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Clinical and biochemical phenotypes, genotypes, and long-term outcomes of individuals with galactosemia type I from a single metabolic genetics center in Alberta

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ABSTRACT

Background: Galactosemia type I is an autosomal recessive disorder of galactose metabolism due to galactose-1-phosphate uridyltransferase deficiency, encoded by *GALT*. To investigate the phenotypes, genotypes and long-term outcomes of galactosemia, we performed a retrospective cohort study in our center.

Methods: All individuals with galactosemia type I were included. We divided individuals into two groups to compare the outcomes of those treated symptomatically (SymX) and asymptomatically (AsymX). We reviewed electronic patient charts for clinical features, biochemical investigations, molecular genetic investigations, treatments, and outcomes.

Results: There were 25 individuals including classic ($n = 17$), clinical variant ($n = 4$), and biochemical variant (Duarte) galactosemia ($n = 4$). Twelve individuals were diagnosed symptomatically (SymX), and 9 individuals were diagnosed asymptomatically (AsymX). We did not include individuals with biochemical variant (Duarte) galactosemia into any of these groups. At the time of the diagnosis, conjugated hyperbilirubinemia was present in 83.3% of SymX group, whereas only 22% of AsymX group. SymX group had hepatomegaly (25%), failure to thrive (33.3%), cataract (16.7%) and sepsis (25%), whereas none of the individuals in the AsymX group had these clinical features. Fourteen variants in *GALT* were identified including pathogenic/likely pathogenic ($n = 12$), and likely benign/benign ($n = 2$) variants. The vast majority of individuals with classic and clinical variant galactosemia were treated with a galactose-lactose-free diet for life ($n = 20/21$). Intellectual disability was present in 54.5% of the SymX group, and in 37.5% of the AsymX group as a long-term outcome. Tremors were present 50% of the SymX group, and in 22% of the AsymX group as a long-term outcome. Although, intellectual disability and tremors seem to be less common in the AsymX group, there was no statistically significant difference between both groups. Primary ovarian insufficiency was present 50% of the SymX group, whereas in 20% of the AsymX group in post-pubertal females. We report a novel hypomorphic *GALT* variant (p.Ala303Ser) in one individual with clinical variant galactosemia. We also report an individual with clinical variant galactosemia with normal urine galactitol levels on a normal diet.

Conclusion: It seems that newborn screening and early administration of a galactose-lactose-free diet decreases the long-term galactosemia-associated complications but does not prevent them completely. It may be that not all individuals with clinical variant galactosemia may need a galactose-lactose-free diet. It is timely to find new therapeutic strategies that can reduce the frequency of late-onset complications in galactosemia.

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1. Introduction

Galactosemia type I (OMIM# 230400) is an inborn error of galactose metabolism. It is characterized by the elevation of galactose-1-phosphate and its metabolites due to a deficiency of galactose-1-phosphate uridyltransferase (GALT) (EC # 2.7.7.12) [1–3]. GALT catalyzes the reaction to form UDP galactose and glucose-1-phosphate from UDP glucose and galactose-1-phosphate [4]. The biochemical phenotypes based on the residual GALT activity include 1) classic galactosemia with residual enzyme activity <1% of normal; 2) clinical variant galactosemia with residual enzyme activity between 1 and 10% of normal; 3) biochemical variant (Duarte) galactosemia with residual enzyme activity >15% of normal [5,6].

The characteristic clinical and biochemical phenotypes of classic and clinical variant galactosemia include poor feeding, jaundice, cataract, cellular and synthetic hepatic dysfunction, renal Fanconi syndrome and *E. coli* sepsis (in some newborns) [7,8]. Long-term complications of classic galactosemia range from global developmental delay, speech delay, intellectual disability, primary ovarian insufficiency, tremors, ataxia, osteopenia, to behavioural problems [9,10]. The presence of elevated red blood cell galactose-1-phosphate, elevated urine galactitol and markedly low red blood cell GALT activity are the diagnostic biomarkers [1,4]. The direct Sanger sequencing of *GALT* confirms the molecular genetic diagnosis of galactosemia type I[9]. Clinical variant galactosemia is reported in African Americans and native Africans in South Africa [11]. Galactosemia type I is one of the newborn screening diseases worldwide in many newborn screening programs [12,13]. Clinical variant galactosemia may be missed by newborn screening as the galactose levels may not be markedly elevated depending on the methodology [1].

The mainstay of treatment is a galactose-lactose-free diet that can prevent or revert the early onset manifestations such as liver insufficiency, liver cirrhosis, cataract, and renal Fanconi syndrome [6,14]. Despite the diet therapy speech apraxia, movement disorder (e.g., ataxia, tremor), and primary ovarian insufficiency [14] have been reported as long-term complications.

To report clinical and biochemical phenotypes, genotypes, and long-term treatment outcomes of individuals with galactosemia type I, we performed a retrospective cohort study from a single-centre metabolic genetics clinic in Alberta. In our study, we compared the outcomes of individuals diagnosed symptomatically (SymX group) and asymptomatically by positive newborn screening or positive family history (AsymX group). This is the first study to be conducted in Alberta to investigate the long-term outcomes of individuals with galactosemia comparing the outcomes between early and late diagnosis cohorts.

2. Methods

This study was approved by the Institutional Research Ethics Board (Approval number Pro00117378, signed consent form was waived). The study was approved by The Northern Alberta Clinical Trials and Research Centre (NACTRC) and Alberta Health Services provided operational and administrative approval (PRJ38569).

