lower levels of achievement and are more likely to be identified as having special educational needs than pupils from other ethnic groups in Ireland [26]. It was not possible to correct for parental educational or socioeconomic status as they were not reported in any of the original studies. Therefore, to prevent bias caused by reduced access to schooling by members of the Traveller community, we excluded the FSIQ data reported in Coss et al. (2013) from this group[1].

Identifying studies
A computerized search was conducted by a clinical librarian and one of the inves-

tigators (LW) in EMBASE, MEDLINE, PsycInfo and the Cochrane Library up to June 2015, who also hand-searched reference lists of included articles, conference abstracts and narrative and systematic reviews for additional relevant articles. Search strategies for each database are presented in Supplement 1.

Study selection process
Two independent investigators (AMB, LW) performed the initial selection of potentially eligible articles, by screening titles of identified records. Thereafter, these investigators read abstracts (or the whole article in case an abstract was lacking), to make a more precise selection of potentially eligible articles. Of these studies, the full-text articles were read and selected based on predefined eligibility criteria. Study selection process for the meta-analysis of FSIQ (Figure 1) and the study selection process of the analysis of PIQ and VIQ were performed separately. Based on the IPD, patients not meeting all inclusion criteria were excluded from the meta-analysis.

Data collection process: Individual Patient Data
Authors of all relevant articles were contacted via e-mail with the request to share IPD. Multiple attempts, contacting different authors, were undertaken to obtain IPD.

Data items: Individual Patient Data
Variables collected at a participant-level were: sex, age at diagnosis and/or initiation of dietary treatment, age at testing, test instrument used, and individual FSIQ. If applicable: if patients were from a Traveller community. If individuals had been tested multiple times, the most recent score was used in the analysis.

Individual Patient Data integrity
All IPD received from the authors were checked for completeness. If data were incomplete, or if data included a sample that differed from the original article, authors were asked to explain these differences. Additional data were incorporated in the dataset only if they met inclusion criteria used for the systematic review, thus preventing selection bias.

Risk of bias assessment in individual studies
The Scottish Intercollegiate Guidelines Network (SIGN) quality appraisal checklists to assess bias were used (for RCT, non-RCT, cohort study and case-control study designs). For cross-sectional studies and case series, no standardized critical appraisal checklists were available, and risk of bias assessment was performed by systematically judging the study population in terms of reliability of the diagnosis of CG. Two
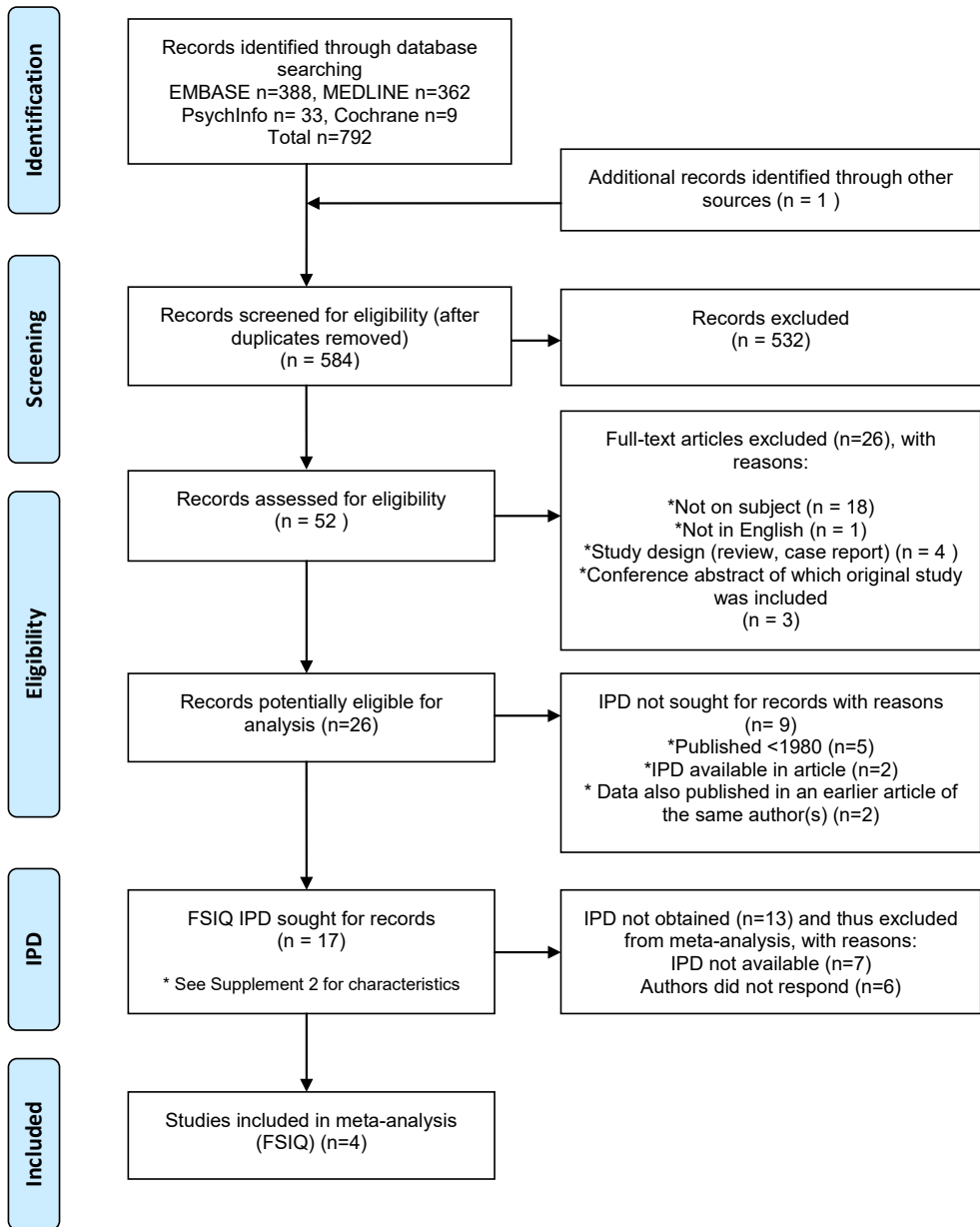
Figure 1. Flow diagram of study selection process for the meta-analysis of full-scale IQ

independent investigators performed risk of bias assessment (AMB, LW).

Specification of outcomes and effect measures
The primary outcome investigated in this systematic review is cognitive ability, expressed as a FSIQ. Secondary outcomes are VIQ and PIQ.

Statistical analysis
We present age in terms of median and range, with no correction for clustering within studies. We tested for homogeneity of variances of FSIQ across the studies using an univariate test for equal distributions [27]. We used linear mixed models [28] with study as a random intercept, to obtain estimates of overall mean FSIQ with 95% confidence intervals (CI) for the means and *p*-values of differences between subgroups based on dichotomized age and sex. We used similar models to assess the association between FSIQ and age in years as a continuous variable. We considered *p*-values less than 0.05 statistically significant. We used R version 3.2.2015-12-04[29] and the packages lme4 [30] and energy [31] to perform the meta-analysis and IBM SPSS Statistics version 22 for descriptive analyses.

Results

Study selection process
A total of 584 articles were identified by our search strategies (without doubles), of which 532 were discarded because they did not address our research question.

Meta-Analysis
The study selection process for the meta-analysis is presented in Figure 1. Of the 26 potentially eligible articles, IPD were sought for a total of 17 articles potentially eligible, and authors of these articles were contacted (for characteristics of these studies see Supplement 2 Data retrieval, via http://www.thesisgalt.nl/). IPD were not sought for five articles published before 1980 [6,7,22,32], for two articles in which IPD was already available [12,13] and for two articles that reported data also published in an earlier article of the same author(s)[33,34]. Finally, IPD from four of these 17 articles were included in the meta-analysis [1,4,17,35]. Due to our inclusion and exclusion criteria, IPD of 87/199 individuals could be included in this meta-analysis. The first and/or last author of each article ascertained that the reported patients were not a selection of individuals tested because of (suspected) cognitive impairment, but were a good representation of the group of patients followed at that time.

Secondary outcomes
No articles addressing PIQ and VIQ (n= 5) met all inclusion criteria, and therefore zero studies were included.

Risk of bias within studies and quality of evidence
None of the selected studies had a study design for which a risk of bias checklist from SIGN was available.

Meta-analysis Full scale Intelligence Quotient
Four studies were included in our meta-analysis [1,4,17,35] with relatively small patient sample sizes in the original studies (varying from 8 to 85 patients tested). Data of 87 individuals were pooled in the meta-analysis, 54 males and 33 females, with a median age of 13 years (range 3-38 years).
Antshel et al. assessed the neuropsychological profile (including FSIQ) in a cross-sectional study in 25 children and adolescents, homozygous for the c.563A>G (p.Gln188Arg) variation in the *GALT* gene (NM_000155.3), and tested with the WISC-III[17]. FSIQ scores of two originally tested patients (age 11 and 13 years) were not reported in the article (because the patients were excluded for further neuropsychological testing in that study), but FSIQ scores were provided for the meta-analysis. Therefore a total of 27 children from this study were included, 17 males and 10

females, median age 11 years (range 8 to 14 years).

In a retrospective study, Coss et al assessed long-term outcome in 130 individuals [1]. At total of 85 individuals, aged 6-39 years, were administered IQ tests (32/63 Travellers and 53/67 non-Travellers). Tests used were the WPPSI-III (Third UK Edition) for children aged 2 years and 6 months to 7 years and 3 months, the WISC-IV generally used for children aged 6 to 16 years and the WAIS-IV for individuals aged 16 years onward. All Travellers were excluded for reasons described in the Methods section. Furthermore, we excluded six non-Traveller individuals who had been diagnosed >4 weeks of life and/or had a variant form of galactosemia. Nine non-Traveller individuals not included in the original article were tested under the same protocol after the study was finished, and one female patient (15 years, FSIQ 90) meeting the inclusion criteria was included in this meta-analysis. A total of 48 non-Traveller individuals were thus included in our meta-analysis, and their median age was 19 years (range 6 to 38 years). All but three of these 48 individuals were homozygous for the c.563A>G (p.Gln188Arg) variation, two individuals were heterozygous for the known pathologic c.563A>G (p.Gln188Arg)/ c.997C>T (p.Arg333Trp), and one individual was heterozygous for c.563A>G (p.Gln188Arg)/c.598delC (p.Gln200Serfs).

Waisbren et al 1983 reported IQ scores in eight individuals, two males and six females, in a cross-sectional study [35]. This was the complete group of children, identified at an early age (by NBS), and followed in their hospital at that time. Tests used were the McCarthy Scales of Children's Abilities, the Stanford-Binet (ages 3-6 years) and the WISC (ages 7 years and older). One patient was excluded because of a 5% residual GALT activity compared to virtually no residual activity in the other individuals. Thus, seven individuals (with a median age of 6 years, range 3-11 years) were included in our meta-analysis.

Waisbren et al 2012 assessed the adult phenotype, including FSIQ, in a cross-sectional study including 33 individuals aged 18-59 years [4]. All had absent GALT activity and two severe or null variations in the *GALT* gene and were administered the WAIS. Of these patients, only five could be included in this meta-analysis, because age at diagnosis was not known for the others. Their median age at testing was 20 years (range 18 to 23 years).

Full-scale Intelligence Quotient in CG

The mean FSIQ in the four separate studies, calculated with the obtained IPD, were Antshel et al 2004 mean 82 (SD 11.6), Coss et al 2013 mean 83 (SD 18.0), Waisbren et al 1983 mean 95 (SD 14.5) and Waisbren et al 2012 mean 100 (SD 20.6)[1,4,17,35]. The univariate test of equal distributions with FSIQ data of all individuals included from the four studies indicated that the distribution of FSIQ scores across the four studies was not homogeneous (*p*=0.02), justifying the use of linear mixed models to obtain mean FSIQ scores.

Following correction for the grouping of patients within studies, data from 87 individuals (median age 13 years, range 3 to 38 years) from these four studies revealed FSIQ range from 47 to 122, and a group mean of 87 (95% CI; 81 to 94). In all, 13 individuals (15%) attained scores lower than two standard deviations below the general population mean (FSIQ <70), 27 individuals (31%) performed between one and two standard deviations below the population mean (FSIQ 70-84), 34 individuals (39%) attained scores within one standard deviation below the population mean (FSIQ 85-100), and 13 individuals (15%) attained scores above 100.

Males and females
The mean FSIQ score of 54 males(median age 13 years, range 3 to 35 years) was 92 (95% CI, 81 to 103). The mean FSIQ score of 33 females (median age 16 years, range 8 to 38 years) was 81 (95% CI, 77 to 86). The difference in mean FSIQ between males and females was not statistically significant (5.5 points, 95% CI -1.4 to 12.5, *p* = 0.1241). The estimate of the difference between mean FSIQ in males and females is different from the difference between the estimates for males and females, because of the correction for the difference between the studies in the meta-analysis.

FSIQ score and age
There was no statistically significant association between FSIQ and age (increase of 0.4 points per year, 95% CI -0.1 to 0.9, *p*=0.1146).

Performance and Verbal IQ scores in Early-Treated CG Individuals
No studies addressing PIQ and VIQ scores that met all our inclusion criteria were identified.

Chapter 5

Discussion

In this systematic review and meta-analysis we demonstrate, to our knowledge in the largest and most homogeneous sample with early-treated individuals with a confirmed diagnosis, that individuals with the classical form of galactosemia are at risk for cognitive impairment. The mean FSIQ in pooled data of 87 children and adults with CG was found to be 87, which is at the 19th percentile of the general population and substantially lower compared to the general population mean. Compared to the general population, a large percentage of individuals perform in the borderline to low average intellectual range (FSIQ 70-85) and in the range of intellectual disability (FSIQ <70). Slightly more individuals perform within one SD below the general population mean (FSIQ 85-100), and fewer individuals attained a FSIQ score above 100. The range of FSIQ in patients is remarkably wide. Thus, developmental and neuropsychological assessments are important in clinical follow-up to identify individuals in need of early intervention, special education or other supportive services. No statistical difference was found between mean FSIQ in males and females (5.5 points, 95% CI -1.4 to 12.5, p = 0.1241). However, the sample sizes were small, consequently the confidence interval was relatively wide, and therefore such a difference between males and females cannot be ruled out. Biological factors (hereditary traits) and environmental factors may influence cognitive development, such as perinatal factors, individual and parental educational opportunities and socioeconomic status. One very important factor was taken into account in this study, by excluding patients who started treatment after the first month of life. Because the other environmental factors were not reported in the original studies, these could not be accounted for in this meta-analysis.

To date, no consensus exists regarding whether CG is a progressive disorder, with deteriorating cognitive abilities. This systematic review and meta-analysis provided no indication of deterioration of FSIQ over time. However, information about FSIQ after the fourth decade of life is lacking. Our finding contradicts results from other cross-sectional studies, which reported a correlation between age and cognition, and supports results from longitudinal studies demonstrating that FSIQ does not correlate with age [8,19,22,23]. This difference may be explained by a cohort effect in these cross-sectional studies, whereby patients born before a certain date did not have access to the modern standard of care and diagnosis techniques. This may have resulted in later diagnosis and start of treatment in the elder patients in these studies, possibly negatively affecting cognitive outcome. In these studies, age at diagnosis and start of treatment was frequently not taken into account or not reported. This may falsely result in the impression that cognitive abilities regress over time. In our study, this effect was eliminated by applying strict inclusion criteria, with all patients being treated soon after birth. Furthermore, additional studies are

needed to assess potential differences in VIQ and PIQ.

This study has several strengths and limitations. Strengths are the large number of patients included compared to previous studies assessing cognitive abilities and the homogeneous sample of early-diagnosed individuals with a confirmed diagnosis of CG, increasing power and precision of the results. Our results confirm and, importantly, fortify the conclusions of previously published studies, settling controversies arising from these previous studies. By only including studies that did not test patients because of (suspected) cognitive impairment, the risk of sampling bias was kept as small as possible. The results of this study are likely the most representative published to date. Limitations are the small patient sample sizes of the included studies, and in some cases not all patients reported in the original paper could be included in our review. We used mixed-effects models to estimate group mean FSIQ. Occasionally, these models may produce biased parameter estimates when the number of groups or individuals within groups is very small [36]. However, we feel that using random effects to account for the group structure is more appropriate than using fixed effects, as the patient selections may have been subtly and functionally different between studies, though studies that tested patients on indication were excluded [37]. The small patient sample in Waisbren et al. 1983 is most likely the cause of the smaller range of FSIQ demonstrated in that group of patients and it is unlikely that this is caused by selection bias because all patients met our definition of CG[35]. Since only cross-sectional data were used in this review and the majority of adult FSIQ scores were from one study, conclusions about FSIQ scores over time must be interpreted with caution.

Future research is needed to determine the factors that contribute to the broad variability in cognitive functioning in CG, predicting which patients are at risk for a low FSIQ [38]. This could lead to interventions or modifications in treatment that may allow each individual to reach his/her full cognitive potential. Neuropsychological testing incorporated in regular clinical follow-up will identify infants and toddlers in need of early intervention and school-age children in need of special education or other supportive services.

Chapter 5

Conclusions

Early-treated individuals with confirmed classical galactosemia, defined by a profound impairment of GALT enzyme activity (absent or barely detectable) and/or the presence of two null or severe missense variations, with age at diagnosis/start of dietary treatment ≤4 weeks of age, are at risk for having impaired cognitive abilities, with almost two-thirds of individuals performing more than a standard deviation below the normative mean of 100 (FSIQ range ≤85), a quarter performing in the low average to average range (FSIQ 85-100) and a minority (15%) attaining scores ≥100. These results confirm the wide range in cognitive functioning in individual patients. There is no indication that FSIQ deteriorates over time, as assessed in patients up to 38 years of age, but prospective longitudinal studies following FSIQ in individual patients, also after the third decade of life, are needed to confirm this impression.

References

1. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis 2013;36:21–7.

2. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis 2006;29:516–25.

3. Ounap K, Joost K, Temberg T, Krabbi K, Tõnisson N. Classical galactosemia in Estonia: selective neonatal screening, incidence, and genotype/phenotype data of diagnosed patients. J Inherit Metab Dis 2010;33:175–6.

4. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis 2012;35:279–86.

5. Jones NL, Leak D. The treatment of congenital galactosaemia. Arch Dis Child 1959;34:307–11.

6. Donnell GN, Collado M, Koch R. Growth and development of children with galactosemia. J Pediatr 1961;58:845–51.

7. Komrower GM, Lee DH. Long-term follow-up of galactosaemia. Arch Dis Child 1970;45:367–73.

8. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis 1990;13:802–18.

9. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr 1993;152:36–43.

10. Kaufman FR, Reichardt JK, Ng WG, Xu YK, Manis FR, McBride-Chang C, et al. Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridyltransferase gene. J Pediatr 1994;125:225–7.

11. Kaufman FR, McBride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. Eur J Pediatr 1995;154:S2–5.

12. Hansen TW, Henrichsen B, Rasmussen RK, Carling A, Andressen AB, Skjeldal O. Neuropsychological and linguistic follow-up studies of children with galactosaemia from an unscreened population. Acta Paediatr 1996;85:1197–201.

13. Rasmussen RK, Andreassen AB, Stromme P, Hansen TW. Learning disabilities and language pathology in patients with galactosemia. Logoped Phoniatr Vocol 1996;21:157–62.

14. Badawi N, Cahalane SF, McDonald M, Mulhair P, Begi B, O'Donohue A, et al. Galactosaemia--a controversial disorder. Screening & outcome. Ireland 1972-1992. Ir Med J 1996;89:16–7.

15. Shield JP, Wadsworth EJ, MacDonald A, Stephenson A, Tyfield L, Holton JB, et al. The relationship of genotype to cognitive outcome in galactosaemia. Arch Dis Child 2000;83:248–50.

16. Widhalm K, Miranda-Da-Cruz B, De Sonneville LMJ. Information processing characteristics and uridine treatment in children with classical galactosemia. Nutr Res 2002;22:257–70.

17. Antshel KM, Epstein IO, Waisbren SE. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology 2004;18:658–64.

18. Doyle CM, Channon S, Orlowska D, Lee PJ. The neuropsychological profile of galactosaemia. J Inherit Metab Dis 2010;33:603–9.

19. Schadewaldt P, Hoffmann B, Hammen HW, Kamp G, Schweitzer-Krantz S, Wendel U. Longitudinal assessment of intellectual achievement in patients with classical galactosemia. Pediatrics 2010;125:e374-81.

20. Rubio-Agusti I, Carecchio M, Bhatia KP, Kojovic M, Parees I, Chandrashekar HS, et al. Movement disorders in adult patients with classical galactosemia. Mov Disord 2013;28:804–10.

21. Hoffmann B, Wendel U, Schweitzer-Krantz S. Cross-sectional analysis of speech and cognitive performance in 32 patients with classic galactosemia. J Inherit Metab Dis 2011;34:421–7.

22. Fishler K, Koch R, Donnell G, Graliker B V. Psychological correlates in galactosemia. Am J Ment Defic 1966;71:116–25.

23. Manis FR, Cohn LB, McBride-Chang C, Wolff JA, Kaufman FR. A longitudinal study of cognitive functioning in patients with classical galactosaemia, including a cohort treated with oral uridine. J Inherit Metab Dis 1997;20:549–55.

24. Haidich AB. Meta-analysis in medical research. Hippokratia 2010;14:29–37.

25. The Cochrane Collaboration. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. 2011.

26. Wilkin A, Derrington C, White R, Martin K, Foster B, Kinder K, et al. Improving the Outcomes for Gypsy, Roma and Traveller Pupils: Final Report: Research Brief. Dep Educ 2010;138.

27. Rizzo M, Székely G. DISCO analysis: A nonparametric extension of analysis of variance. Ann Appl Stat 2010;4:1034–55.

28. Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. Chapter 5. Mixed Effects Modelling for Nested Data. In: Mixed Effects Models and Extensions in Ecology with R. New York, NY: Springer New York; 2009.

29. R Core Team. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. 2014;

30. Bates D, Maechler M, Bolker B, Walker S. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://CRAN.R-project.org/package=lme4. 2014;

31. Rizzo M, Székely G. Energy: E-statistics (energy statistics). R package version 1.6.2. http://CRAN.R-project.org/package=energy. 2014;

32. Fishler K, Donnell GN, Bergren WR, Koch R. Intellectual and personality development in children with galactosemia. Pediatrics 1972;50:412–9.

33. Waggoner DD, Buist NRM. Long-term complications in treated galactosemia. 175 US cases. Int Pediatr 1993;8:97–100.

34. Hughes J, Ryan S, Lambert D, Geoghegan O, Clark A, Rogers Y, et al. Outcomes of siblings with classical galactosemia. J Pediatr 2009;154:721–6.

35. Waisbren SE, Norman TR, Schnell RR, Levy HL. Speech and language deficits in early-treated children with galactosemia. J Pediatr 1983;102:75–7.

36. Maas CJM, Hox JJ. Sufficient sample sizes for multilevel modeling. Methodology 2005;1:86–92.

37. Borenstein M, Hedges L V, Higgins JPT, Rothstein HR. Introduction to Meta-Analysis. John Wiley and Sons Ltd; 2009. page 83–4.

38. Coss KP, Treacy EP, Cotter EJ, Knerr I, Murray DW, Shin YS, et al. Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism? Mol Genet Metab 2014;113:177–87.

Supplement 1. Search strategies

* Search strategies were created in Embase. In order to replicate search, please use Embase

Medline June 2015*

1	galactosemias/
2	(galactosem* or ((galt or utp hexose 1 phosphate uridyltransferase or gale or galk or galactokinase) adj3 deficien*)).tw.
3	1 or 2
4	exp psychologic test/
5	((intelligence or psychological or language or neuropsychological) adj3 test*).tw,kw.
6	speech therapy/
7	((language or speech or logoped*) adj3 (therap* or educat* or train*)).tw,kw.
8	exp hearing disorder/
9	(((auditor* or hearing) adj3 (disorder* or problem*)) or apraxia or dyspraxia).tw,kw.
10	executive function/ or (executive adj3 function*).tw,kf.
11	exp intelligence/
12	exp memory disorder/
13	(iq or intelligence quotient or amnesia or memory or problem solving).tw,kw.
14	exp *galactosemia/co
15	(long term adj5 (outcom* or therap* or treatment*)).tw,kw.
16	clinical study/ or case control study/
17	exp language disorders/
18	exp "rehabilitation of speech and language disorders"/
19	exp Cognition Disorders/
20	exp Mental Disorders/et [Etiology]
21	Developmental Disabilities/et [Etiology]
22	exp epidemiologic studies/ or (prospective or retrospective or cohort or case control*).tw,kf.
23	or/4-22
24	3 and 23

EMBASE June 2015*

1	galactosemia/
2	(galactosem* or ((galt or utp hexose 1 phosphate uridyltransferase or gale or galk or galac-tokinase) adj3 deficien*)).tw.
3	1 or 2
4	exp psychologic test/
5	exp psychometry/
6	((intelligence or psychological or language or neuropsychological) adj3 test*).tw,kw.
7	exp language disability/
8	speech therapy/
9	((language or speech or logoped*) adj3 (therap* or educat* or train*)).tw,kw.
10	exp hearing disorder/
11	(((auditor* or hearing) adj3 (disorder* or problem*)) or apraxia or dyspraxia).tw,kw.
12	cognitive defect/ or (cognitive adj3 (defect* or disorder*)).tw,kw.
13	executive function/ or (executive adj3 function*).tw,kw.
14	exp intelligence/
15	exp memory disorder/
16	(iq or intelligence quotient or amnesia or memory or problem solving).tw,kw.
17	exp *galactosemia/co
18	exp mental development/
19	developmental disorder/et [Etiology]
20	(long term adj5 (outcom* or therap* or treatment*)).tw,kw.
21	exp case control study/ or exp longitudinal study/ or prospective study/ or retrospective study/ or (prospective or retrospective).tw.
22	or/4-21
23	3 and 22

PsychInfo June 2015

1	(galactosem* or ((galt or utp hexose 1 phosphate uridyltransferase or gale or galk or galac-tokinase) adj3 deficien*)).tw.

6

Chapter 6

Bone health in classic galactosemia: systematic review and meta-analysis

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Abstract

Introduction Previous studies have reported an association between classic galactosemia (CG) and decreased bone mass. The primary objective of this systematic review with meta-analysis was to determine the extent of bone mineral density (BMD) Z-score reduction. Low BMD was defined as a Z-score ≤-2 standard deviations (SD). The secondary objective was to evaluate other indicators of bone status through a descriptive analysis.

Methods Systematic search strategies were developed by an experienced clinical librarian. Selection of relevant manuscripts, risk of bias assessment and data-extraction were performed independently by two investigators.

Results Four studies were included in the meta-analysis. BMD Z-scores in children and adults with CG measured at the lumbar spine (LBMD; 4 studies; n = 112), total hip (HBMD; 2 studies; n = 58), and femoral neck (FBMD; 2 studies; n = 73) were assessed. Mean BMD Z-scores in the CG population were: LBMD -0.70 (95% CI: -0.88, -0.52); HBMD -0.89 (95% CI: -1.14, -0.64); FBMD -0.63 (95% CI -1.29, 0.02). Results from studies included in the descriptive analysis (n=7) show that vitamin D levels were frequently in the low reference range, whereas serum calcium levels were within reference range.

Conclusion The mean BMD Z-score in the CG population is -0.7, which is lower than in the general population, though still within two SD of the reference mean of zero. This indicates that bone health is mildly affected in CG and that more patients, compared to the general population, are at risk for a BMD Z-score ≤-2 SD. In conclusion, clinicians should ensure appropriate preventive and therapeutic measures for CG patients.

Introduction

Classic galactosemia (CG, MIM 230400), a genetic disorder of galactose metabolism due to deficiency of galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12), is characterized by the occurrence of late complications in spite of early diagnosis and lifelong dietary treatment. The first report of an association between CG and decreased bone mass dates back to 1993 [1] and a bone mineral density (BMD) Z-score more than two standard deviations (SD) below the mean was found in 25-30% of adult patients [2,3]. Thorough evaluation of the frequency and severity of impaired bone health in CG patients, compared to the non-galactosemia population, is crucial for determining its extent and relevance for patient care.

CG patients could be at risk for compromised bone health due to diet restrictions, ovarian insufficiency in women, limited physical activity in some cases, and possibly unknown intrinsic factors associated with the disease. Sufficient intake of calories, protein and micronutrients is essential for acquiring an optimal bone mass, and the life-long galactose restriction may predispose patients to nutritional deficiencies [3–6]. Furthermore, ovarian damage resulting in low estrogen concentrations is present in over 80% of female patients with CG [7], which increases their susceptibility to the development of low bone mass. Remarkably, supplementation of calcium, vitamins and estrogen only seem to partially improve bone mass [1,8,9], which may point to the presence of an underlying intrinsic bone defect. Aberrant glycosylation of collagen and other glycoproteins related to bone metabolism, which is also seen in patients with other glycosylation defects such as phosphomannomutase 2 deficiency (PMM2-CDG) [10], has been suggested as a potential intrinsic abnormality [1,11] and the decreased IGF-I and IGFBP-3 concentrations that are found in patients might reflect this [12]. Furthermore, reduced physical activity due to motor abnormalities [13] and cognitive impairment [14–17] may affect bone health as well. Clinical practice, focused on reducing the risk of impaired bone health in those with CG, includes routine monitoring of BMD, which is an important determinant of bone strength and has predictive value in assessing fracture risk [18], and optimization of exogenous factors affecting bone mass (nutrition, estrogen concentrations, physical activity)[19].

The primary aim of our study is to evaluate the extent of impaired bone health in patients with CG, and the need of monitoring and treatment. Few studies assessing bone health in CG are published. These studies have small patient sample sizes, and results vary from one study to another. As the current conjecture of low BMD in CG is based on single studies, further evaluation is needed to confirm the validity of this hypothesis [20]. Meta-analysis is the most commonly used statistical technique to pool results from two or more separate studies. The added value of a meta-analysis

may include increased power and improved precision of the results.

Therefore, we performed a systematic review with meta-analysis of BMD in children and adults with CG. As recommended by the International Society for Clinical Densitometry (ISCD), low BMD is defined here as a BMD Z-score ≤ -2.0 SD [21,22]. We also explored the usefulness of other indicators of bone status as potential diagnostic or monitoring tools.

Methods

Research question

The primary outcome for our meta-analysis was bone mass reported as BMD (areal BMD), either as Z-score or as absolute measurement, assessed with dual energy X-ray absorptiometry (DXA) since this is the preferred tool for evaluating BMD/bone mass in both children and adults [21,22]. BMD is a major determinant of bone strength and its assessment is considered the cornerstone in the diagnostics of low bone mass [23]. Only areal BMD measurements were included in the analysis since these are most commonly used in clinical practice; results of estimated volumetric BMD were not included.

The secondary outcomes for our systematic review, reported in a descriptive way, were bone mineral content (BMC), parameters involved in bone metabolism such as vitamins, minerals, hormones, bone turnover markers (BTM), and fracture risk.

Inclusion and exclusion criteria for study selection

We included all original studies (cross-sectional study design, randomized controlled trial [RCT], cohort study, case-control study) as well as conference abstracts on bone health in children and adults with CG. If there was (potential) overlap between study cohorts, only the first/original article was included. Articles in a language other than English, and animal and cell studies were excluded.

Search strategy

A computerized literature search was conducted in MEDLINE, EMBASE and the Cochrane Library by one of the investigators (LW) and a trained clinical librarian from University of Amsterdam (see Supplement 1, Search strategies). Databases were searched initially in February 2015 and a final search was completed in July 2015 to ensure the inclusion of recently published articles. No limits were used in these searches.

Study selection

Titles and abstracts generated by literature searches were screened by two separate researchers (BvE and LW) to select potentially eligible studies. Studies not relating to the research question were excluded. Selected conference abstracts and full text articles of selected studies were independently reviewed by two authors (BvE and LW) for inclusion in the systematic review. In case of exclusion, the reason was reported. Additionally, reference lists from included trials and excluded narrative or systematic reviews were hand-searched to identify additional relevant studies. Consensus was reached between the two authors regarding the eligibility assessments.

Chapter 6

Data extraction
Data were extracted by two separate researchers (BvE and LW) according to predefined criteria (see Supplement 2a and 2b, Summary of evidence tables, via http://www.thesisgalt.nl/). Outcome measures of interest were BMD at any site measured with DXA; BMC; incidence of fractures; BTM reflecting bone formation (bone-specific alkaline phosphatase, under-carboxylated osteocalcin [ucOC], carboxylated osteocalcin [cOC]), bone resorption (N-terminal telopeptide, C-terminal telopeptide) or bone modeling (insulin-like growth factor-1 [IGF-1]); and vitamins (vitamin D), minerals (calcium) and hormones (estradiol, parathormone) important for bone metabolism.

Data collection processes
If a mean BMD Z-score and/or standard deviation (SD) was not reported for the entire cohort in an article, the authors were requested to share either these variables together with mean age at testing and SD, or individual patient data (IPD). IPD included individual mean BMD Z-score with SD, age at testing and gender. The corresponding author was contacted first, and if there was no reply within four weeks, one of the other authors was contacted.

Individual patient data integrity
IPD were checked for integrity by reviewing completeness; in case of incompleteness the data were not included in the systematic review. If more data were received from the authors than originally published, these were only included if patient characteristics matched those from the original article. In case of any discrepancies, the authors were contacted.

Assessment of quality and risk of bias in individual studies
Quality appraisal and assessment of bias were performed with an appropriate checklist from the 'Scottish Intercollegiate Guidelines Network' (SIGN), if available (available for: RCT, non-RCT, cohort study, case-control study). Quality appraisal and risk of bias analysis were performed and discussed by two independent investigators (BvE and LW). Articles were appraised as low, acceptable or high quality; those assessed as low quality were excluded from the review.

Data analysis
We performed a meta-analysis of BMD to evaluate whether the mean BMD Z-score in the CG population differs from normative data (a mean BMD Z-score of zero in the general population). We used Review Manager 5.3 version 5.3.5 for the analysis. The inverse-variance method was used to pool study data, and the individual effect sizes were weighted according to the reciprocal of their variance [24]. I^2 was used

as a measure of heterogeneity [25]. The *p*-value used to reject the null hypothesis of homogeneity was 0.1 (p-value of Q; Q=chi squared statistic). In case of low heterogeneity (0-30%) a fixed effects model was used to pool data. In case of moderate to high heterogeneity (30-100%), both a fixed and random effects model were applied, resulting in a sensitivity analysis with description of differences between the fixed and random effects models and selection of the most appropriate model. Aggregate data and IPD were analyzed together using a two-stage approach: for IPD, a mean BMD Z-score and SD were calculated first, and were then included in the next step as aggregate data. BMD Z-scores were pooled based on the measurement site (lumbar spine, total hip, femoral neck and total body). For the overall effect size, a *p*-value of 0.05 was considered statistically significant. Yet, in case of multiple testing, adjustment for Bonferroni correction was applied.

In order to assess clinical relevance of the mean BMD Z-score of the CG population, the normal distribution was used to find the percentiles of Z-scores, and thus the estimated proportion of patients with a BMD Z-score ≤-2 SD (low bone mass) using SPSS version 23. For this, two approaches were used, one with the SD of normative data (mean of zero, SD 1), another with the SD of the mean calculated in our meta-analysis.

The secondary outcome measures (BMC, minerals, vitamins, hormones and BTM) were presented in a descriptive manner.

Results

Study selection
The literature search identified 138 potential publications of which 94 remained after removing duplicates (n=44). After screening the titles and abstracts of the unique publications, 71 were excluded because they did not relate to the research question. Detailed evaluation of the 23 selected publications led to the exclusion of 10 studies for various reasons (see Figure 1. Flow diagram selection process).

For the meta-analysis, eight studies were selected because they encompassed BMD measurements. Only one of these studies reported all data required for inclusion [26]; the authors of the other 7 studies were contacted for additional information. Three authors provided the required data at request [3,27,28] (Supplement 3, Data collection process, via http://www.thesisgalt.nl/). Accordingly, a total of four articles were included in our meta-analysis on BMD. A total of seven studies reported on our secondary outcome measures and were therefore included in the descriptive analysis. The complete study selection process is presented as a flow diagram, separately for the meta-analysis and the descriptive analysis (Figures 1a and 1b).

Chapter 6
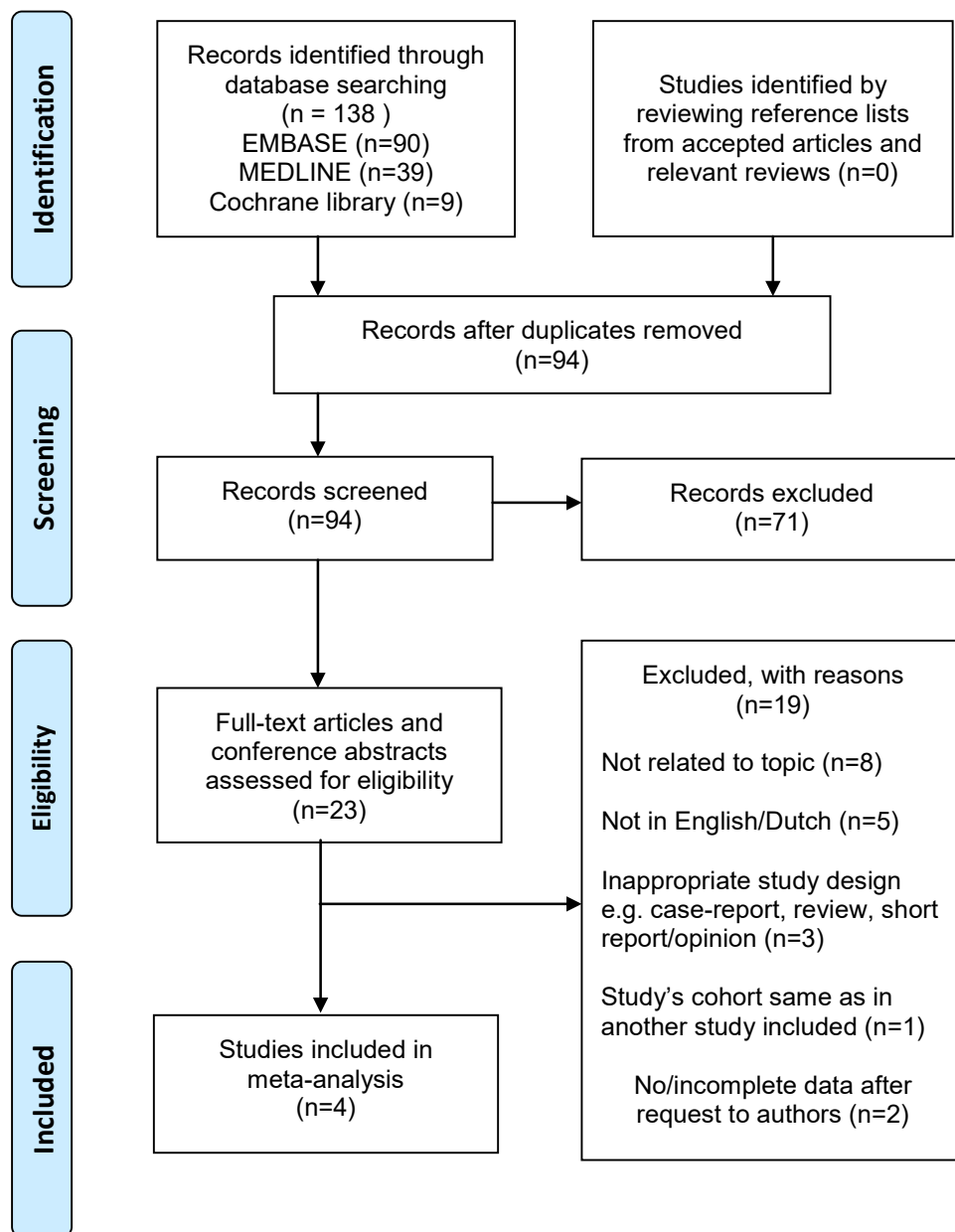
Figure 1a. Flow diagram selection process meta-analysis.

Identification

Records identified through database searching
(n = 138)
EMBASE (n=90)
MEDLINE (n=39)
Cochrane library (n=9)

Studies identified by reviewing reference lists from accepted articles and relevant reviews
(n=0)

Records after duplicates removed
(n=94)

Screening

Records screened
(n=94)

Records excluded
(n=71)

Eligibility

Full-text articles and conference abstracts assessed for eligibility
(n=23)

Excluded, with reasons
(n=16)

Not related to topic (n=7)

Not in English/Dutch (n=5)

Inappropriate study design e.g. case-report, review, short report/opinion (n=3)

Study's cohort same as in another study included (n=1)

Included

Studies included in descriptive analysis
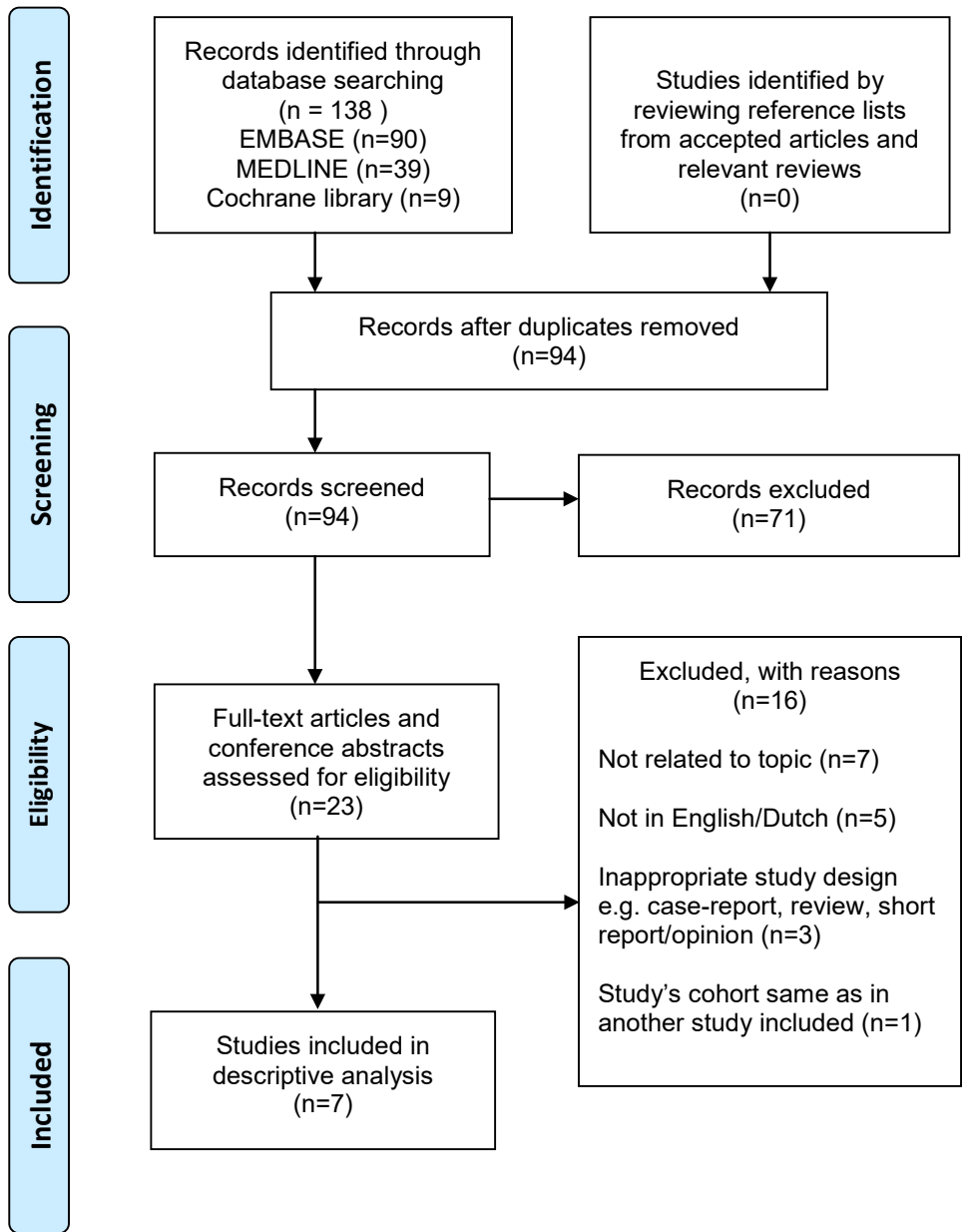(n=7)

Figure 1b. Flow diagram selection process meta-analysis.

Chapter 6

Included studies
Characteristics of the studies included in the meta-analysis or descriptive analysis are presented in Supplement 2a and 2b, Summary of evidence tables (via http://www.thesisgalt.nl/). Age of the patients in the included studies ranged from 2.5-59 years.

Individual patient data integrity
No issues with IPD integrity were detected.

Assessment of quality and risk of bias in individual studies
A quality appraisal with the standardized checklist was applicable for only one study, a randomized controlled trial [8]. The paper was graded high quality as defined by the risk of bias checklist for randomized-controlled trial of SIGN.

Meta-analysis on BMD in classic galactosemia patients
Four studies (three cross sectional studies and one retrospective case series) were included in this meta-analysis of BMD Z-score [3,26–28]. Panis et al. (2004) assessed 40 patients with a mean age of 8.9 years (range 3.0-17.3). Waisbren et al. (2012) evaluated BMD in 33 patients with a mean age of 32.6 years (range 18-59). Doulgeraki et al. (2014) reported BMD Z-scores of 14 patients with a mean age of 13.16 years (range 6.17-16.58 years). Tan et al. (2014) provided the individual patient data reported in their conference abstract as well as additional new data (n=5). The mean age in these 25 patients was 13.5 years (range 5-41 years). Mean BMD Z-scores were calculated and are presented in Supplement 2a (via http://www.thesisgalt.nl/).

Complete cohort (children and adults)
A total of 112 CG patients assessed in 4 studies were included in this part of the meta-analysis. At the site of the lumbar spine, a mean BMD Z-score of -0.70 (95% CI: -0.88, -0.52) was found [3,26–28]. Heterogeneity was zero (I^2 = 0%); a fixed effects model was used. The overall effect was statistically significant (p < 0.00001) when compared to the mean of normative data (BMD Z-score of zero). One of the studies crossed the line of no effect (Figure 2).
In 58 pooled patients included in 2 studies, mean total hip BMD Z-score was -0.89 (95% CI: -1.14, -0.64)[3,27]. Heterogeneity was zero (I^2 = 0%); a fixed effects model was used. The overall effect was significantly different from the mean of normative data (p < 0.00001) and none of the studies crossed the line of no effect (Figure 3).
At the site of the femoral neck, 73 CG patients from 2 studies were pooled [3,26]. The heterogeneity was high (I^2 = 91%), leading to determination of the mean BMD Z-score with both a fixed as well as a random effects model (Figures 4 and 5, respectively). A mean BMD Z-score of -0.63 was found with both models, with a difference
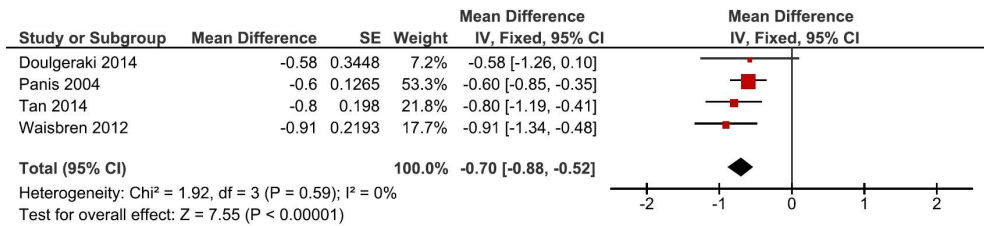
Figure 2. Forest plot of lumbar spine BMD Z-score in complete CG cohort.
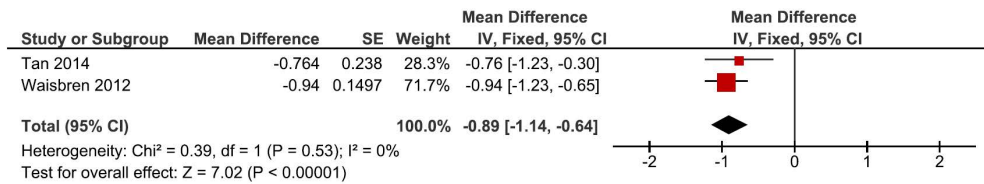
Figure 3. Forest plot of total hip BMD Z-score of the complete CG cohort.

in 95% confidence interval (95% CI fixed effects model: -0.83, 0.44; 95% CI random effects model: -1.29, 0.02). As heterogeneity was very high and both studies are of adequate quality with comparable cohort sizes, it was chosen to base conclusions on the results of the random effects model. Even though both studies did not cross the line of no effect, the overall effect was not significantly different from the mean of normative data when using the random effects model (p = 0.06) (Figure 5).

Figure 4. Forest plot of femoral neck BMD Z-score in complete CG cohort (fixed effects model).

Figure 5. Forest plot of femoral neck BMD Z-score in complete CG cohort (random effects model).

Chapter 6

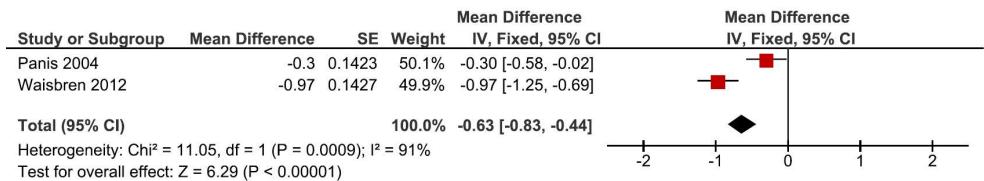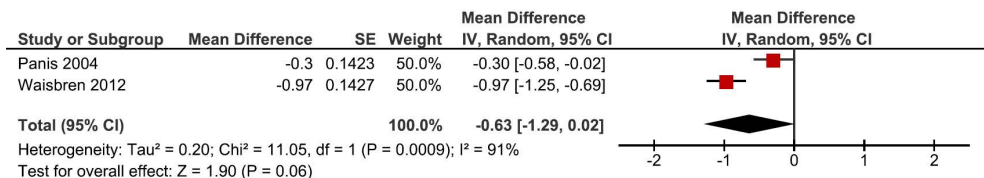| Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Fixed, 95% CI	Mean Difference IV, Fixed, 95% CI
Doulgeraki 2014	-0.58	0.3448	9.2%	-0.58 [-1.26, 0.10]	
Panis 2004	-0.6	0.1265	68.5%	-0.60 [-0.85, -0.35]	
Tan 2014	-0.78	0.2217	22.3%	-0.78 [-1.21, -0.35]	
Total (95% CI)			**100.0%**	**-0.64 [-0.84, -0.43]**	

Heterogeneity: Chi² = 0.53, df = 2 (P = 0.77); I² = 0%
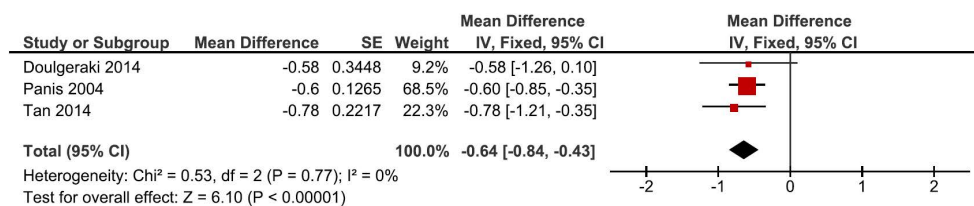Test for overall effect: Z = 6.10 (P < 0.00001)

Figure 6. Forest plot of lumbar spine BMD Z-score in children with CG.

Children with CG

In pooled data from 76 children from 3 studies, mean lumbar spine BMD Z-score was -0.64 (95% CI: -0.84, -0.43)[26–28]. Heterogeneity was zero (I² = 0%); a fixed effects model was used. This was overall significantly different from the mean of normative data ($p < 0.00001$). One study crossed the line of no effect (same study as in lumbar spine BMD Z-score analysis of the entire patient group) (Figure 6).

Adults with CG

In pooled data from 36 adults with CG included in 2 studies, mean lumbar spine BMD Z-score was -0.94 (95% CI: -1.30, -0.57)[3,27]. Heterogeneity was zero (I² = 0%); a fixed effects model was used. The overall effect was statistically significant from the mean of normative data ($p < 0.00001$), and none of the studies crossed the line of no effect (Figure 7).

Mean BMD Z-score at the total hip in this group of adults was -0.91 (95% CI: -1.20, -0.63)[3,27]. Heterogeneity was zero (I² = 0%); a fixed effects model was used. One study crossed the no effect line, but the overall effect was significantly different from the mean of normative data ($p < 0.00001$) (Figure 8).

We could not perform a subgroup analysis on BMD Z-scores for adult males and adult females separately since one study included data on only one or two patients.

Clinical relevance

The normal distribution was used to find the percentiles of Z-scores, and thus the estimated proportion of patients with a BMD Z-score ≤-2 SD (low bone mass). Two approaches were used: one with an SD of 1.9 (resulting from the 95% CI of the calculated mean lumbar spine BMD Z-score of -0.7 in the complete CG group) and one with an SD of 1.0 (according to the normal distribution curve of BMD in the general population). Accordingly, 10-25% of CG patients are estimated to be at risk for a BMD Z-score ≤ -2 SD, and thus a low bone mass, whereas this is only 2.3% in the general population (normally distributed parameter).
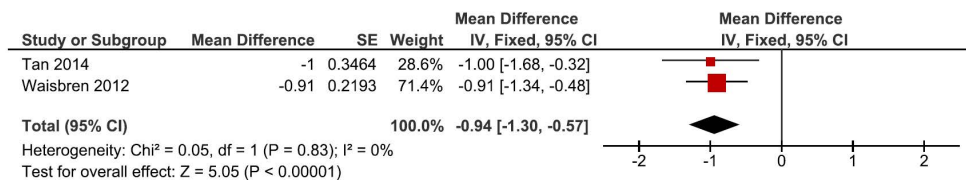
Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Fixed, 95% CI	Mean Difference IV, Fixed, 95% CI
Tan 2014	-1	0.3464	28.6%	-1.00 [-1.68, -0.32]	
Waisbren 2012	-0.91	0.2193	71.4%	-0.91 [-1.34, -0.48]	
Total (95% CI)			100.0%	**-0.94 [-1.30, -0.57]**	

Heterogeneity: Chi² = 0.05, df = 1 (P = 0.83); I² = 0%
Test for overall effect: Z = 5.05 (P < 0.00001)

Figure 7. Forest plot of lumbar spine BMD Z-score in adults with CG.

Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Fixed, 95% CI	Mean Difference IV, Fixed, 95% CI
Tan 2014	-1	0.3464	28.6%	-1.00 [-1.68, -0.32]	
Waisbren 2012	-0.91	0.2193	71.4%	-0.91 [-1.34, -0.48]	
Total (95% CI)			100.0%	**-0.94 [-1.30, -0.57]**	

Heterogeneity: Chi² = 0.05, df = 1 (P = 0.83); I² = 0%
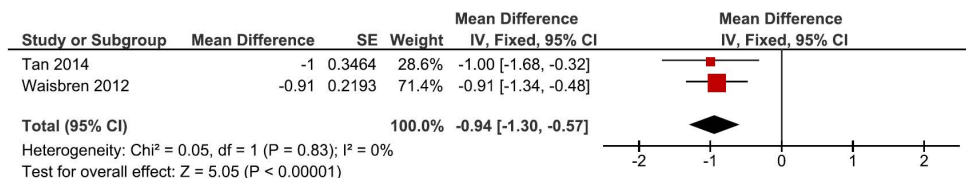Test for overall effect: Z = 5.05 (P < 0.00001)

Figure 8. Forest plot of total hip BMD Z-score in adults with CG.

Descriptive analysis

Other indicators of bone status in classic galactosemia

Bone mineral content
Another indicator of bone health is BMC, which reflects other aspects of bone mass acquirement than BMD and might therefore be of additional value in children [29]. BMC of femoral neck, lumbar spine and total body was assessed in a single study by Panis et al. (2006) (40 patients, age range 3-17 years)[8]. In this randomized-controlled trial, in which the effect of supplementation of calcium, vitamin K1 and vitamin D3 on bone health was studied, baseline measurements of BMC Z-scores in 40 children with CG (age 3-17 years) were conducted. Mean BMC Z-scores (varying between -0.3 and -1.1 for different subgroups) were lower than in controls.

Vitamins, minerals and hormones
Six studies evaluated vitamin D status in CG patients (n=197)[3,26,30–33]. One study assessed 1,25-OH-D concentrations only [26], four measured 25-OH-D only, and one evaluated both [32]. The studies varied with regard to vitamin D reference ranges and units, and in some the specified range of desired vitamin D concentrations was not stated. Two studies in adults with CG reported that most patients had levels in the low reference range [3,33]. Compliance to vitamin D supplements differed between the two cohorts. The remaining four studies, performed in children with CG, found vitamin D levels to be within reference range.
Four studies with a total of 148 patients assessed serum calcium levels [26,30–32]. In all studies calcium levels were found to be within reference range. Rubio-Gozalbo et al.

(2002) found no correlation between BMD results and calcium intake in their cohort of children with CG [32].

Parathormone was also measured in the studies by Rubio-Gozalbo et al. (2002) and Panis et al. (2004) and revealed values within reference range. Only one study measured 17-beta estradiol levels in a cohort of 40 children aged 3-17.3 years (mean age 8.9 years)[26]. Mean levels did not differ from reference values.

Bone turnover markers

Four cross-sectional studies examined BTM in CG patients [26,30–32], three studies included children only (<18 years) and one study included patients up to 20 years. A total of 148 patients were included in these studies (range 11-62 patients/study). There were no studies reporting on markers of bone status in adult patients.

Carboxy-terminal telopeptide of type 1 collagen, measured in all four studies, was found to be significantly reduced in children but not in adolescents when compared to controls. Panis et al. (2004) found it to be inversely correlated with BMD Z-score of the femoral neck and lumbar spine [26]. Amino terminal telopeptide of type I collagen levels were measured in two study cohorts and levels were found to be significantly low in both cohorts of CG children [26,32]. Gajewska et al. (2008) reported about 30% higher values of bone-specific alkaline phosphatase in adolescents than in controls [31], whereas normal values were found in children. In addition, Gajewska et al. found increased osteocalcin (OC; sum of carboxylated [cOC] and under-carboxylated osteocalcin [ucOC]) levels in adolescents with CG [31], whereas Panis et al. (2004) reported significantly decreased cOC levels with normal ucOC levels in children [26]. Normal values of OC [30,31] and cOC and ucOC [32] in children were reported in three studies. Furthermore, Panis et al. (2004), the only study measuring IGF-1 levels, reported reduced IGF-1 Z-scores in children [26] and found that IGF-1 Z-score was a strong positive predictor of femoral neck and lumbar spine BMD.

Fracture history

Waisbren et al. (2012) reported that 45% of 33 patients (mean age 32.6 years, range 18-59) had broken a bone, six during childhood and the others at ages 20 to 46 years [3].

Discussion

In this systematic review we evaluated bone mass in patients with CG through a meta-analysis of BMD. The results of our meta-analysis indicate that mean bone mass in the CG population, reflected by mean BMD Z-score, is more than a half-standard deviation lower than in the general population. While this is still within two SD of the normative mean, this result indicates that an increased proportion of individuals with CG will have a BMD Z-score ≤ -2 SD, and thus a low bone mass for age, as compared to individuals in the general population. Based on the meta-analysis, estimated prevalence of BMD Z-score ≤-2 SD in patients with CG is 10% to 25%, which is higher than in the general population (2.3%). Mean BMD Z-scores in pooled data of adults and children with CG were reduced at all sites (lumbar spine -0.70, total hip -0.89, femoral neck -0.63), of which only the latter was not statistically significant, probably due to heterogeneity between included study cohorts. Findings from our descriptive analysis support the need for improved evaluation and optimization of vitamin D concentrations. Though serum calcium levels were within reference range in all studies that addressed calcium status, ensuring sufficient intake of calcium remains a point of attention in this population. Data on the role of BMC and BTM as other indicators of bone status are very limited and, in the case of BTM, highly ambiguous. Therefore, routine screening of these indicators in CG patients does not seem of additional value at present. Literature on bone mass in CG is scarce and study cohorts are small as a result of the rarity of the disease. This limits the number of studies and patients included in our meta-analysis, which hampers extensive subgroup analyses and solid conclusions. However, this systematic review is currently the most comprehensive study evaluating bone health in CG patients, thereby providing results that are more representative for the whole population of CG patients.

This study was limited in that not all original patient data could be obtained. In addition, data used in this study were all cross-sectional, and conclusions about progression over time must therefore be interpreted with caution. Longitudinal studies following patients in time are needed to enable firm conclusions on this. Moreover, ISCD recommendations for pediatric DXA [34] were not unanimously followed by the researchers, as they used different sites of measurement and did not take into account the presence of short stature or growth delay, with the exception of only one study [28]. Furthermore, data on fracture history were obtained in only one study [3], though this is required to establish a diagnosis of osteoporosis. Future studies should consider the ISCD recommendations to further improve insights on bone health in CG.

Conclusions

BMD Z-scores in individual CG patients are within two SD of the normative mean in the majority of patients with CG. However, results from our meta-analysis demonstrate that the mean BMD Z-score in the CG population is lower than the mean BMD Z-score in the general population. These results suggest that bone health in general is mildly affected, and that an estimated proportion of 10-25% of patients with CG could be at risk for a low bone mass (BMD Z-score ≤-2) as compared to the general population. With the currently available literature, which lacks data on fracture prevalence, it is impossible to draw conclusions about osteoporosis risk. Vitamin D levels are low in many patients, emphasizing the need for monitoring of 25(OH)D levels and vitamin D supplementation. Optimization of calcium intake remains important. Evaluation of the importance of other parameters of bone health (BCM, BTM, hormones) was inconclusive due to a limited number of studies with inconsistent results. Concluding, it is important that treating physicians are aware that patients with CG are at risk for having or developing low bone mass, so that patients will be screened and treated appropriately.

References

1. Kaufman FR, Loro ML, Azen C, Wenz E, Gilsanz V. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. J Pediatr 1993;123:365–70.

2. Batey LA, Welt CK, Rohr F, Wessel A, Anastasoaie V, Feldman HA, et al. Skeletal health in adult patients with classic galactosemia. Osteoporos Int 2013;24:501–9.

3. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis 2012;35:279–86.

4. Rutherford PJ, Davidson DC, Matthai SM. Dietary calcium in galactosaemia. J Hum Nutr Diet 2002;15:39–42.

5. El-Bassyouni HT, Ashour AM, Ezzat A, Bassiouni R, Fateen EM. The effect of diet on antioxidant status in patients with galactosemia. J Med Sci 2006;6:452–7.

6. Wiesmann UN, Rosé-Beutler B, Schlüchter R. Leguminosae in the diet: the raffinose-stachyose question. Eur J Pediatr 1995;154:93–6.

7. Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo ME. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357–66.

8. Panis B, Vermeer C, van Kroonenburgh MJPG, Nieman FHM, Menheere PPCA, Spaapen LJ, et al. Effect of calcium, vitamins K1 and D3 on bone in galactosemia. Bone 2006;39:1123–9.

9. Renner C, Razeghi S, Uberall MA, Licht P, Wildt L, Dorr HG, et al. Hormone replacement therapy in galactosaemic twins with ovarian failure and severe osteoporosis. J Inherit Metab Dis 1999;22:194–5.

10. Bons JA, Michielsen EC, de Boer D, Bouwman FG, Jaeken J, van Dieijen-Visser MP, et al. A specific immunoprecipitation method for isolating isoforms of insulin-like growth factor binding protein-3 from serum. Clin Chim Acta 2008;387:59–65.

11. Coss KP, Doran PP, Owoeye C, Codd MB, Hamid N, Mayne PD, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis 2013;36:21–7.

12. Panis B, Gerver W-JM, Rubio-Gozalbo ME. Growth in treated classical galactosemia patients. Eur J Pediatr 2007;166:443–6.

13. Rubio-Agusti I, Carecchio M, Bhatia KP, Kojovic M, Parees I, Chandrashekar HS, et al. Movement disorders in adult patients with classical galactosemia. Mov Disord 2013;28:804–10.

14. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr 1993;152:36–43.

15. Hoffmann B, Wendel U, Schweitzer-Krantz S. Cross-sectional analysis of speech and cognitive performance in 32 patients with classic galactosemia. J Inherit Metab Dis 2011;34:421–7.

16. Rasmussen RK, Andreassen AB, Stromme P, Hansen TW. Learning disabilities and language pathology in patients with galactosemia. Logoped Phoniatr Vocol 1996;21:157–62.

17. Shield JP, Wadsworth EJ, MacDonald A, Stephenson A, Tyfield L, Holton JB, et al. The relationship of genotype to cognitive outcome in galactosaemia. Arch Dis Child 2000;83:248–50.

18. Marchall D, Johnell O, H W. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ 1996;18:1254–9.

19. Van Erven B, Römers MM, Rubio-Gozalbo ME. Revised proposal for the prevention of low bone mass in patients with classic galactosemia. JIMD Rep 2014;17:41–6.

Chapter 6

20. Haidich AB. Meta-analysis in medical research. Hippokratia 2010;14:29–37.

21. Schousboe J, Shepherd JA, Bilezikian JP, Baim S. Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. J Clin Densitom 2013;16:455–66.

22. Gordon C, Leonard MB, Zemel BS, International Society for Clinical Densitometry. 2013 Pediatric Position Development Conference: executive summary and reflections. J Clin Densitom 2014;17:219–24.

23. Kanis JA, McCloskey E V, Johansson H, Cooper C, Rizzoli R, Reginster JY, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 2013;24:23–57.

24. Deeks JJ, Higgins JPT, on behalf of the Statistical Methods Group of The Cochrane Collaboration. Statistical algorithms in Review Manager 5 [Internet]. 2010 [cited 2016 Oct 24];Available from: RevMan-5.pdf

25. The Cochrane Collaboration. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. 2011.

26. Panis B, Forget PP, Van Kroonenburgh MJPG, Vermeer C, Menheere PP, Nieman FH, et al. Bone metabolism in galactosemia. Bone 2004;35:982–7.

27. Tan C, Stonestreet K, Winstone R, van Wyk K, Champion M, Mundy H, et al. Management and long-term outcomes of classical galactosaemia-a single centre experience in the UK. Conf Annu Symp Soc Study Inborn Errors Metab SSIEM 2014 Innsbruck Austria Conf Start 20140902 Conf End 20140905 Conf Publ (var.pagings) 37 (1 SUPPL 1) (pp S57), 2014 Date Pub 2014;37:S57.

28. Doulgeraki A, Monopolis I, Deligianni D, Kalogerakou M, Schulpis KH. Body composition in young patients with galactose metabolic disorders: a preliminary report. J Pediatr Endocrinol Metab 2014;27:81–6.

29. Mølgaard C, Thomsen BL, Prentice a, Cole TJ, Michaelsen KF. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child 1997;76:9–15.

30. Gajewska J, Ambroszkiewicz J, Radomyska B, Laskowska-Klita T. Bone turnover markers in prepubertal children with classical galactosemia. Indian J Gastroenterol 2006;25:221–2.

31. Gajewska J, Ambroszkiewicz J, Radomyska B, Chelchowska M, Oltarzewski M, Laskowska-Klita T, et al. Serum markers of bone turnover in children and adolescents with classic galactosemia. Adv Med Sci 2008;53:214–20.

32. Rubio-Gozalbo ME, Hamming S, van Kroonenburgh MJ, Bakker JA, Vermeer C, Forget PP. Bone mineral density in patients with classic galactosaemia. Arch Dis Child 2002;87:57–60.

33. Sirrs S, Bosdet T, Branov J, O'Riley M, Paquin W, Rosen A, et al. High prevalence of vitamin D insufficiency in galactosemic adults despite compliance with supplementation. J Inherit Metab Dis Conf Annu Symp Soc Study Inborn Errors Metab Istanbul Turkey Conf Start 20100831 Conf End 20100903 Conf Publ (var.pagings) 33 (pp S64), 2010 2010;33:S64.

34. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, El-Hajj Fuleihan G, Kecskemethy HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom 2014;17:225–42.

Supplement 1. Search strategies

Searches in all databases were performed in February 2015.

Search strategy MEDLINE

1	galactosemias/
2	(galactosem* or ((galt utp hexose 1 phosphate uridyltransferase or gale or galk or galactokinase) adj3 deficien*)).tw.
3	1 or 2
4	Bone Density/
5	Bone Diseases, Metabolic/
6	exp bone resorption/
7	exp "Bone and Bones"/
8	(Calcium/ or vitamin d/ or vitamin k/) and (me or bl).fs.
9	Absorptiometry, Photon/
10	Osteocalcin/
11	((bone adj3 density) or bone mass or fractures or fracture or bone metabolism or bone resorption or osteolysis or bone loss or bone mineral* or osteoporosis or osteolysis or skeletal health or bone turn over or dxa or dual energy x ray).mp,kf.
12	exp Fractures, Bone/
13	or/4-12
14	3 and 13

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Search strategy EMBASE

1	galactosemias/
2	(galactosem* or ((galt utp hexose 1 phosphate uridyltransferase or gale or galk or galactokinase) adj3 deficien*)).tw.
3	1 or 2
4	Bone Density/
5	Bone Diseases, Metabolic/
6	exp bone metabolism/
7	exp "Bone and Bones"/
8	Calcium/ec or vitamin d/ec or vitamin k/ec
9	Absorptiometry, Photon/
10	Osteocalcin/
11	((bone adj3 density) or fracture or fractures or bone mass or bone resorption or bone metabolism or bone loss or bone mineral* or osteoporosis or osteolysis or skeletal health or bone turn over or dxa or dual energy x ray).mp,kw.
12	exp Fractures, Bone/
13	calcium blood level/
14	or/4-13
15	3 and 14

Cochrane Library

Search in Title, Abstract, Keywords: galactosemias (MeSH term)

7

The need for additional care in patients with classical galactosemia

Lindsey Welling, Anke Meester-Delver, Terry G. Derks, Mirian C.H. Janssen, Maaike de Vries, Carla E.M. Hollak and Annet M. Bosch

Abstract

Aim Classical galactosemia (CG) is an inborn error of galactose metabolism which may lead to impairments in body functions and accordingly, need for additional care. The primary aim of this study was to establish the type and intensity of this additional care.

Method CG patients aged ≥2 years were evaluated with the Capacity Profile, a standardized method to classify additional care needs according to type and intensity. Based on a semi-structured interview, current impairments in five domains of body functions were determined. The intensity of additional care was assessed (from 0, usual care, to 5, total dependence).

Results Forty-four CG patients, 18 males and 26 females (median age 15 years, range 2 to 49 years), were included. There was a wide spectrum of impairments in mental functions. Motor function impairments were present in four patients, and mild speech impairments in eight patients. Additional care for sensory functions was uncommon. All patients needed a diet, which care is scored in the physical health domain.

Interpretation Apart from the diet all patients need, CG leads to the need for additional care mainly in the domains of mental functions and speech and voice functions.

Introduction

Classical galactosemia (CG, OMIM 230400) is an inborn error of galactose metabolism, caused by a severe deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT, EC 2.7.7.12). Affected newborn infants who are fed galactose-containing milk, develop a life-threatening illness (liver failure, kidney failure, sepsis-like symptoms, *E*. Coli sepsis) that quickly resolves after start of treatment: a life-long galactose-restricted diet [1]. Despite early diagnosis and treatment, patients are at risk for long-term complications, including decreased cognitive abilities, neurological complications (movement disorders), speech and language problems (verbal dyspraxia), and bilateral cataracts presenting in the neonatal period [2]. Though there is a high frequency of these different complications, the consequences of these impairments in body functions for daily life and participation have not been studied. These impairments may limit the capacities of the affected person, leading to the need for ongoing additional care. Recognition of impairments is important for planning of individual care, and for the implementation of adequate and timely medical interventions. For parents, information about the future need for care is important to help them to set realistic goals, and to make adequate arrangements for the child's future requirements. The Capacity Profile (CAP) is a standardized method for classifying additional care needs in permanent conditions, indicated by current impairments in five domains of body functions: physical health, motor (neuromusculoskeletal and movement-related), sensory, mental, and voice and speech functions. The intensity of care in each domain is defined from 0 (no need for additional care) to 5 (needs help with every activity), and indicates the CAP for the individual patient [3]. By scoring the dependency on additional care in each separate domain irrespective of the need for care in the other domains, and not combining the five scores into one single CAP score, insight is obtained about the contribution of the additional care to the various domains. In the development of the CAP the International Classification of Functioning, Disability and Health (ICF) was the frame of reference.

The aim of this study was to assess the current impairments in body functions in patients with CG, and the subsequent need for additional care, using the Capacity Profile as a test instrument.

Chapter 7

Methods

Research design
We performed a cross-sectional study, in which CAP scores for the five domains of body function were determined in a single semi-structured interview with patients and/or parents. Additional questions were asked concerning: time of start of dietary treatment and dietary compliance, current and past interventions (speech and language therapy, physiotherapy and mental health care), educational attainment, work, living situation, and relationships. Residual GALT enzyme activity, and genetic analysis were retrospectively collected from the patients' medical charts.

Participants

Eligibility criteria
In the Netherlands, all patients aged ≥2 years, with a residual GALT enzyme activity of <15% (compared to healthy controls) and/or two known pathogenic variations in the *GALT* gene, are treated as if they are classical galactosemia patients, and were eligible for inclusion in the study. This is in line with the international guideline [1] defines CG as a profound impairment of GALT enzyme activity (absent or barely detectable), but acknowledges that newborn screening detects patients with low but not profoundly deficient GALT enzyme activities up to 15%. If the diagnosis of CG was not confirmed, patients were excluded from the analysis.

Sample size
Sample size of this study was based on feasibility.

Recruitment strategies
Patients were invited for this study via multiple routes. First, all members (n=131) of the Dutch Galactosemia Patient Society were invited through e-mail in March 2016. This invitation was linked to an invitation for a family weekend of the society. Before this invitation, all treating physicians in the Netherlands were informed about this research.
Second, all patients who were not recruited after this invitation, who attended the outpatient clinics of the Academic Medical Center (Amsterdam, The Netherlands) (n=20) or Radboud University Medical Center (Nijmegen, the Netherlands)(n=20) were invited. These patients were invited between March and September 2016. By inviting all patients of two large centers, in addition to the invitation via the patient society, risk of sampling bias was minimalized.

Ethical approval and consent procedure

The Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands, confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this research and that an official approval of this study by the Ethical Committee was not required. All patients and/or parents provided written informed consent.

Measurement

The intensity of care in each domain of the CAP is defined from 0 (no need for additional care) to 5 (needs help with every activity). A score of 1 or higher means there is impairment to an extent that adaptations in the daily program or additional care are needed. See Table 1 for a detailed description of the definitions of the intensity of additional care in each of the domains. The CAP aims to provide insight into the need for additional care, and it does not aim to compare patients to the general population, and therefore no control group is necessary. The CAP has been validated in pre-school children and adolescents with variable non-progressive neurodevelopmental disorders, such cerebral palsy [4,5]. The CAP has not been validated for adults, and has not been separately validated for CG.

Procedure

Patients and/or their parents were interviewed once in a quiet and private environment. If the patient was <18 years, or if the patient was a non-capacitated adult, at least one of the parents was present during the interview. All interviews were performed by the same investigator (LW) who was trained in the use of the CAP.

Statistical analysis

All results were presented in a descriptive manner. We used SPSS version 22 to perform the descriptive statistics. Because of a non-normal distribution, age is presented as a median with range.

Chapter 7

Results

Participants
Participation rate
After the invitation through the Dutch Galactosemia Society, 32/131 invited individuals were recruited. In addition, 14 patients who had not responded after this invitation, were recruited after invitation through their treating physicians at the Academic Medical Center (12 of 20 individuals invited) and the Radboud University Medical Center (2 of 20 individuals invited). A total of 46 patients were thus interviewed, and 44 patients were included in our study; two patients were excluded afterwards because CG had not been confirmed with enzyme measurement or DNA analysis. All participants were interviewed between March and September 2016.

Characteristics of the respondents
Of the 44 patients included, 18 were male and 26 were female, with a median age of 15 years (range 2 to 49 years). This sample included 23 children and adolescents (2 to 17 years), and 21 adults (range 18 to 49 years). All patients had either a GALT enzyme deficiency (range 0% to 7.1% of healthy controls), and/or two known pathogenic variations in the *GALT* gene.

Capacity profile scores
Frequency of domain scores are presented separately for each of the five domains in Figure 1.

Physical health:
At time of the study, all patients adhered to a galactose-restricted diet, and therefore all participants scored at least 1 at this scale (see Table 1). Three of 44 patients needed more care (all had a score of 2); in two adults this was due to further adaptation of their daily program because of tiredness during the day, and in one adult patient due to epilepsy that was not fully controlled with anti-epileptic medication (this patient suffered from severe motor retardation and cognitive impairment).
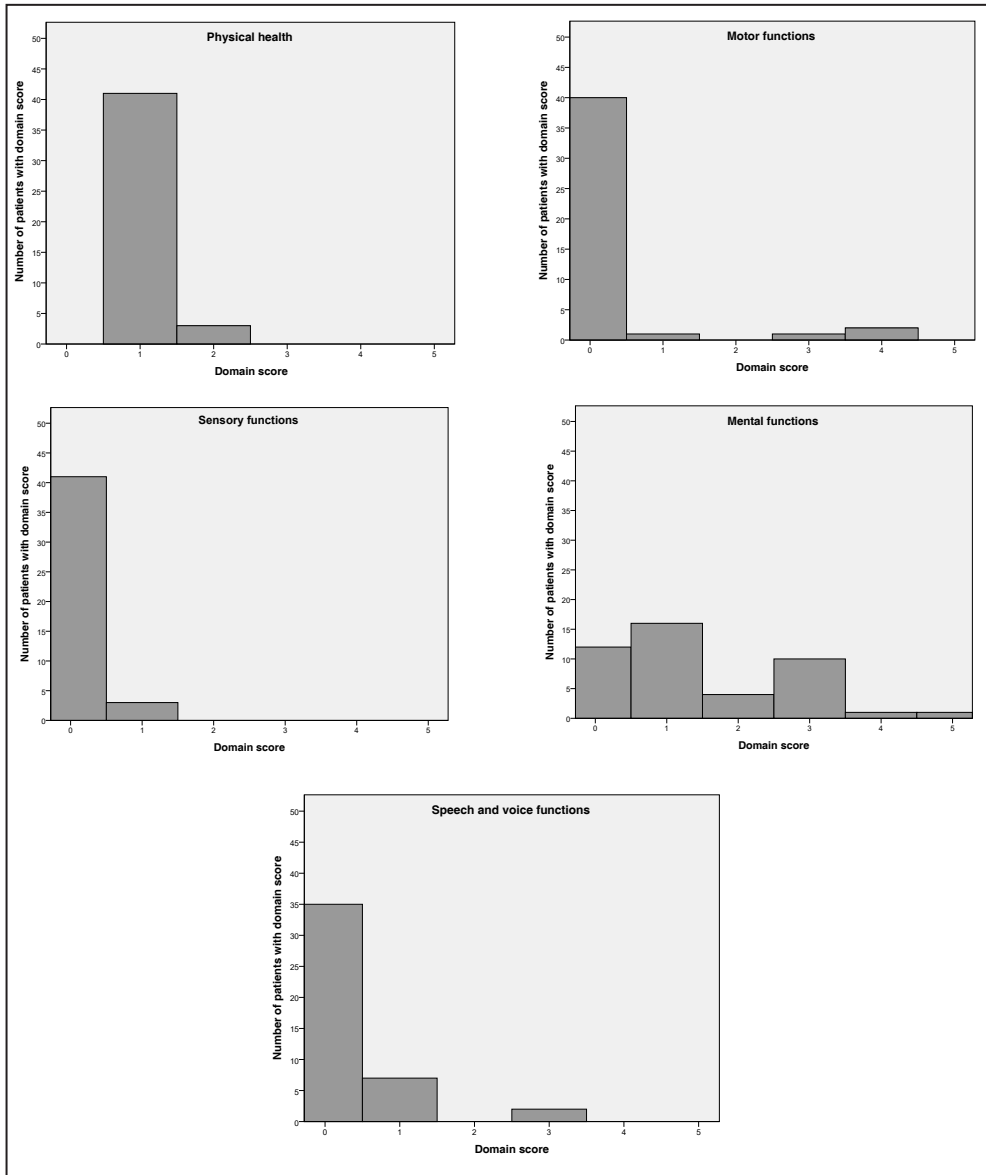
Figure 1. Histogram of scores of 44 patients, for each domain of the Capacity Profile

Motor functions:

Four of 44 patients (aged 11 to 19 years) suffered from impairment of motor functions, scoring 1 to 4 on the CAP scale. These patients suffered from mild to severe tremors in their extremities, necessitating adaptations in their daily program or extra personal help in all. All four demonstrated moderate to severe cognitive impair-

ment with scores of 3 or higher on the mental functions scale. Two of these four patients additionally were wheelchair bound because of motor impairments. Of the complete group, many patients/parents reported abnormalities in fine and gross motor skills which did not lead to additional care. Reported abnormalities were: difficulties with handwriting (and other fine motor skills), stiff/clumsy gross motor skills, difficulties in learning new (sets of) movements (such as a new swim stroke or dancing moves) until automated, struggles with games and activities using a ball.

Sensory functions:
Three of 44 patients (all adults) reported abnormal sensory functions (vision, hearing and tactile sense). One patient suffered from glares in his vision due to cataract. The other two patients suffered from hearing impairment, unrelated to the galactosemia, which indicated adaptations in their daily life(score of 1).

Mental functions:
Scores on the mental functions scale ranged from 0 to 5, with a median of 1. Of the adults, 6/21 patients had a score of 0, and were thus not in need of any additional help or coaching, and received normal primary and secondary education. Nine of 21 adult patients had a score of 1, mainly due the fact that they were socially vulnerable and needed some coaching in their daily life. Most of these patients had difficulties with social interaction, such as making contact with others and maintaining friendships. Of these patients, four received special education in primary school, of which one also received special education in secondary school. Three patients had a score of 2, due to learning problems and need of frequent coaching and support from others. One of three patients received special primary education, but all received normal secondary education. Two patients had a score of 3, because they had severe learning problems and/or were in need of daily support from others. One of these two received special primary and secondary education, the other patient received normal education but suffered from autism. One patient had a score of 4 (severely impairment intellectual disability), and was fully dependent on the care.
Of all adults, 18/21 were living independently (all had a score of either 0, 1 or 2 on the mental functions domain) or would be able to live independently in the near future, as four patients were aged <22 years and lived with their parents. The three patients who were not able to live independently had domain scores ranging from 2, 3 and 4 respectively on the mental functions domain.
Of the children, 6/23 patients had a score of 0, and thus received normal education (two patients were not in school yet). Seven of 23 patients had a score of 1, mainly because they were socially vulnerable and parents reported these children to be-

have young for their age. One patient was not in school yet, two were in primary school and received normal education. The five others were in secondary school, of whom one received special education. One of 23 patients had a score of 2, and this patient was in kindergarten at the age of six and not ready yet to receive primary education. Eight of 23 patients had a score of three, because of severe learning problems and/or need of daily support from others. Three patients were in primary school and received special education, and five were in secondary school and all received special education. Zero patients had a score of 4, and one patient had a score of five (severely impairment intellectual disability), and was fully dependent on care.

Many patients who received normal education, or had received normal education in childhood/adolescence, reported extra help in school specifically for math, reading and languages, both in primary and secondary education.

Speech and voice functions:
A total of 9 of 44 patients (of whom five children and four adults) suffered from impairment on the speech and voice functions scale. Reported problems differed in severity and included articulation errors, speech sound errors (phonemes), disturbed voice quality and speech difficult to understand (mainly mumbled speech). The majority had a score of 1, meaning that listeners had to concentrate more, and understanding in telephone conversation was difficult. Many parents of children reported difficulties with phonemes, but these difficulties did not always result in clear impairment of speech function, therefore not leading to a score above zero on the scale. A total of 22 of 44 patients received speech and language therapy in the past, and four received speech and language therapy at time of the study.

Chapter 7

Discussion

The aim of this study was to assess the type and intensity of the need for additional care in patients with CG using the Capacity Profile. The importance of identifying such impairments lies in achieving early recognition and timely initiation of appropriate extra help, care or treatment, to facilitate participation. Moreover, defining the severity of disorders in this way, provides insight in the efforts that parents, other caregivers, and society, should make. Ranging from 0 to 5, a CAP score of 1 or higher indicates the need for adaptations in the daily program or need for additional care.

The most frequent impairment besides the dietary adaptations, with also the widest spectrum, was found in the domain of mental functions, which is in line with the spectrum of cognitive abilities found in patients with CG, which ranges from severely decreased to above average. The mental functions domain also includes social elements. Remarkably, there is are large group of patients with a score of 1 (16 of 45 patients), with (low)normal cognitive abilities, and usually normal education, but in need of additional care/help in the form of coaching due to social vulnerability difficulties in social interaction. Because the CAP is not designed to specify the type and severity of problems in social functioning, future research is warranted to further explore social functioning in patients with CG.

Also in line with previously published results about childhood apraxia of speech and other speech and language defects in CG, 9 of 45 patients had some impairment of speech. Almost half of all patients received speech and language therapy in childhood. Most patients did not have impairment of motor functions leading to adaptations in their daily life, but four patients suffered from invalidating tremors and two patients were wheelchair dependent. All suffered from intellectual disability as well, scoring 3, 3, 4 and 5 respectively on the mental functions scale. Many other patients reported difficulties regarding fine motor skills (handwriting), learning new sets of movements, clumsiness etc. At the time of the study, these patients did not report adaptations in daily life or need for additional care for these problems, but this seemed to have an effect on their self-esteem. In the domain of physical health, all patients had some adaptation in their daily program because they all need to adhere to a galactose-restricted diet, but the vast majority of patients did not have further problems leading to impairment of physical health, felt healthy and had normal daily programs. Only one patient suffered from impairment in sensory functions as a result of CG, as he reported glares in his vision due to residual cataract. Two other patients suffered from hearing impairment, which is not a known complication of CG. The difficulties in motor skills in combination with the social difficulties, suggests the possibility of developmental coordination disorder (DCD), which has not been described before in CG. Recognition of such problems is important because different therapeutic interventions for children

with (characteristics of) DCD are available: task-orientated therapy (aims to improve specific tasks through practice) and process-oriented therapy (concentrating on developing sensory modalities involved in motor performance)[6]. Also therapies focusing on self-esteem have been developed. Future studies into this are warranted.

This study has several strengths and limitations. This is the first study to systematically evaluate the consequences of impairments in body functions in patients with CG. The Capacity Profile has shown to be a helpful and effective tool in evaluating the need for additional care as the consequence of the current impairments in individual patients with CG. A strength of this study is the representativeness and size of the patient sample included. By not only inviting patients through the patient society, but also all patients followed by two major metabolic centers in the Netherlands, risk of sampling bias was kept as small as possible. The patient sample includes about one fourth of all patients with CG known in the Netherlands.

A limitation of this study is the fact that a few adult patients started dietary treatment after the first two months of life, possibly affecting cognitive outcome [7,8], which may influence their score on the mental functions domain. On the other hand, patients with enzyme activities <15% are identified through newborn screening, who may have a relatively mild phenotype [9]. Furthermore, the CAP is not a diagnostic tool, but a tool to classify the need for additional care. If impairments are reported by a patient or parents, additional assessment should be performed to further determine the type and extent of the impairments. In galactosemia, the regular follow-up as advised by the international guideline, should be performed [1]. To date, it is not known if initiation of additional care for the impairments, such as identified by the CAP, will lead to a better quality of life in the individual patient.

In conclusion, we demonstrated the need for additional care as the consequence of impairments in body functions in CG. These impairments occurred most frequently in the mental functions domain, with specific problems in social functioning, which have not been reported in such detail before. Ten percent of patients with CG had motor function impairment due to tremors, and some may have characteristics of DCD, however not leading to impairments. Impairment of speech and voice function was common, but was usually mild. Impairment of physical health (above the need for compliance to a galactose-restricted diet) and sensory functions were uncommon. The CAP instrument is an effective tool in evaluating impairments and need for additional care in individual patients with CG. It is also a helpful tool to obtain an overview of the capacities and impairments of a larger group of patients, and thus the results of this study provide professionals more insight in the broad phenotypic spectrum of CG.

Chapter 7

References

1. Welling L, Bernstein LE, Berry GT, Burlina AB, Eyskens F, Gautschi M, et al. International clinical guideline for the management of classical galactosemia: diagnosis, treatment, and follow-up. J Inherit Metab Dis 2017;40:171–6.

2. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis 2006;29:516–25.

3. Meester-Delver A, Beelen A, Hennekam R, Nollet F, Hadders-Algra M. The Capacity Profile: a method to classify additional care needs in children with neurodevelopmental disabilities. Dev Med Child Neurol 2007;49:355–60.

4. Meester-Delver A, Beelen A, Ketelaar M, Hadders-Algra M, Nollet F, Gorter GW. Construct validity of the Capacity Profile in preschool children with cerebral palsy. Dev Med Child Neurol 2009;51:446–53.

5. Meester-Delver A, Beelen A, Van Eck M, Voorman J, Dallmeijer A, Nollet F, et al. Construct validity of the Capacity Profile in adolescents with cerebral palsy. Clin Rehabil 2010;24:258–66.

6. Gibbs J, Appleton J, Appleton R. Dyspraxia or developmental coordination disorder? Unravelling the enigma. Arch Dis Child 2007;92:534–9.

7. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr 1993;152:36–43.

8. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis 1990;13:802–18.

9. Welling L, Boelen A, Derks TGJ, Schielen PCJI, de Vries M, Williams M, et al. Nine years of newborn screening for classical galactosemia in the Netherlands: Effectiveness of screening methods, and identification of patients with previously unreported phenotypes. Mol Genet Metab 2017;120:223–8.

Table 1. Definitions of the intensity of additional care in each of the domains (Meester-Delver et al. 2007[3])

Physical health functions

0 = No additional care: no conditional impairments (disorders), an age-appropriate daily pro-gramme can be followed.
1 = An age-appropriate programme is possible, but only with the necessary medication, diet, or regimens.
2 = Owing to limited capacity, amendment of the daily programme is necessary. Example: self-catheterization can be learnt, but this takes more time, so that the daily programme must be amended accordingly.
3 = In spite of aids and adaptations there are limitations in daily functioning and daily assistance is needed; this can be arranged at regular times.
4 = Needs assistance from other all day long, but is actively involved
5 = Is totally dependent on assistance.

Neuromusculoskeletal and movement-related functions

0 = No motor impairments (disorders).
1 = Slight motor impairments (disorders), but no limitations in daily functioning; possibly there is a need for elbow crutches, orthopaedic footwear, braces, etc., but no adaptations in the environment.
2 = The patient experiences no limitations in daily functioning, but aids and adaptations in the environment are necessary.
3 = In spite of aids and adaptations there are limitations in daily functioning and daily assistance is needed; this can be arranged at regular times.
4 = Needs assistance from others all day long, but is actively involved.
5 = Is totally dependent on assistance.

Sensory functions

0 = No sensory impairments (disorders); situations can be assessed age-appropriately.
1 = Some sensory impairments (disorders). Sometimes aids are needed, but there are no limita-tions in daily functioning.
2 = The individual can function independently without personal assistance, but aids and adapta-tions in the environment are necessary.
3 = In spite of aids and adaptations there are limitations in daily functioning and daily assistance is needed; this can be arranged at regular times.
4 = Needs assistance from others all day long, but is actively involved.
5 = Is totally dependent on assistance.

Mental functions

0 = Age-adequate functioning.
1 = Takes adequate initiatives in decisions him/herself and is able to live independently, but needs coaching or intermittent support and asks for assistance.
2 = Able to live independently, needs intermittent support, but is mainly guided by the care-pro-viders, who also have the necessary authorization.
3 = Takes initiatives and makes plans that contribute to independent functioning. Mainly has to learn social skills. Needs daily feedback on activities and can be left alone for part of the day. Needs limited support.
4 = Needs assistance with every decision that is taken; is able to make simple choices, but unable to carry them out. Learns mainly from constant repetition. Needs extensive support.
5 = Has no insight into his/her own life, cannot manage his/her own life, and is unable to make de-cisions or choices; the care-providers know what is needed through observation and experience. Needs pervasive support.

Chapter 7

Voice and speech functions

0 = The requirements concerning mouth movement ability, vocal tone, breathing, and sensitivity of the oral cavity are met, so that good, understandable speech should be possible. Whether or not the individual actually does talk, and the content of speech depends on the level of mental functions.

1 = As 0, but vocalization and articulation are difficult, so that the listener needs to concentrate more and understanding in telephone conversation is difficult.

2 = The above requirements are met with such difficulty that an augmentative form of communication is needed, but there is no need for assistance from other people.

3 = As 2; the individual is able to use an additional form of communication, but needs assistance to install the device in order to use it.

4 = The individual can only communicate if personal assistance is available, e.g. because only eye movements are possible.

5 = No communication possible.

Chapter 8

Social responsiveness in classical galactosemia

Lindsey Welling, Marieke de Vries, Rebecca Holman, Carla E.M. Hollak, Kim J. Oostrom and Annet M. Bosch

Abstract

Classical galactosemia (CG) is a rare inborn error of metabolism, leading to complications in many patients. Because patients and parents often report social problems, we aimed to objectify the occurrence of difficulties in social responsiveness (SR). Patients aged ≥4 years with CG participated. Patients or parents completed the Social Responsiveness Scale questionnaire. A T-score ≥61 indicates deficiencies in SR that result in mild to moderate interference in everyday social interactions and a T-score ≥76 indicates deficiencies that result in severe interference in everyday social interactions. Thirty-three patients were included (13 children, 20 adults, median age 19 years, range 6 - 46 years). Five patients (15%) had a score of ≥76. This is a statistically significant difference when compared to the expected frequency of 0.6% based on a T-distribution. The percentage of patients with a T-score of ≥61 was not significantly higher than expected (27%; versus expected 16%). Higher T-scores were associated with lower levels of educational attainment.

Conclusions Relatively many patients with CG have severe deficiencies in SR. The patient sample was too small for definitive conclusions about mild to moderate deficiencies in SR in this group of patients. Co-occurring intellectual disability may contribute to these deficiencies.

Introduction

Classical galactosemia (CG, OMIM 230400) is a rare inborn error of metabolism, caused by deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT, EC 2.7.7.12). A life-threatening illness occurs after neonates ingest galactose, but symptoms quickly resolve once a galactose-restricted diet is initiated, which is currently the only available treatment. Despite early start of treatment and good compliance, patients are at risk to develop long-term complications including impaired cognitive abilities and primary ovarian insufficiency. In previous studies, evaluating Health-Related Quality of Life, it was shown that patients are less frequently married or living in stable partnership compared to peers and experience lower social functioning [1–6]. In our recent study (unpublished work, evaluating patient capacities) social vulnerability and other problems in social functioning (such as difficulties to make friends and maintain friendships) were strongly expressed by parents and patients of all ages. Social responsiveness (SR) is the ability to engage in emotionally appropriate reciprocal social interactions, which is key in developing and maintaining relationships and in community participation [7]. Reciprocal social behavior requires one to recognize emotional and interpersonal cues of others, to appropriately interpret those cues, to respond appropriately to what he or she interprets and to be motivated to engage in social interactions [8]. Intellectual disability (ID) is a known co-occurring factor of problems in reciprocal social behavior, though widely discrepant numbers in overlap have been reported and it has not been unraveled yet if they are related in terms of their etiology [9]. Occurrence and type of problems in social functioning have not been systematically studied in patients with CG.

The primary aim of this pilot study is to objectify the occurrence and nature of difficulties in SR in children and adults with CG, using the Social Responsiveness Scale (SRS) questionnaire.

Chapter 8

Methods

Research design
We performed a cross-sectional study in which patients (≥18 years) or their parents (<18 years) completed the SRS questionnaire. This validated questionnaire takes a quantitative approach in measuring social abilities, as well as broader autistic symptomatology (e.g. preoccupations/traits). It provides an index of deficiency in SR, and higher scores on the SRS indicate greater social difficulties.

Participants

Inclusion and exclusion criteria
Individuals aged ≥4 years with confirmed CG (residual GALT enzyme activity of <15% and/or two known pathogenic variations in the *GALT* gene) were eligible to participate.

Exclusion criteria: severe mental impairment (*i.e.* highly dependent on professional care, and unable to attend school), adults who were unable to complete the questionnaire independently.

Sample size
No formal power calculation was performed. Since CG is a rare disorder, we decided to include as many patients as possible.

Recruitment strategies
All eligible patients who participated in a previous study (unpublished work) were invited to participate (between March and September 2016). In the previous study, patients were invited via multiple routes. First, all members (n=131) of the Dutch Galactosemia Patient Society were invited through e-mail in March 2016. This invitation was linked to an invitation for a family weekend of the society. Second, all patients who were not recruited after this invitation, who attended the outpatient clinics of the Academic Medical Center (Amsterdam, The Netherlands)(n=20) or Radboud University Medical Center (Nijmegen, the Netherlands)(n=20) were also invited between March and September 2016. By inviting all patients of two large centers, in addition to the invitation via the patient society, risk of sampling bias was minimalized. A total of 46 patients were included in the previous study, of whom 39 were invited for this research (7 patients were not invited because they were not eligible). For the current research, patients were additionally invited through a presentation about this research at a patient society meeting (November 2016) and seven patients expressed interest in participation.

Ethical approval and consent procedure
The Ethical Committee of the Academic Medical Center, Amsterdam, The Nether-lands, confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this research and that an official approval of this study by the Ethical Committee was not required. All procedures in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its lat-er amendments or comparable ethical standards. Informed consent was obtained from all individual patients (and/or their parents) included in this study.

Measurement

Social responsiveness questionnaire
The SRS is a quantitative measure of SR, with separate questionnaires for children and adults. Both versions were found to have an excellent sensitivity and specificity [10,11].
For children, the validated Dutch version SRS-2 parent questionnaire was used, which consists of 65 items [11]. Each item was scored by the parent on a 4-point scale Likert-scale, ranging from 1 (not true) to 4 (almost always true). For adults, the vali-dated Dutch version of the SRS-A self-report questionnaire was used, which consists of 65 items, scored by the patients on a 4-point scale Likert-scale similar to the SRS-2 [10]. T-scores were generated, based on raw scores, for the total scale as well as for several treatment subscales: Social Awareness, Social Cognition (children only), Social Communication, Social Motivation, and Restricted Interests and Repetitive Behavior. T-scores are used to tell how far an individual's score is from the mean. A T-distribution has a mean of 50 and a standard deviation of 10. T-scores of ≥76 are interpreted as indicating severe deficiencies in social reciprocal behavior that are clinically highly significant and lead to substantial interference in everyday social interactions, T-scores of 61 to 75 are in the mild to moderate range and T-scores of <61 are considered to be within typical limits.

Educational attainment
Neuropsychological testing was not systematically performed. To be able to analyze outcomes in relation to mental capacity, we used educational attainment of pa-tients. Patients were categorized based on highest achieved educational attainment. Adults were categorized using the International Standard Classification of Education (ISCED) 2011 [12]. Children were categorized as: 1) Mainstream primary or secondary education, 2) Mainstream primary or secondary education with additional teaching support, 3) Special primary or secondary education, and 4) Education for mentally and multi-handicapped children.

Statistical analysis

SPSS version 22 was used to perform all statistical analyses. Because of a non-normal distribution and because of the relatively small patient sample, age and T-scores of the Social Responsiveness Scale (SRS) are presented as the median and range. Median T-scores of children and adults, as well as males and females, were compared using a Mann-Whitney U test. Median T-scores of the different subscales were compared using a Friedman test. We report the percentage of patients with a score indicating a clinically significant deficiency in SR, resulting in interference in everyday social interactions. A T-score of ≥61 (1 SD above the norm mean) indicates mild to moderate deficiencies in every day social interaction, a T-score of ≥76 (2.5 SD above the norm mean) indicates severe deficiencies in SR as determined in the SRS. For comparison of our patient sample with the norm population, we determined the expected percentages of individuals with a T-score of >1 SD and >2.5 SD respectively in a T-distribution critical values table, which were 17% and 0.6% respectively. We compared the percentage of patients with CG with a T-score of ≥61 and ≥76 to the expected percentage of individuals having a T-score of ≥61 and ≥76 in the norm population using a one sample Chi-square test. A correlation between educational attainment and total T-scores was assessed using a Spearman rank correlation test and 95% confidence intervals were obtained using 1000 bootstrap samples. For all tests we reported the exact significance and considered p-values less than 0.05 as statistically significant.

Results

Participants

A total of 33 of 46 patients (72%) who were invited to participate were included in this study, 11 males and 22 females, with a median age of 19 years (range 6 to 46 years). A total of 13 patients were aged 4 to 17 years and 20 patients were aged ≥ 18 years.

Social Responsiveness Scale

The median total T-score of all 33 patients was 54 (range 37 to 92). Nine patients (27%) had a score of ≥61. Based on a normal T-distribution, the expected frequency of a score ≥61 is 16%. The difference between the frequency in our sample compared to the expected frequency is not statistically significant (p = 0.093). Five patients (15%) had a T-score ≥76, indicating severe deficiencies in reciprocal responsiveness, compared to an expected frequency of 0.6%. This difference is highly statistically significant ($p \leq 0.001$). The difference between median total T-scores for children (59, range 40 to 84; n=13) and adults (52, range 37 to 92; n=20) was not statistically significant (p = 0.147). The difference between the median T-score of males (56, range 40 to 92; n=11) and females (50.5, range 37 to 85; n=22) was also not statistically significant (p = 0.200).

Subscales

For the whole group, median T-scores of the subscales (social awareness, social communication, social motivation and restricted interests and repetitive behavior) were compared and no statistically significant difference was found (*p* = 0.572). There were also no statistically significant differences between median T-scores of the subscales in the group of children (p = 0.916) and adults (*p* = 0.680), nor between the subscales in the group with a total median T-score of <61 (p= 0.618), T-score ≥61 (0.992), T-score <76 (p = 0.736) and T-score ≥76 (p = 0.709).

Educational attainment

Educational attainment was known for 32/33 patients (see Table 1; missing data for one adult patient). Higher total T-scores were associated with lower levels of educational attainment in both children (correlation coefficient 0.65, 95% confidence interval 0.13 to 0.95, *p* = 0.017) and adults (correlation coefficient 0.50, 95% confidence interval 0.06 to 0.81, *p* = 0.031).

Table 1. Highest level of educational attainment relative to median total T-scores on the Social Responsiveness Scale

Highest level of education	Number of patients	Median total T-score (range)
Children[a], *	13	
Category 1	3	48 (46 – 51)
Category 2	6	59.5 (40 – 71)
Category 3	0	-
Category 4	4	80.5 (49 – 85)
Adults[b], *	19	
ISCED category 666	2	39 (37 – 41)
ISCED category 354	4	49.5 (39 – 65)
ISCED category 353	8	54.5 (46 – 76)
ISCED category 254 and lower	5	55 (50 – 92)

[a] Category 1: Mainstream primary or secondary education; Category 2: Mainstream primary or secondary education with additional teaching support; Category 3: Special primary or secondary education; Category 4: Education for mentally and multi-handicapped children.
[b] ISCED category 666: Bachelor or equivalent, orientation unspecified; ISCED category 354: as 353 with direct access to firs tertiary programs; ISCED category 353: Upper secondary vocational education, without direct access to first tertiary programs; ISCED category 254: Lower secondary education level completion with direct access to upper secondary programs.
* Statistically significant correlation between level of education and total T-score with Spearman rank correlation test.

Discussion

In this pilot study the aim was to objectify the occurrence and severity of impairments in social functioning and social interaction in children and adults with CG, using the SRS questionnaire to quantitate deficiencies in SR. Relatively many patients with CG show severe deficiencies in SR (15%), significantly more than the expected frequency of 0.6% in a normal T-distribution.

No less than 27% of patients had a total T-score indicating mild to moderate deficits in social interaction. The difference between this 27% in patients with CG and the expected 16% in a normal distribution escaped conventional levels of significance, likely due to the small sample size included, which is the main limitation of this study. There was no indication for differences in SR between children and adults, nor between males and females. There was also no indication of more problems in one specific domain of SR. Educational attainment was structurally categorized and higher total T-scores were associated with lower levels of educational attainment in both children and adults. Because ID is a known co-occurring factor of SR (though the exact relation has not been unraveled [9]), co-occurrence of ID in patients with CG may thus contribute to clinically relevant problems in SR. This should be further evaluated in a larger group of patients in whom intellectual abilities are formally tested. In conclusion, this study provides a first indication that severe deficiencies in SR occur more frequently in patients with CG. A larger sample of patients should be studied for definitive conclusions about the occurrence of mild to moderate deficiencies in SR in this patient group. Further research is necessary to determine if deficiencies in SR are mainly determined by ID or if these patients have deficiencies in SR irrespective of ID.

References

1. Bhat M, Haase C, Lee PJ. Social outcome in treated individuals with inherited metabolic disorders: UK study. J Inherit Metab Dis. 2005;28:825–30.

2. Waisbren SE, Potter NL, Gordon CM, Green RC, Greenstein P, Gubbels CS, et al. The adult galactosemic phenotype. J Inherit Metab Dis. 2012;35:279–86.

3. Hoffmann B, Dragano N, Schweitzer-Krantz S. Living situation, occupation and health-related quality of life in adult patients with classic galactosemia. J Inherit Metab Dis. 2012;35:1051–8.

4. Bosch AM, Maurice-Stam H, Wijburg FA, Grootenhuis MA. Remarkable differences: the course of life of young adults with galactosaemia and PKU. J Inherit Metab Dis. 2009;32:706–12.

5. Gubbels CS, Maurice-Stam H, Berry GT, Bosch AM, Waisbren S, Rubio-Gozalbo ME, et al. Psychosocial developmental milestones in men with classic galactosemia. J Inherit Metab Dis. 2011;34:415–9.

6. Bosch AM, Grootenhuis MA, Bakker HD, Heijmans HSA, Wijburg FA, Last BF. Living with classical galactosemia: health-related quality of life consequences. Pediatrics. 2004;113:e423-8.

7. Beauchamp MH, Anderson V. SOCIAL: an integrative framework for the development of social skills. Psychol Bull. 2010;136:39–64.

8. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, et al. Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the Autism Diagnostic Interview-Revised. J Autism Dev Disord. 2003;33:427–33.

9. Matson JL, Shoemaker M. Intellectual disability and its relationship to autism spectrum disorders. Res Dev Disabil. 2009;30:1107–14.

10. Constantino JN, Gruber C, Noens I, De la Marche W, Scholte E. Handleiding SRS-A Screeningslijst voor autismespectrumstoornissen. Amsterdam: Hogrefe Uitgevers B.V.; 2012.

11. Constantino JN, Gruber C, Roeyers H, Thys M, Druart C, De Schryver M, et al. Handleiding SRS-2 Screeningslijst voor autismespectrumstoornissen. Amsterdam: Hogrefe Uitgevers B.V.; 2015.

12. Statistics OI for. ISCED 2011 overview. In: ISCED 2011 Operational Manual: Guidelines for Classifying National Education Programmes and Related Qualifications. 2015.

Chapter 8

9

Chapter 9

Summary, discussion and future perspectives

Summary, discussion and future perspectives

Since the identification of classical galactosemia (CG), an autosomal recessive in-born of metabolism error caused by a deficiency of the enzyme galactose-1-phos-phate uridyltransferase (GALT), only one treatment has been introduced and this treatment remained essentially unchanged over the years: a galactose-restricted diet. It was soon discovered that after initiation of this diet the critical illness of the intoxication phase in CG quickly resolves. However, already between 1969 and 1980, the first disappointing reports on long-term outcome in CG patients treated with a galactose-restricted diet since diagnosis were published, leading to great concerns [1–3]. In 1982, an article by an anonymous author, with the intriguing ti-tle 'Clouds over galactosaemia', was published in the Lancet[4]. In this paper it was concluded that the success of dietary treatment is indeed disappointing, as cogni-tive impairment was common in these long-term treated patients. Over three de-cades later, other long-term complications have been identified, occurring despite early start of treatment and good dietary compliance[5]. These include neurological problems and primary ovarian insufficiency in most female patients. Unfortunately at this moment no other disease-modifying therapies are available. It is clear that much more insight needs to be gained into the pathophysiological processes under-lying these complications in order to design such therapies.

Meanwhile, it is essential to study the prevalence and clinical significance of com-plications in more detail and to further explore the phenotypic spectrum of the disease, in order to improve patient care. Furthermore, new methods should be developed which help to predict clinical outcome early in life and individualize cur-rent treatment protocols.

Part I Newborn screening and patients with a variant presentation of classical galactosemia

Newborn screening
Many countries have not (yet) implemented CG into their newborn screening (NBS) programs, due to uncertainty about the balance between advantages and harms. A potential risk of all screening programs is the detection of false-positive (FP) cases, which may cause severe anxiety and even depression in parents, which may per-sist long after the disease was ruled out. Second, in screening programs for other diseases, patients are identified with clinical and biochemical phenotypes and gen-otypes that have not been reported, for whom it is unclear if they need treatment and follow-up[6]. Furthermore, it has been demonstrated that early treatment does not prevent long-term complications in CG. In the Netherlands, NBS for CG was

introduced in 2007, with the aim to prevent critical illness and death in the first weeks of life.

In **Chapter 2** the primary aims were to assess the effectiveness of all five screening methods used in the Netherlands between 2007 and 2015, and to identify and discuss patients with previously unreported clinical and biochemical phenotypes and genotypes.

The screening method which was initially introduced comprised of only marker: total galactose (TGAL: galactose-1-phosphate (Gal-1-P) plus galactose) measured in dried blood spots (DBS). Patients were referred for further testing when TGAL was >700 μmol/l blood. Due to an excessive number of false-positive (FP) cases, a two-tier system was subsequently introduced with GALT activity (cut-off value (COV) ≤20%) in DBS as a primary marker, and TGAL as a second tier, only measured when GALT was ≤20%. 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 the period up to July 2014 all screening laboratories switched to a new GALT essay, with the COV's set at GALT ≤ 2.7 U/dl and TGAL≥900 μmol/l. Finally, from July 1st 2015 the COV's were changed to GALT≤2.0 U/dl and TGAL≥1100 μmol/l, again with the purpose to further reduce the number of FP cases. Despite ongoing efforts to improve effectiveness of screening, high numbers of FP cases and relatively low positive predictive values were found with every screening method (with an average positive predictive value of 3.6%). For the future, it is important to further reduce the number of FP cases, by continuously evaluating and adapting COV's.

Between 2007 and 2015, 31 patients were diagnosed with CG in the Netherlands, of whom 28 through NBS (out of 1.637.733 screened newborn infants) and three were diagnosed antenatally because of a sibling with CG. After a cross-check with the Dutch Diagnosis Registration Metabolic Diseases (DDRMD), there was no indication that patients were missed by NBS (i.e. diagnosed by clinical signs and symptoms). In almost all patients detected with NBS, critical illness was prevented and the mortality rate was 0%. The mortality rate before introduction of CG into the NBS program in another country (Sweden) was reported to high, though exact numbers are not available[7]. Of the 31 patients, six had a previously unreported clinical and biochemical phenotype and genotype. Because three of them were from one family and had the same genotype and phenotype, they were considered as one case. Therefore, four individuals in a cohort of 29 patients, were considered to have a previously unreported phenotype and genotype (14%) and are referred to as atypical CG for the following reasons. In contrast to 'typical' patients with CG, these individuals:
1) Did not demonstrate signs and symptoms of CG at time of diagnosis while on a

diet containing galactose.

2) Demonstrated a very rapid decrease of Gal-1-P to levels below the detection limit, within months after start of diet.

3) Did not suffer from cataract in the neonatal period.

4) Had higher residual GALT enzyme activities.

5) Had previously unreported genotypes.

They most likely have a higher residual capacity to metabolize galactose, and may benefit from a less severely restricted diet or may later even be prescribed a normal diet. It is therefore important to identify patients with atypical CG, to prospectively follow them up and to develop methods for individual prognostication and treatment. This is discussed in more detail in the Future Perspectives section at the end of this chapter.

Galactose oxidation measurement and galactose metabolites profiling in fibroblasts of patients with a classical and variant presentation of galactosemia

In **Chapter 3** the aim was to develop a method to provide more insight into whole-cell galactose metabolism, which allows quantitative assessment of residual galactose metabolism in galactosemia patients. Radioisotope labelled galactose oxidation measurements (using $U^{14}C$-galactose-1-phosphate as a substrate) were performed and a method for galactose metabolites profiling (GMP) in fibroblasts using $U^{13}C$-galactose as a substrate was developed, followed by metabolite analysis with tandem mass spectrometry. Both methods were studied in patients with a classical and variant presentation of galactosemia (referred to as classical patients and variant patients, who were referred to as atypical patients in **Chapter 2** of this thesis).

Though there is great variability in outcome of patients with the same genotype and residual GALT enzyme activity, it is not possible to predict outcome at the time of diagnosis with current methods and markers. Uncertainty about the prognosis not only puts a heavy burden on patients and their families, but may also lead to unnecessary stringent treatment. This becomes even more important since after introduction of CG in the NBS program in the Netherlands in 2007, individuals are identified with a variant presentation of galactosemia with potentially a more beneficial outcome (see **Chapter 2** of this thesis). Patients with a better clinical outcome may have a higher capacity to metabolize galactose compared to patients with more complications of the disease, due to higher residual GALT enzyme activities in both erythrocytes and other tissues. However, this can currently not be reliably assessed with the GALT essays used to diagnose galactosemia. Alternatively, galactose may be metabolized in these patients through alternative, currently unknown,

pathways, or these patients may have a different tolerance of the body to (toxic) galactose metabolites. In very-long-chain acyl-CoA dehydrogenase deficiency, flux of fatty acid oxidation in fibroblasts correlated much better with clinical outcome than residual enzyme activity [8]. In galactosemia, the individual residual capacity to metabolize galactose in fibroblasts may be a predictor of individual clinical outcome and dietary galactose tolerance.

In this study radioactive ^{14}C-labeled galactose oxidation measurements and stable isotope ^{13}C-labeled GMP were performed in fibroblasts of three classical patients, three variant patients and three healthy controls. The following metabolites were extracted: $^{14}CO_2$ for the oxidation measurements and U$^{13}C_6$-galactose, (U$^{13}C_6$)-galactose-1-phosphate and (U$^{13}C_6$)-UDP-galactose for GMP. The ratio of (U$^{13}C_6$)-galactose-1-phosphate/ (U$^{13}C_6$)-UDP-galactose was determined and labeled the galactose index.

Results show that the galactose oxidation capacity was markedly reduced in classical patients and was significantly different from patients with a variant presentation and healthy controls. The galactose oxidation capacities of variant patients were not deficient and were comparable to classical patients, at least in two of the three variant patients. With GMP analysis, U^{13}C-galactose and U^{13}C-Gal-1-P were increased in fibroblasts of classical patients and variant patients, but $^{13}C_6$-UDP-galactose was decreased in these patients. The galactose index (GI), defined as the ratio of U^{13}C-Gal-1-P/ $^{13}C_6$-UDP-galactose, was determined for all groups and was increased in all patients compared to the controls but was significantly higher in classical patients ($p<0.001$) than in variant patients.

In conclusion, our results demonstrate that the developed GMP is a sensitive method for discrimination of classical patients from variant patients, and the latter from healthy controls. These results indicate that variant patients have a higher residual capacity to metabolize galactose compared to classical patients and that the GI can be used as a measure for the severity of the GALT deficiency and residual galactose metabolism in galactosemia patients. The developed GMP method in fibroblasts needs further validation in a larger group of galactosemia patients representing the whole clinical outcome spectrum. Furthermore it is essential to determine if patients with a classical presentation, but with different outcomes, have different profiles of galactose metabolites and if GMP can thus be used as predictor of outcome.

Part II Management of classical galactosemia

Patients with CG need monitoring of dietary treatment and long-term complications, and counselling of the patient and their families. Due to the rarity of the disease, little evidence is available to support decision making in clinical practice. Strategies concerning treatment and follow-up differ significantly between countries and even

between treatment centers within the same country [9,10]

International clinical guideline for diagnosis, treatment and follow-up
In **Chapter 4** the objective was to develop an international, evidence-based guideline addressing diagnosis, treatment and follow-up of patients with CG, aiming to help professionals worldwide to provide the same standard of care to each patient. In this process, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used. A 21-member expert panel was formed after an invitation to all members of the Society for the Study of Inborn Errors of Metabolism (SSIEM). Experts participated in working groups, focusing on topics related to their field of interest. The expert panel formulated key questions, after which search strategies were developed to identify publications of interest. All experts were involved in extraction of evidence from relevant publications. Recommendations were formulated by experts from working groups through multiple consensus rounds. Based on the evidence, recommendations were labelled as "supported by evidence" or as "expert opinion". Furthermore, a strength of recommendation was assigned to each recommendation (either discretionary, or strong).

A total of 40 recommendations were formulated concerning diagnosis, dietary treatment, biochemical monitoring and follow-up of long term complications including problems of cognitive development, speech and language, neurological functioning, psychosocial development, fertility, bone health and the eyes. A 100% consensus was reached for 39 of the 40 recommendations and a 93% consensus was reached on the recommendation concerning timing of bone density screening. Almost half of all recommendations were expert opinion, due to lack of supporting evidence. The other recommendations were mainly supported by studies with an observational study design.

Not surprisingly, substantial gaps in knowledge were identified, foremost in the field of clinically effective treatment strategies and in the field of follow-up of long-term complications. Next to the need to develop a satisfying disease-modifying therapy that effectively prevents late-onset symptoms, an important topic for future research is the development of methods that enable early prognostication and individualized treatment. Both topics are discussed in the Future Perspectives section at the end of this chapter. Future research with regard to long-term complications should be targeted on more extensive assessment of bone health (including clinical relevance assessment of bone turnover makers) and clinical consequences (if patients have a higher risk of fractures), on exploration of new markers concerning reproductive function in females as currently there are no markers to predict chances of pregnancy, and on evaluation of psychosocial and executive functioning with structured questionnaires or testing tools.

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Part III Long-term complications studied in more depth

It is a difficult to reliably and precisely determine the prevalence and extent of long-term complications in CG and their clinical relevance, as the literature primarily consists of studies including relatively small numbers of patients and with variable methodological approaches. However, in order to better understand the natural course of the disease, data on larger and unbiased cohorts are needed. The Galactosemia Network (www.galactosemianetwork.org) has therefore recently developed an international registry which will, in time, supply essential data.

Cognitive abilities

Cognitive functioning of patients with CG has been the subject of many studies. All reported a broad spectrum of functioning and most studies concluded that the majority of patients have a low to low average intelligence quotient (IQ)[11–26]. However, it is difficult to draw conclusions from these single studies, as results typically vary from one study to another. Frequently, it was not reported how CG was diagnosed (based on which criteria), inclusion or exclusion criteria were not defined and age at start of treatment was not reported. Also, patient sample sizes were small, outcomes were presented in different manners and various test instruments were used. A meta-analysis allows to pool individual patient data or aggregate data in a statically correct manner and may include a more precise estimate of the outcome than the single studies which contributed to the meta-analysis [27].

In **Chapter 5** the aim was to assess cognitive functioning of patients with a confirmed diagnosis of CG using full-scale IQ (FISQ) as the primary outcome, in a representative sample of early-treated (<4 weeks of life) patients of all ages. Secondary variables of interest were performance IQ and verbal IQ. We performed a systematic review with meta-analysis based on individual patient data (IPD). After search strategies were created, relevant articles were selected and authors of these articles were contacted to obtain IPD of patients meeting the inclusion criteria. IPD were obtained only for FSIQ. Individual patient data were pooled for meta-analysis using linear mixed-effect models, including covariates (age or gender) as fixed effects where appropriate. Data of 87 patients with a median age of 13 years (range 3 to 38 years) demonstrated a mean FSIQ of 87 (95% CI; 81 to 94), with a broad range of 47 to 122. Almost half of all patients attained scores of less than one standard deviation below the mean (FSIQ <85), almost 40% attained scores of 85-100 and a minority (15%) attained scores above 100. No statically significant difference between males and females was found and there was no indication of deterioration of cognitive abilities over time. No articles addressing performance IQ and verbal IQ were identified that met the inclusion criteria.

The results of this study fortify earlier findings and conclusions about the broad spectrum of cognitive outcome patients, also if treated very early in life, and the fact that these patients are at risk for having impaired cognitive function.

Bone health
Several studies have assessed bone health in CG and indicated mild to moderate impairment of bone mineral density in CG. All studies, however, used different methods and outcome measures and measured bone density at different sites[25,28–34].

In **Chapter 6** the primary aim was to determine the extent of bone health impairment in CG more precisely, by pooling bone densitometry (dual-energy x-ray absorptiometry or DEXA) data in a meta-analysis, using aggregate data (mean bone mineral density (BMD) Z-scores). Also, other indicators of bone status were evaluated, including bone mineral content, bone turnover markers, hormones, vitamins and minerals, and fracture history. After search strategies were formulated, relevant articles were selected and data was extracted. If insufficient data was reported in an article, the authors were contacted. A total of four articles were included in the analysis, including 112 patients. BMD-Z-scores were calculated for separate measurement sites (lumbar spine, total hip, and femoral neck) in different groups (in the complete patient sample, as well as in children and adults separately). In the complete group, mean BMD Z-score was decreased at all measurement sites to a similar extent, with measurements performed at the lumbar spine in all 112 patients, resulting in a lumbar spine BMD Z-score of -0.70 (95% CI: -0.88, -0.52). Data of 76 children from three studies resulted in a mean lumbar spine BMD Z-score of -0.64 (95% CI: -0.84, -0.43), and data of 36 adults included in two studies resulted in a mean lumbar spine BMD Z-score of -0.94 (95% CI: -1.30, -0.57).
Seven studies contributed to the analysis of other indicators of bone status and demonstrated that vitamin D levels were in the low range for the majority of patients and calcium levels were frequently within the reference range. Only a limited number of studies assessed bone mineral content, bone turnover markers, minerals, other hormones and fracture risk, yielding inconsistent results.
In conclusion, the mean BMD Z-score in patients with CG is only moderately decreased (-0.7 SD for the whole group), but remains within two SD of the reference mean of zero. This indicates that more patients (10% to 25%) compared to the general population (2.3%) have BMD Z-scores ≤-2 SD, and appropriate preventive and therapeutic measures are warranted in all patients. For the future, it is most essential to collect data on fracture history in patients of all ages, with special attention for postmenopausal females with primary ovarian insufficiency, to determine if bone health problems in CG also has relevant clinical consequences.

Chapter 9

Capacities, impairments and need for additional care

Despite a high frequency of various complications in CG, consequences of these impairments in body functions have not been studied. Impairments in body functions (as defined in the International Classification of Functioning, Disability, and Health (ICF)[35]) may lead to ongoing need for adaptation in the daily program or additional help or care. The Capacity Profile (CAP) is standardized method to classify need for additional help or care in chronic conditions[36]. Via a semi-structured interview, current impairments in five domains of body functions are determined: physical health, motor (neuromusculoskeletal and movement-related), sensory, mental, and voice and speech functions. The intensity of care is defined separately for each domain and ranges from no need to additional care (score 0), to help with every activity (score 5). A score of 1 and higher thus indicates need for additional care.

In **Chapter 7** current impairments in body functions in 44 patients with CG, and the subsequent need for additional care, were assessed using the CAP. The patient sample included 23 children and 21 adults. Patients and/or their parents were interviewed once and CAP scores for each of the five domains were determined. Need for additional care was most frequently reported in the domain of mental functions, with a wide spectrum of intensity of care with CAP scores ranging from 0 to 5. These results are in line with the range of cognitive functioning in patients with CG. Surprisingly, many patients had a score of 1 due to additional help because of problems with social interaction and social vulnerability. Also in line with the speech disorders reported previously in CG [37,38], one fifth of patients were found to be in need of additional care for speech and voice impairment. Additional need for care in the domain of motor functions was not frequently reported, but four patients suffered from tremors with need for care and two were additionally wheelchair bound. Many patients and/or parents reported problems with fine motor skills (handwriting), learning new sets of movements, clumsiness, symptoms that did not lead to need for additional care at time of the study, but sometimes had a negative effect on self-esteem. These problems, in combination with difficulties in social functioning, may indicate developmental coordination disorder (DCD). DCD has not been described before in CG. However, as therapeutic interventions are available for DCD, this may need more attention. Besides the adaptation in daily programs in all patients because they need to comply to a diet, other impairments in physical health leading to additional care were uncommon. This was also the case for sensory functions.

Concluding, the CAP instrument is an effective tool in evaluating impairments and need for additional care in individual patients with CG. This research provides professionals more insight in the broad phenotypic spectrum of CG. Future studies

evaluating social skills and possible signs of DCD are needed to elucidate the impairments reported and to provide specific therapeutic interventions.

Social functioning

In previous studies, it was shown that patients with CG are less frequently married or living in stable partnership compared to peers. Patients and parents also frequently report social vulnerability and other problems in social functioning, such as difficulties to make friends or to maintain friendships (**Chapter 7**). The Social Responsiveness Scale (SRS) enquires about specific and observable elements of social behavior and the ability to engage in emotionally appropriate reciprocal social interactions, and provides an index of deficiency in social responsiveness (SR)[39,40]. Higher scores on the SRS indicate greater severity of social impairment.

In **Chapter 8** we performed a pilot study with the aim objectify the occurrence and nature of difficulties in SR in children and adults with CG, using the validated Dutch version of the SRS questionnaire[41,42]. For children (<18 years) parents completed the questionnaire and adult patients (≥18 years) completed the questionnaire themselves. With the SRS, raw scores are obtained that are converted to T-scores, based on a norm population. A T-score of 61 to 75 indicates mild to moderate deficiencies in in every day social interaction, a T-score of >75 indicates severe deficiencies in SR. Total T-scores as well as T-scores for several subscales were obtained: social awareness, social cognition (children only), social communication, social motivation and restricted interests and repetitive behavior. Because intellectual disability (ID) is a known co-occurring factor of problems in social reciprocal behavior, information on highest level of educational attainment was collected as a measure of intellectual abilities. Adults were categorized with the International Standard Classification of Education (ISCED) 2011 [12]. Children were categorized as follows: 1) Mainstream primary or secondary education, 2) Mainstream primary or secondary education with additional teaching support, 3) Special primary or secondary education, and 4) Education for mentally and multi-handicapped children.

In total, questionnaires of 33 patients with a median age of 19 years (range 6 to 46 years), were included in the analysis. The median total T-score for all patients was 54 (range 37 to 92), with no statistically significant difference between males and females, nor between children and adults or any of the subscales. Almost 30% of patients (n=9) had a score indicating clinically relevant deficiencies in SR (T-sore >60), whilst the expected frequency of a score >60 in a normal T-distribution is 16%. This difference is not statistically significant, which is most likely due to the small patient sample included. Five of 33 patients (15%), however, had a T-score of >75, compared to an expected frequency of 0.6%, which is a highly statistically signif-

icant difference. Higher T-scores (indicating more severe deficiencies in SR) were significantly associated with lower educational attainment.

This study indicates that severe deficiencies in SR occur more frequently in patients with CG. Further research in a larger patient sample is necessary to determine if mild to moderate deficiencies in SR occur more frequently in CG as well and to assess if deficiencies in SR are mainly determined by ID or if other factors are involved. Such knowledge may help to identify patients with problems in SR timely and to design counseling procedures for this group of patients.

Future perspectives

In this thesis we primarily focussed our studies on improving knowledge of several aspects of CG and improving patient care, by developing an international guideline for management of CG, by more precisely determining the extent and consequences of several long-term complications, by evaluating effectiveness of newborn screening and the identification of previously unreported phenotypes of the disease, and by developing a method that could be helpful in predicting the outcome and personalising treatment. The gaps in the current knowledge and topics for future research, as identified in this thesis, have been summarized in the Summary and Discussion section. Those gaps that we consider most important to fill, will be highlighted and discussed separately below.

First and foremost, as the galactose-restricted diet has been proven to be ineffective in preventing the long-term complications, the ultimate goal of future research is to develop a therapy that sufficiently prevents and/or treats all complications of the disease in each patient. Development of novel therapies may be focussed on substrate reduction (decreasing accumulation of galactose-1-pohosphate, e.g. by inhibiting galactokinase, the upstream enzyme of GALT)[43], or increasing the activity of GALT, for example by the use of small-molecule chemical or pharmacological chaperones [44–46], as well as enzyme replacement therapy. The most effective treatment option is probably increasing GALT enzyme production by gene therapy. As the brain is the main target for long-term therapy in this disease, the route and place of administration of the therapy will be crucial. For example, intravenously administered enzyme has been demonstrated to inadequately target the brain in other enzyme deficiencies. Potential therapies will only be successful if, at the time of treatment initiation, the targeted organs have not yet suffered irreversible damage. It is therefore of major importance to unravel the timing and nature of the damage to separate organs and organ systems (mainly the central nervous system and the ovaries), specifically assessing if damage already occurs prenatally. However, such studies are very difficult to do in human patients. This, and the lack

of valid biomarkers to predict disease severity (which are essential for evaluation of effectiveness of new treatments) pose major difficulties in the development of new treatment strategies. New insights may be gained from the available animal models (the GALT knock-out mouse[47], the GALT-deficient Drosophila melanogaster (fruit fly)model[48], and the recently developed GALT knock-out zebrafish model[49]), though none of these animal models demonstrate all relevant complications of CG in humans.

Second, until a disease-modifying treatment is available which effectively prevents long-term complications, improvement of the current treatment strategy, the ga-lactose-restricted diet, is urgently needed. The large variations in residual enzyme activity as well as in clinical outcomes, suggest that there may be inter-individual variations in the capacity to metabolize galactose. However, at this time the degree of galactose restriction is the same for each patient and remains the same through-out life. The previously mentioned lack of biomarkers for follow-up, also hampers adjustment of the diet based on individual capacities. Future research should be targeted at who is in need of treatment and to what extent, as galactose over-re-striction may be harmful and the burden of treatment may even be higher than burden of disease in some patients.

The individual clinical outcome and need for dietary restriction may correspond to the overall capacity to metabolize galactose in that patient. This, however, needs to be confirmed. Results of in vitro galactose metabolites profiling in fibroblasts (see Chapter 3), and whole-body galactose oxidation capacity as assed by in vivo ga-lactose oxidation tests (breath test with labelled galactose), may provide insight in an individual's capacity to metabolize galactose. Galactosylation patterns in serum and effects of various amounts of galactose intake on glycosylation abnormalities, have been demonstrated to occur in CG, and may correlate with disease severity and treatment effects and may thus serve as a biomarker [50]. Combination of data from the above mentioned tests, together with detailed information on clinical out-come, may allow the construction of a model that can be used to predict clinical outcome early in life. Such a model should be validated in a large group of patients representing the whole outcome spectrum. Results of such an approach may help to unravel who is in need of treatment and to what extent.

Third, it is important to unravel why phenotypes often vary substantially between patients with the same genotype and residual erythrocyte GALT activity. These dif-ferences may be explained by differences in the overall residual capacity to metab-olize galactose (which is not well-reflected by currently available biomarkers), or by mechanisms altering the response and tolerance of the body to (toxic) galactose

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metabolites. The explanation of such differences may be found in epigenetic factors, such as alterations in gene expression caused by changes in DNA methylation and histone modification. Studies should therefore focus on the methylation of the full epigenome in patients of whom the phenotype has been well-defined, as well as the methylation of the genes and pathways that have been demonstrated by microarray analysis of the transcriptome to be dysregulated[51]. In the Dutch patient cohort, multiple sets of siblings demonstrate outcomes on opposites of the spectrum. It is likely that, next to epigenetic influences, also variants in other genes influence galactose metabolism. This may be elucidated by performing whole exome sequencing in sets of CG affected siblings, to search for variants in genes that may explain the outcome differences.

References

1. Donnell GN, Koch R, Bergen WR. Observation on results of management of galactosemic patients. In: Hsia DYY, ed. Galactosemia. Springfield: Charles C Thomas; 1969:247-268.

2. Donnell GN, Koch R, Fishler K, Ng WG. Clinical aspects of galactosemia. In: Burman D, Holton JB, Pennock CA, eds. Inherited Disorders of Carbohydrate Metabolism. Lancaster: MTP Press; 1980:103-115.

3. Sardharwalla IB. Inherited Disorders of Carbohydrate Metabolism. (Burman D, Holton JB, Pennock CA, eds.). Lancaster: MTP Press; 1980.

4. Anonymous. Clouds over galactosaemia. Lancet. 1982;2:1379-1380.

5. Bosch AM. Classical galactosaemia revisited. J Inherit Metab Dis. 2006;29:516-525.

6. Varela-Lema L, Paz-Valinas L, Atienza-Merino G, Zubizarreta-Alberdi R, Villares RV, López-García M. Appropriateness of newborn screening for classic galactosaemia: a systematic review. J Inherit Metab Dis. April 2016:1-17.

7. Ohlsson A, Guthenberg C, von Dobeln U. Galactosemia screening with low false-positive recall rate: the Swedish experience. JIMD Rep. 2012;2:113-117.

8. Diekman EF, Ferdinandusse S, van der Pol L, et al. Fatty acid oxidation flux predicts the clinical severity of VLCAD deficiency. Genet Med. 2015;17(12):989-994.

9. Jumbo-Lucioni PP, Garber K, Kiel J, et al. Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes. J Inherit Metab Dis. 2012;35(6):1037-49.

10. Adam S, Akroyd R, Bernabei S, et al. How strict is galactose restriction in adults with galactosaemia? International practice. Mol Genet Metab. 2015;115(1):23-6.

11. Donnell GN, Collado M, Koch R. Growth and development of children with galactosemia. J Pediatr. 1961;58(6):845-851.

12. Komrower GM, Lee DH. Long-term follow-up of galactosaemia. Arch Dis Child. 1970;45(241):367-373.

13. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis. 1990;13(6):802-818.

14. Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr. 1993;152(1):36-43.

15. Kaufman FR, Reichardt JK, Ng WG, et al. Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridyltransferase gene. J Pediatr. 1994;125(2):225-7.

16. Kaufman FR, McBride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. Eur J Pediatr. 1995;154(0340-6199 (Print)):S2-S5.

17. Hansen TW, Henrichsen B, Rasmussen RK, Carling A, Andressen AB, Skjeldal O. Neuropsychological and linguistic follow-up studies of children with galactosaemia from an unscreened population. Acta Paediatr. 1996;85(10):1197-1201.

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18. Rasmussen RK, Andreassen AB, Stromme P, Hansen TW. Learning disabilities and language pathology in patients with galactosemia. Logoped Phoniatr Vocol. 1996;21:157-162.

19. Badawi N, Cahalane SF, McDonald M, et al. Galactosaemia--a controversial disorder. Screening & outcome. Ireland 1972-1992. Ir Med J. 1996;89(0332-3102 (Print)):16-17.

20. Shield JP, Wadsworth EJ, MacDonald A, et al. The relationship of genotype to cognitive outcome in galactosaemia. Arch Dis Child. 2000;83(3):248-50.

21. Widhalm K, Miranda-Da-Cruz B, De Sonneville LMJ. Information processing characteristics and uridine treatment in children with classical galactosemia. Nutr Res. 2002;22:257-270.

22. Antshel KM, Epstein IO, Waisbren SE. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology. 2004;18(4):658-664.

23. Doyle CM, Channon S, Orlowska D, Lee PJ. The neuropsychological profile of galactosaemia. J Inherit Metab Dis. 2010;33(5):603-609.

24. Schadewaldt P, Hoffmann B, Hammen HW, Kamp G, Schweitzer-Krantz S, Wendel U. Longitudinal assessment of intellectual achievement in patients with classical galactosemia. Pediatrics. 2010;125(2):e374-81.

25. Coss KP, Doran PP, Owoeye C, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis. 2013;36(1):21-7.

26. Rubio-Agusti I, Carecchio M, Bhatia KP, et al. Movement disorders in adult patients with classical galactosemia. Mov Disord. 2013;28(6):804-10.

27. The Cochrane Collaboration. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]. (Higgins J, Greens S, eds.).; 2011.

28. Waisbren SE, Potter NL, Gordon CM, et al. The adult galactosemic phenotype. J Inherit Metab Dis. 2012;35(2):279-286.

29. Batey LA, Welt CK, Rohr F, et al. Skeletal health in adult patients with classic galactosemia. Osteoporos Int. 2013;24(1433-2965 (Electronic)):501-509.

30. Rubio-Gozalbo ME, Hamming S, van Kroonenburgh MJ, Bakker JA, Vermeer C, Forget PP. Bone mineral density in patients with classic galactosaemia. Arch Dis Child. 2002;87(1468-2044 (Electronic)):57-60.

31. Panis B, Forget PP, Van Kroonenburgh MJPG, et al. Bone metabolism in galactosemia. Bone. 2004;35(4):982-987.

32. Doulgeraki A, Monopolis I, Deligianni D, Kalogerakou M, Schulpis KH. Body composition in young patients with galactose metabolic disorders: a preliminary report. J Pediatr Endocrinol Metab. 2014;27(1-2):81-6.

33. Kaufman FR, Loro ML, Azen C, Wenz E, Gilsanz V. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. J Pediatr. 1993;123(0022-3476 (Print)):365-370.

34. Karadag N, Zenciroglu A, Eminoglu FT, et al. Literature review and outcome of classic galactosemia diagnosed in the neonatal period. Clin Lab. 2013;59(9-10):1139-1146.

35. World Health Organization (WHO). International Classification of Funcitoning, Disability and Health. World Heal Organ. 2003;(Version 2.1a, Clinician Form):1-15.

36. Meester-Delver A, Beelen A, Hennekam R, Nollet F, Hadders-Algra M. The Capacity Profile: a method to classify additional care needs in children with neurodevelopmental disabilities. Dev Med Child Neurol. 2007;49(5):355-360.

37. Nelson CD, Waggoner DD, Donnell GN, Tuerck JM, Buist NR. Verbal dyspraxia in treated galactosemia. Pediatrics. 1991;88:346-350.

38. Potter NL, Lazarus JA, Johnson JM, Steiner RD, Shriberg LD. Correlates of language impairment in children with galactosaemia. J Inherit Metab Dis. 2008;31:524-532.

39. Constantino J, Gruber C. Social Responsiveness Scale (SRS-2). Torrance, CA: Western Psychological Services; 2012.

40. Constantino JN, Gruber C. Social Responsiveness Scale. Los Angeles, CA: Western Psychological Services; 2005.

41. Constantino JN, Gruber C, Roeyers H, et al. Handleiding SRS-2 Screeningslijst Voor Autismespectrumstoornissen. Amsterdam: Hogrefe Uitgevers B.V.; 2015.

42. Constantino JN, Gruber C, Noens I, De la Marche W, Scholte E. Handleiding SRS-A Screeningslijst Voor Autismespectrumstoornissen. Amsterdam: Hogrefe Uitgevers B.V.; 2012.

43. Liu L, Tang M, Walsh MJ, et al. Structure activity relationships of human galactokinase inhibitors. Bioorg Med Chem Lett. 2015;25(3):721-727.

44. Coelho AI, Trabuco M, Ramos R, et al. Functional and structural impact of the most prevalent missense mutations in classic galactosemia. Mol Genet genomic Med. 2014;2(6):484-96.

45. McCorvie TJ, Timson DJ. Structural and molecular biology of type I galactosemia: disease-associated mutations. IUBMB Life. 2011;63(11):949-54.

46. McCorvie TJ, Gleason TJ, Fridovich-Keil JL, Timson DJ. Misfolding of galactose 1-phosphate uridylyltransferase can result in type I galactosemia. Biochim Biophys Acta. 2013;1832(8):1279-93.

47. Tang M, Siddiqi A, Witt B, et al. Subfertility and growth restriction in a new galactose-1 phosphate uridylyltransferase (GALT) - deficient mouse model. Eur J Hum Genet. February 2014.

48. Kushner RF, Ryan EL, Sefton JMI, et al. A Drosophila melanogaster model of classic galactosemia. Dis Model Mech. 2010;3(9-10):618-627.

49. Vanoevelen JM, van Erven B, Bierau J, et al. Impaired fertility and motor function in a zebrafish model for classic galactosemia. In: Classic Galactosemia: A Zebrafish Model and New Clinical Insights. ; 2017:19-40.

50. Coss KP, Byrne JC, Coman DJ, et al. IgG N-glycans as potential biomarkers for determining galactose tolerance in Classical Galactosaemia. Mol Genet Metab. 2012;105(2):212-220.

51. Coss KP, Treacy EP, Cotter EJ, et al. Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism? Mol Genet Metab. 2014;113(3):177-187.

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Samenvatting (Dutch summary)

Chapter 10 Samenvatting (Dutch summary)

Klassieke galactosemie (vanaf hier galactosemie genoemd) is een zeer zeldzame, aangeboren afwijking van de melksuikerstofwisseling. Ongeveer 1 op de 40.000 pasgeboren baby's heeft galactosemie, en op dit moment zijn er in Nederland ongeveer 200 patiënten bekend met deze ziekte. Door foutjes in het erfelijk materiaal (DNA), kan een enzym (eiwit betrokken bij afbraak, aanmaak of omzetting van stoffen) genaamd galactose-1-fosfaat uridyltransferase (GALT) niet, of onvoldoende, worden aangemaakt in de cellen van het lichaam. Dit enzym is noodzakelijk voor de afbraak van galactose, een onderdeel van lactose (melksuiker). Pasgeborenen met galactosemie die lactose (en dus galactose) via de voeding binnenkrijgen (borstvoeding of de normaal gebruikte flesvoedingen), worden vaak levensbedreigend ziek, waarbij de leverfunctie verstoord raakt. Hierdoor krijgen ze geelzucht, kunnen er voedingsproblemen en bloedvergiftiging ontstaan en komt het kind niet goed aan in gewicht. Ook ontstaat er meestal staar aan beide ogen (troebel worden van de ooglenzen). Als de ziekte niet tijdig wordt herkend en behandeld, is de kans op overlijden groot. Daarom is galactosemie in Nederland sinds 2007 opgenomen in het hielprikscreeningprogramma, waardoor in principe alle kinderen met de ziekte in de eerste levensweek kunnen worden opgespoord. Al sinds galactosemie voor het eerst als ziekte herkend werd, is de behandeling hetzelfde: een galactosebeperkt (vrij van melk en melkproducten) dieet, voor pasgeborenen bestaande uit aangepaste zuigelingenvoeding. Dit dieet is momenteel de enige beschikbare behandeling en patiënten worden geadviseerd zich levenslang aan dit dieet te houden. Zieke kinderen knappen vrijwel altijd heel snel op na start van het levensreddende dieet. Wat helaas erg tegenvalt is dat veel mensen met galactosemie, ondanks dat zij zich houden aan het strenge dieet, toch op latere leeftijd blijken te lijden aan lange termijncomplicaties zoals een lager IQ, spraak- en taalproblemen, bewegingsstoornissen, (sterk) verminderde vruchtbaarheid in het overgrote deel van de vrouwelijke patiënten, en een verlaagde botdichtheid.

Er is momenteel nog onvoldoende kennis over hoe en op welk moment de complicaties ontstaan (wellicht al voor de geboorte). Het uiteindelijke doel van onderzoek bij galactosemie is dan ook om deze mechanismen goed te begrijpen en om een behandeling te ontwikkelen die alle complicaties van de ziekte goed behandelt. Daarnaast is het van groot belang om een methode te ontwikkelen die vroeg in het leven kan voorspellen hoeveel last een patiënt krijgt van complicaties, en zo goed mogelijk uit te zoeken hoe vaak de complicaties voorkomen, bij welke patiënten, en hoe relevant de complicaties zijn. Daarnaast is het belangrijk de hielprikscreening voor galactosemie zo betrouwbaar en goed mogelijk uit te voeren.

Deel 1 Hielprikscreening en patiënten met klassieke en atypische (variant) presentatie van galactosemie

Hielprikscreening

In **Hoofdstuk 2** van dit proefschrift wordt de hielprikscreening voor galactosemie in Nederland geëvalueerd vanaf de start van screening (2007) tot en met 2015. Als er bij onderzoek van de hielprikkaart in het laboratorium blijkt dat er bij de pasgeborene sprake kan zijn van galactosemie, wordt het kind direct verwezen naar een afdeling voor stofwisselingsziekten in een academisch ziekenhuis voor verder onderzoek. Soms blijkt dan dat de hielprik 'fout-positief' was: de merkstofjes in bloed waren te hoog, maar het kind heeft geen galactosemie. Er was dan een andere, onschuldige, reden voor de hoge waarden. Het doel van screenen is om álle kinderen met de ziekte te vinden en niemand met de ziekte te missen. Hierbij is het uiteraard ook de bedoeling om zo min mogelijk van deze fout-positieven te hebben. Immers, het blijkt tot veel stress te leiden bij ouders als zij horen dat de hielprik afwijkend was, ook al blijkt na aanvullend testen dat het kind zeker geen galactosemie heeft. In Nederland zijn er tussen 2007 en 2015 verschillende manieren van screenen in de screeningslaboratoria gebruikt (verschillende merkstofjes en afkapwaarden), en in ons onderzoek hebben wij bekeken hoe effectief die screeningsmanieren waren: zijn alle kinderen met galactosemie gevonden? Is er iemand gemist? Waren de kinderen bij wie de ziekte is vastgesteld erg ziek? Hoeveel fout-positieven waren er, en kunnen we de screening verbeteren?

Het onderzoek toonde aan dat met alle screeningsmethoden alle kinderen met galactosemie opgespoord zijn, en er dus geen één is gemist. Deze kinderen waren vaak wel al een beetje ziek, maar niet ernstig, en geen kind is overleden. Dit toont aan hoe succesvol deze screening is. Echter werden er helaas relatief veel 'fout-positieven' opgespoord met alle screeningsmanieren: gemiddeld ca. 90% van alle naar het academische ziekenhuis verwezen kinderen bleek geen galactosemie te hebben. Het is belangrijk om dit aantal te verminderen, omdat dit zeer belastend is voor de ouders van het kind. Dit is mogelijk door de afkapgrenzen voor de merkstofjes te blijven evalueren en verder aan te scherpen.

Door kennis van andere stofwisselingsziekten die onderdeel zijn van de hielprikscreening, weten we dat er met de hielprikscreening regelmatig kinderen worden gevonden met een andere vorm of presentatie van de ziekte dan de klassieke (ernstige) vorm en presentatie die het meest bekend is. Kinderen met een andere presentatie, die 'milder' lijkt dan de klassieke presentatie, maken vaak nog wel een beetje goed werkzaam enzym aan en hebben waarschijnlijk minder last van complicaties van de ziekte. Het is voor die kinderen soms onduidelijk of ze behandeling nodig hebben, en zo ja hoe streng. In het tweede deel van dit onderzoek is dan ook onderzocht of bij de hielprikscreening voor klassieke galactosemie ook

kinderen worden gevonden met een andere presentatie van de ziekte en resultaten toonden aan dat dit het geval is. Zij hebben een atypische, variant presentatie van galactosemie om meerdere redenen: 1) ze hebben een hogere restwaarde van het enzym tot wel 9% (bij patiënten met een klassieke presentatie van galactosemie is dit vaak maar 0-2%), 2) als ze worden gevonden met screening zijn ze niet ziek, terwijl kinderen met klassieke galactosemie allemaal wel al een beetje ziek waren, 3) in tegenstelling tot bij kinderen met klassieke galactosemie, dalen na start van het dieet de giftige waarden in bloed (merkstofjes) héél snel tot onmeetbaar laag, zoals ook bij gezonde mensen wordt gevonden. Deze bloedwaarden blijven bij mensen met klassieke galactosemie, ondanks een streng dieet, het hele leven verhoogd. Dit wijst erop dat deze kinderen beter galactose kunnen verwerken. Deze kinderen moeten nader bestudeerd worden. Het is nu nog niet mogelijk op jonge leeftijd te voorspellen hoe het met hen zal gaan, en er moet worden gezocht naar nieuwe manieren om deze voorspelling mogelijk te maken, en als mogelijk, een dieet of andere behandeling op maat. Zowel in huidcellen als in uitademingslucht kan gemeten worden hoeveel galactose iemand kan verbranden, en dit een zou een manier kunnen zijn om vroeg in het leven zo'n voorspelling te doen.

Galactoseverwerking in patiënten met een klassieke en variant presentatie van galactosemie

In **Hoofdstuk 3** hebben we een methode ontwikkeld om in huidcellen te kunnen meten hoeveel galactose zij kunnen afbreken (analyse van de tussenstoffen van de galactosestofwisseling). Het doel hiervan was om te kijken of huidcellen van kinderen met een variant presentatie van galactosemie, zoals beschreven in **Hoofdstuk 2** (daar atypische galactosemie genoemd), inderdaad meer galactose kunnen afbreken dan mensen met de klassieke presentatie.

Er is een groot verschil tussen patiënten in hoeveel complicaties zij hebben, zelfs tussen patiënten die dezelfde foutjes hebben in hun erfelijk materiaal en met ongeveer evenveel aanmaak van enzym. Met de huidige technieken is het niet mogelijk om bij diagnose al een voorspelling te doen over of een patiënt (ernstige) complicaties zal krijgen. Patiënten en families zijn daardoor in grote onzekerheid, en bovendien wordt er bij sommige patiënten wellicht een te streng dieet gegeven, of is er voor een aantal zelfs helemaal geen dieetbehandeling nodig. Dit is helemaal van belang omdat er, sinds er in Nederland wordt gescreend op galactosemie (2007), kinderen worden gevonden met een variant presentatie van galactosemie die mogelijk weinig tot geen complicaties van de ziekte zullen ontwikkelen. Patiënten die minder complicaties van de ziekte hebben, kunnen waarschijnlijk beter galactose afbreken. Bij een andere stofwisselingsziekte bleek dat de hoeveelheid van de stof (zoals bij galactosemie de galactose is) die omgezet kon worden door huidcellen, goed past bij de ernst van de complicaties. Wel-

licht kan het meten van de omzetting van galactose door huidcellen bij mensen met galactosemie dus voorspellen of zij last krijgen van complicaties, en zou deze meting kunnen bijdragen aan het individueel bepalen van hoe streng het dieet moet zijn.

In dit onderzoek werden in het laboratorium metingen gedaan om dit te bepalen in gekweekte huidcellen van 3 patiënten met de klassieke presentatie van galactosemie, van 3 patiënten met een variant presentatie, en van 3 mensen die geen galactosemie hebben. Aan de huidcellen werd galactose met een vlaggetje toegevoegd gedurende twee uur. Daarna werden drie belangrijke (tussen)stoffen in de galactoseverwerking gemeten (allen met een vlaggetje): galactose, galactose-1-fosfaat en UDP-galactose . Ook werd de galactose index berekend: galactose-1-fosfaat gedeeld door UDP-galactose. Daarnaast werd de galactose oxidatie gemeten in de huidcellen: het proces waarbij normaal gesproken uiteindelijk CO_2 wordt gevormd. Dit werd gemeten met behulp vaneen radioactief vlaggetje.

De resultaten van dit onderzoek laten zien dat mensen met een variant presentatie inderdaad beter galactose kunnen verwerken omdat zij in verhouding tot mensen met de klassieke vorm lagere waarden hadden van galactose en galactose-1-fosfaat (de stoffen die door de ziekte niet goed kunnen worden omgezet). Daarnaast hadden ze hogere waarden van UDP-galactose (de stof die bij klassieke galactosemie niet tot nauwelijks gevormd kan worden) vergeleken met mensen met de klassieke vorm. De galactose index is bij mensen met een variant presentatie lager dan bij patiënten met de klassieke presentatie van de ziekte, maar niet zo laag als bij mensen zonder galactosemie. Dit wijst erop dat deze analyse van de tussenstoffen van de galactosestofwisseling een goede methode is om deze groepen patiënten van elkaar te onderscheiden. Dit was niet het geval bij de galactose oxidatie meting. Bij mensen met klassieke galactosemie werd een gestoorde galactose oxidatie gezien (geen vorming van CO_2). In huidcellen van gezonde mensen en mensen met een variant presentatie van galactosemie werd wel CO_2 gevormd , maar er werd geen verschil tussen de twee groepen gevonden.

Concluderend toont dit onderzoek aan dat we met analyse van de tussenstoffen van de galactosestofwisseling goed in staat zijn om mensen met een variant presentatie van galactosemie en klassieke presentatie van galactosemie van elkaar te onderscheiden. In de toekomst zal het nodig zijn om deze methode verder te valideren, door metingen te doen in een grote groep patiënten. Uiteindelijk zal deze methode mogelijk kunnen bijdragen aan het vroeg in het leven voorspellen van de ernst van de galactosemie en hoe streng de behandeling voor iedere patiënt moet zijn.

Deel 2 Management van klassieke galactosemie

In **Hoofdstuk 4** wordt (de ontwikkeling van) een internationale richtlijn voor galactosemie beschreven, met aanbevelingen voor het stellen van de diagnose, de behandeling en het vervolg van de ziekte. Zo'n richtlijn is van groot belang, omdat er wereldwijd nog grote verschillen zijn in de zorg voor patiënten. Een richtlijn waarin de aanbevelingen zoveel mogelijk zijn gebaseerd op wetenschappelijk bewijs, en die internationaal gebruikt kan worden, leidt tot betere en gelijke zorg voor patiënten in de hele wereld.

Er namen 21 experts op het gebied van galactosemie, afkomstig uit acht Europese landen en Amerika, deel aan het ontwikkelen van deze richtlijn. Door hen werden belangrijke onderwerpen vastgesteld om aanbevelingen over te maken. Om de aanbevelingen te onderbouwen met wetenschappelijk bewijs, werd de medische literatuur doorzocht. Aan de hand van dit bewijs, of op basis van de meningen van de experts (als er geen of onvoldoende bewijs was), werden aanbevelingen gemaakt, waarbij het doel was dat zoveel mogelijk experts het volledig eens waren met deze aanbevelingen. Er werden totaal 40 aanbevelingen opgesteld over hoe de diagnose het beste gesteld kan worden, over de behandeling (het dieet), welke laboratoriumwaarden vervolgd moeten worden, en over het vervolg van de (complicaties van) de patiënt: mentale ontwikkeling, spraak en taal, neurologisch functioneren, psychosociale ontwikkeling, vruchtbaarheid, botgezondheid en staar. Over alle behalve één aanbeveling waren alle experts het volledig eens.

Zoals was verwacht, werd er vastgesteld dat er veel bewijs ontbrak, en dat er nog veel onderzoek nodig is in de toekomst, met name ten aanzien van vinden van een goede behandeling en het vinden van methoden om vroeg in het leven te voorspellen hoe de ziekte zal verlopen en wie wel of geen behandeling nodig heeft en hoe streng. Qua onderzoek op het gebied van lange termijncomplicaties is het voornamelijk van belang om merkstofjes te vinden die meer inzicht kunnen geven in het ontstaan van de lange termijncomplicaties.

Deel 3 Meer inzicht in de lange termijncomplicaties

Omdat galactosemie zeldzaam is, worden er in veel onderzoeken relatief weinig patiënten betrokken. Het is heel lastig om uit deze onderzoeken goede conclusies te trekken over hoe vaak een complicatie bij patiënten met galactosemie voorkomt, en of de patiënt er zelf ook echt last van heeft.

Intelligentie Quotiënt (IQ)

In **Hoofdstuk 5** is onderzocht hoe vaak een lager IQ voorkomt bij patiënten die al vroeg in het leven gestart zijn met het dieet (in de eerste vier levensweken). Eerdere onderzoeken laten zien dat de meeste patiënten een laag of laag-normaal IQ hebben, maar dat er ook mensen zijn met een gemiddeld of wat hoger IQ. Dit is

echter de minderheid. In deze onderzoeken werden vaak weinig patiënten betrokken, werd op verschillende manier het IQ getest (waardoor de IQ scores niet goed met elkaar vergelijkbaar zijn), en was het vaak onduidelijk of de patiënten wel echt de klassieke vorm van galactosemie hadden (of wellicht een vorm van galactosemie met sowieso minder complicaties). Daarnaast was het vaak onduidelijk op welke leeftijd er gestart was met dieetbehandeling. Dit is belangrijk om te weten omdat bij later starten van de behandeling er mogelijk een groter risico is op het hebben van een lager IQ.

In dit hoofdstuk zijn IQ scores van patiënten uit verschillende eerdere studies verzameld. Alleen de IQ scores van patiënten die echt de klassieke vorm van galactosemie hadden, met het dieet waren gestart in de eerste vier levensweken, en een IQ test hadden gedaan die goed vergelijkbaar is met andere tests, werden in ons onderzoek betrokken. De IQ scores van deze patiënten werden samengevoegd, om een beeld te krijgen van het IQ in deze grote groep patiënten. Het gemiddelde IQ van 87 patiënten (in leeftijd variërend van 3 tot 38 jaar, gemiddeld 13 jaar), was 87 (het gemiddelde van de normale bevolking is 100). Er was een grote variatie in IQ scores, van 47 to 122. Bijna de helft van de patiënten had een lage score (onder de 85), bijna 40% had een laagnormale score van 85-100, en een minderheid (15%) had een bovengemiddelde score van >100. Mannen en vrouwen haalden gemiddeld ongeveer dezelfde scores. In ons onderzoek was er geen aanwijzing dat het IQ daalt in de loop van het leven.

Dit onderzoek bevestigt dat ook bij patiënten die al snel na de geboorte zijn gestart met dieetbehandeling, er een grote variatie is in IQ scores (en dus het mentaal functioneren), en dat patiënten met galactosemie een groot risico hebben op een lager IQ. Voor de toekomst is het belangrijk uit te zoeken wat precies de oorzaak is van de lagere IQ scores, en welke patiënten dit zullen ontwikkelen.

Botgezondheid

In **Hoofdstuk 6** is onderzocht of een verlaagde botdichtheid een veel voorkomend probleem is bij mensen met galactosemie en of patiënten vaker botbreuken hebben. Verder werd er gekeken naar stoffen (zoals vitaminen en hormonen) die belangrijk zijn voor de botgezondheid. Eerdere onderzoeken toonden aanwijzingen voor een verlaagde botdichtheid bij mensen met galactosemie, maar ook in deze onderzoeken waren maar relatief weinig patiënten betrokken. In dit hoofdstuk zijn botdichtheidsgegevens van patiënten uit verschillende eerdere studies verzameld en samengevoegd, om uit gegevens van een groter aantal patiënten preciezere conclusies te kunnen trekken. Voor dit onderzoek moest de botdichtheid zijn gemeten met een DEXA-scan, wat tegenwoordig de meetmethode van voorkeur is. De botdichtheid kan daarmee op verschillende plaatsen in het skelet worden gemeten:

de wervelkolom, de heup en het dijbeen. De botdichtheid van elke patiënt wordt uitgedrukt in een Z-score. Een Z-score van 0 is gemiddeld, een Z-score die tussen de -2 en +2 valt is respectievelijk iets verlaagd en iets verhoogd ten opzichte van het gemiddelde maar valt binnen het normale, scores daarbuiten worden als afwijkend gezien. Daarbij is vooral een Z-score lager dan -2 van belang, omdat er dan een grotere kans is op botontkalking en breuken.

Voor elk van de meetplaatsen (wervelkolom, heup, dijbeen) werden gegevens van de patiënten samengevoegd. Bij alle 112 patiënten (dit waren zowel kinderen als volwassenen) was er in elk geval een Z-score gemeten voor de wervelkolom. Gemiddeld was er (ten opzichte van het gemiddelde) een wat verlaagde Z-score van -0.7. De scores van de kinderen (totaal 76) zijn ook apart bekeken, en zij hadden gemiddeld ook een wat verlaagde Z-score van -0.64. De gemiddelde Z-score van 36 volwassenen was -0.94. Scores op de andere meetplaatsen (heup en dijbeen) waren vergelijkbaar. Na bekijken van een heel aantal hormonen, vitamines en andere stoffen in bloed, bleken veel patiënten een te laag gehalte van vitamine D in het bloed te hebben, maar een normale waarde van calcium. Vitamine D en calcium werken, als ze voldoende in het bloed aanwezig zijn, beschermend tegen botontkalking. Helaas waren er nauwelijks gegevens over het vóórkomen van botbreuken.

Uit ons onderzoek blijkt dat er gemiddeld een wat lagere waarde van botdichtheid is in kinderen en volwassenen met galactosemie. Dit betekent dat veel patiënten een normale botdichtheidsscore hebben, maar dat wel iets meer patiënten een Z-score zullen hebben lager dan Z-score -2. Om die reden is het belangrijk om bij alle mensen met galactosemie de botdichtheidsscore te bepalen, om per patiënt te beoordelen of hij of zij een verhoogd risico heeft op botontkalking en botbreuken. Daarnaast is het belangrijk om het vitamine D en calcium in bloed regelmatig te controleren, en om dit aan te vullen met tabletten als het te laag is. Verder onderzoek kan worden gericht op de consequenties van deze verlaagde score in een deel van de patiënten, waarbij moet worden onderzocht of er meer botbreuken voorkomen bij mensen met galactosemie.

Capaciteiten, beperkingen en extra zorg
Veel kinderen en volwassenen met galactosemie hebben complicaties van de ziekte (lager IQ, bewegingsstoornissen, staar). Het is nooit onderzocht en vastgelegd of mensen met galactosemie daardoor beperkingen ervaren in het dagelijks leven, en of daardoor extra zorg, hulp of aanpassingen nodig zijn.

In **Hoofdstuk 7** worden zowel de capaciteiten als beperkingen van mensen met galactosemie onderzocht, en wordt er gekeken of er door zulke beperkingen extra zorg nodig is. Het Capaciteitenprofiel is een methode om beperkingen, en daar-

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uit voortkomende noodzaak tot extra zorg, vast te leggen voor vijf verschillende domeinen van zogenaamde lichaamsfuncties: algehele conditie, motorische functies, zintuiglijke functies, mentale functies (waaronder ook sociaal functioneren), en spraak- en stemfuncties. De zwaarte van extra zorg of benodigde aanpassingen wordt bepaald door een interview met de patiënt, en wordt per domein gescoord van 0 tot 5. Een score van 0 betekent geen extra zorg nodig, en een score van 5 betekent volledig afhankelijk van zorg.

In ons onderzoek werden 44 patiënten (waarvan 23 kinderen en 21 volwassenen) geïnterviewd. Extra zorg of hulp was meest nodig in het domein van mentale functies, onder andere vanwege mentale beperkingen, wat past bij de IQ scores van patiënten (zoals onderzocht in hoofdstuk 4). Opvallend genoeg hebben veel patiënten ook extra zorg of hulp nodig in dit domein door problemen op sociaal gebied (moeite met contact maken, vriendschappen onderhouden en sociale kwetsbaarheid). Een vijfde van de patiënten had extra hulp nodig door spraak- en stemfunctieproblemen (zoals zacht en slordig praten, bepaalde klanken niet goed kunnen maken). Dit past bij wat gevonden werd in eerder onderzoek, waarbij werd gezien dat een groot deel van patiënten spraakproblemen heeft. Op gebied van motorische functies waren er weinig beperkingen, en was er dus niet vaak extra zorg nodig. Vier patiënten hadden tremoren (trillen van de ledematen) waarvoor aanpassingen of zorg nodig waren. Veel patiënten (of hun ouders) meldden problemen met fijne motoriek (zoals schrijven), het leren van nieuwe bewegingen, en onhandigheid. Hiervoor waren op het moment van het onderzoek vaak geen aanpassingen of extra zorg nodig, maar deze problemen hadden soms wel een negatieve invloed op het zelfvertrouwen. Deze problemen, in combinatie met de gemelde problemen op sociaal gebied, zouden kunnen passen bij 'developmental coordination disorder' (DCD), een overkoepelende term voor dit soort problemen. Dit is nog niet eerder beschreven bij galactosemie. Op gebied van algehele conditie hadden alle patiënten een aanpassing in het dagelijks leven door hun dieet, maar verder waren er in het overgrote deel van de patiënten geen andere beperkingen waarvoor extra zorg nodig was. Dit gold ook voor de zintuiglijke functies.

Concluderend is uit dit onderzoek gebleken dat Capaciteitenprofiel een behulpzame methode kan zijn om beperkingen en extra zorg bij mensen met galactosemie in kaart te brengen. Dit onderzoek geeft zorgverleners een breder inzicht in het beloop van de ziekte. Voor de toekomst is het belangrijk dat de problemen op sociaal gebied, en de problemen die mogelijk passen bij DCD, verder worden onderzocht.

Sociale wederkerigheid

In **Hoofdstuk 8** wordt geëvalueerd of kinderen en volwassenen met galactosemie méér problemen hebben op gebied van sociaal functioneren, vergeleken met mensen zonder galactosemie. Zoals aangegeven in hoofdstuk 6, maar ook al in eerdere onder-

zoeken, zijn er mogelijk meer problemen op sociaal gebied, zoals moeite met contact maken, het onderhouden van vriendschappen en relaties, en sociale kwetsbaarheid. In dit hoofdstuk werden problemen middels een vragenlijst, ingevuld door de patiënt zelf (of door ouders, ingeval van kinderen), geïnventariseerd. Deze vragenlijst beoordeelt met name de sociale wederkerigheid, waarbij wordt bekeken of mensen in staat zijn normale sociale interactie te hebben, en geeft een maat voor problemen op dit gebied. Des te meer problemen, des te hoger de score (T-score): een T-score van >60 duidt op milde tot matige problemen in de sociale wederkerigheid, een T-score van >75 op ernstige problemen, met grote gevolgen voor dagelijks functioneren.

In totaal werden 33 patiënten, met een gemiddelde leeftijd van 19 jaar (range 6 tot 46 jaar), in dit onderzoek betrokken. De gemiddelde T-score was 54, zonder verschil tussen mannen en vrouwen, of tussen kinderen en volwassenen. Er waren geen aanwijzingen voor méér problemen op één specifiek gebied van sociale wederkerigheid. In zijn geheel genomen was het aantal patiënten met een afwijkende score (T-score >60, 9 patiënten) méér dan je zou verwachten, echter kon dit verschil statistisch niet worden aangetoond. Dit komt waarschijnlijk door het relatief kleine aantal patiënten in dit onderzoek. Echter, zijn er wel significant meer mensen met galactosemie met een T-score van >75 (5 patiënten; score duidt op ernstige problemen in sociale wederkerigheid). Er werden hogere T-scores (wijzend op meer problemen in de sociale wederkerigheid) gevonden bij patiënten met een lager hoogst behaald schoolniveau. Dit wijst er mogelijk op dat intellectueel vermogen van invloed is op problemen in sociale wederkerigheid, al moet dit in de toekomst verder worden onderzocht.

Concluderend wijst dit onderzoek uit dat een relatief groot deel van patiënten met galactosemie ernstige problemen heeft op gebied van sociale wederkerigheid, en mogelijk een groter deel ook milde tot matige problemen, al moet dat laatste worden bevestigd in een onderzoek met een groter aantal patiënten. Deze kennis is belangrijk omdat zorgverleners hierdoor meer aandacht hebben voor problemen op sociaal gebied en sneller gepaste hulp kunnen inschakelen.

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APPENDICES

List of co-authors

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PhD Portfolio

	Year(s)	Workload (ECTS)
1. General courses		
BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2013	0.9
Introduction in practical biostatistics	2013	1.4
AMC World of Science	2014	0.7
2. Seminars, workshops and master classes		
MPS Masterclass in Istanbul	2015	1
MPS Masterclass in Dubai	2016	1
3. Presentations		
Amsterdam Kindersymposium	2014	0.5
Galactosemiedag Galactosemie Vereniging Nederland	2014	0.5
Amsterdam Kindersymposium	2015	0.5
Poster presentation SSIEM annual conference 2016 (three poster presentations)	2016	1.5
Galactosemiedag Galactosemie Vereniging Nederland	2016	0.5
4. (Inter)national conferences		
Amsterdam Kindersymposium	2014	0.5
ESN in Rotterdam	2015	0.5
Amsterdam Kindersymposium	2015	0.5
SSIEM in Lyon	2015	0.5
International Conference Sanfilippo Syndrome and Related Lysosomal Storage Diseases (Geneva, Switzerland)	2015	1.2
14th International Symposium on MPS and Related Diseases (Bonn, Germany)	2016	1.2
13th Annual WORLD Symposium (San Diego, USA)	2017	1.5
5. Other		
Sub-investigator HGT-SAN-067-trial	2013 – 2017	16
Sub-investigator HGT-SAN-093-trial/SHP-610-201-trial	2014 – 2017	4
Sub-investigator NGLU-NH01-trial	2014	1
Sub-investigator LAL-CL06-trial	2016 - 2017	0.25
6. Lecturing		
Werkcolleges Diabetes Mellitus first year students	2014	1
Pre-IHK teaching	2014	0.2
Pre-IHK teaching	2015	0.2
Pre-IHK teaching	2016	0.2
7. Tutoring, Mentoring		
Mentoring bachelor thesis student	2013-2014	1
8. Awards and Prizes		

February 2014	Group winner presentations Amsterdam Kindersymposium
November 2014	Clinical Research Site Award from INC Research – NH01 Study
September 2016	SSIEM annual conference 2016, two abstracts best scored

List of publications

1. Welling L, van Weeghel M, Treacy EP, Ferdinandusse S, Bosch AM.
   Profiling of intracellular metabolites produced from galactose and its potential for galactosemia research. In preparation.

2. Welling L, Meester-Delver A, Derks TG, Janssen MC, Hollak CE, Bosch AM.
   The need for additional care in patients with classical galactosemia. Submitted.

3. Welling L, de Vries M, Hollak CE, Oostrom KJ, Bosch AM.
   Social responsiveness in classical galactosemia. Submitted.

4. Welling L, Waisbren SE, Antshel KM, Colhoun HO, Gautschi M, Grünewald S, Holman R, van der Lee JH, Treacy EP, Bosch AM.
   Systematic Review and Meta-analysis of Intelligence Quotient in Early-Treated Individuals with Classical Galactosemia. JIMD Rep. 2017 Apr 9. [Epub ahead of print].

5. Welling L, Boelen A, Derks TG, Schielen PC, de Vries M, Williams M, Wijburg FA, Bosch AM.
   Nine years of newborn screening for classical galactosemia in the Netherlands: Effectiveness of screening methods, and identification of patients with previously unreported phenotypes. Mol Genet Metab. 2017 Mar;120(3):223-228.

6. Welling L, van Erven B, van Calcar SC, Doulgeraki A, Eyskens F, Gribben J, Treacy EP, Vos R, Waisbren SE, Rubio-Gozalbo ME, Bosch AM.
   Bone Health in Classic Galactosemia: Systematic Review and Meta-Analysis. JIMD Rep. 2016 Dec 20. [Epub ahead of print].

7. Welling L, Bernstein LE, Berry GT, Burlina AB, Eyskens F, Gautschi M, Grünewald S, Gubbels CS, Knerr I, Labrune P, van der Lee JH, MacDonald A, Murphy E, Portnoi PA, Õunap K, Potter NL, Rubio-Gozalbo ME, Spencer JB, Timmers I, Treacy EP, Van Calcar SC, Waisbren SE, Bosch AM; Galactosemia Network (GalNet).
   International clinical guideline for the management of classical galactosemia: diagnosis, treatment, and follow-up. J Inherit Metab Dis. 2017 Mar;40(2):171-176.

8. Meijer OL, Welling L, Valstar MJ, Hoefsloot LH, Brüggenwirth HT, van der Ploeg AT, Ruijter GJ, Wagemans T, Wijburg FA, van Vlies N.
    Residual N-acetyl-α-glucosaminidase activity in fibroblasts correlates with disease severity in patients with mucopolysaccharidosis type IIIB. J Inherit Metab Dis. 2016 May;39(3):437-45.

9. Welling L, Marchal JP, van Hasselt P, van der Ploeg AT, Wijburg FA, Boelens JJ.
    Early Umbilical Cord Blood-Derived Stem Cell Transplantation Does Not Prevent Neurological Deterioration in Mucopolysaccharidosis Type III. JIMD Rep. 2015;18:63-8.

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