All individuals diagnosed with galactosemia type I and followed in our center, were included in our study. Individuals were categorized based on their residual enzyme activity levels into three groups: 1) Classic galactosemia (GALT activity <1% of normal; 2) Clinical variant galactosemia (GALT activity 1–10% of normal); 3) Biochemical variant (Duarte) galactosemia with residual enzyme activity >15% of normal. The percentage of the residual GALT activity was calculated using the reference value provided by the clinical biochemical genetic laboratories. If there was a reference interval, average normal enzyme activity was used to calculate the percentage of the residual GALT activity. We grouped individuals into two groups to compare their outcomes: 1) Group 1: symptomatically diagnosed (SymX); 2) Group 2: diagnosed by positive newborn screening or by positive family history (AsymX). Biochemical variant (Duarte) galactosemia individuals were excluded from this comparison as they are not treated and are not expected to have long-term complications.

Electronic patient charts were reviewed for clinical features, biochemical and molecular genetic investigations, treatments, and outcomes. All data were entered into a Microsoft Excel Database.

The GALT activity measurement was performed by accredited clinical biochemical genetics laboratories as per their established methods. Sanger sequencing of *GALT* was performed as per clinical molecular genetic laboratories established methods. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant classification guidelines were applied for interpretation of variants [15]. All variants were searched in the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/about) for their allele frequency in the general population [16].

Neuropsychological assessments were performed using Wechsler Intelligence Scale for Children —Fourth Edition (WISC–IV) [17].

For statistical analysis, a non-parametric Fisher's exact test was used to compare the groups, with an alpha level of 0.05 as the predetermined threshold for statistical significance. All analyses were conducted using R (v.4.1.0) statistical software.

3. Results

Between 1985 and 2022, there were 25 individuals diagnosed with galactosemia (9 males, 16 females) from 21 unrelated families including classic galactosemia ($n = 17$), clinical variant galactosemia ($n = 4$) and biochemical variant (Duarte) galactosemia ($n = 4$). Their current average age was 24 ± 17.5 standard deviation (SD) years (age range 5 months- 56 years) including 14 adults (>18 years of age; their average age 36.4 ± 10.6 SD years; age range 21–56 years) and 11 children (their average age 6.4 ± 6 SD; age range 6 months −16 years). We summarized their clinical features and outcomes in Tables 1, 2, and 3.

GALT activity was measured in four different accredited clinical biochemical genetics laboratories as per their established methods. The GALT activity was 0 in all individuals with classical galactosemia and ranged between 3.6 and 6.3% of normal activity in all individuals with clinical variant galactosemia.

All variants in *GALT* were re-classified using ACMG/AMP criteria (Supplemental Table 1) [2,3,5,8–10,18–30]. Four individuals had the common p.Gln188Arg *GALT* variant and three of them were heterozygous for this common *GALT* variant. Unfortunately, none of these individuals had full Sanger sequencing of *GALT*. Three individuals did not have a molecular genetic investigation due to limited public funding for molecular genetic investigations at the time of the diagnosis. There were 14 different variants in 22 individuals including 12 pathogenic or likely pathogenic (all individuals with classic or clinical variant galactosemia) and two likely benign or benign variants which were identified in individuals with biochemical variant (Duarte) galactosemia. The most common variant was the p.Gln188Arg *GALT* variant which was identified in 44% of the alleles in the groups with classic and clinical variant galactosemia. To the best of our knowledge, the p.Ala307Ser (clinical variant galactosemia) *GALT* variant was novel.

All individuals with classic galactosemia and three individuals with clinical variant galactosemia were on a galactose-lactose-free diet. One individual with clinical variant galactosemia was on a galactose-lactose-free diet for a short period of time. All individuals were compliant with the dietary restrictions. All individuals with biochemical variant (Duarte) galactosemia were on an unrestricted diet.

3.1. Clinical and biochemical features and treatment outcomes of individuals with galactosemia type I in the SymX group ($n = 12$)

There were 12 individuals in this group: classic ($n = 9$) and clinical variant ($n = 3$) galactosemia. There were eight females and 4 males in

this group. Individuals with classic galactosemia type I had undetectable GALT activity while individuals with clinical variant galactosemia had markedly low GALT activity ranging between 3.6% to 6.3% of normal. The average age of onset and the average age of diagnosis were 38 ± 43 SD days (range 6 days- 5 months). Their average current age was 29.2 ± 16 SD years (range 4–56 years).

The most common initial presenting symptom was jaundice in 10/12 individuals (83.3%). The most common galactosemia-associated long-term complication was intellectual disability in 6/11 individuals (54.5%), two of those individuals had clinical variant galactosemia. We excluded one individual (Gal007) with clinical variant galactosemia in the SymX group who had a history of birth asphyxia. We think that the severity of intellectual disability was most likely associated with this event. For this reason, we have 11 instead of 12 individuals for intellectual disability as long-term complication associated with galactosemia in this group. Tremor was the second most common symptom and was reported in 50% of individuals (6/12) and three of them had clinical variant galactosemia. One individual with classic galactosemia was deceased at 6 weeks of age due to cerebral edema, hepatic failure, and hepatic veno-occlusive disease. All neuropsychology assessments, all bone mineral density results, all biochemical investigations, long-term follow-up investigation results of the individuals in the SymX group are summarized in Supplemental Tables 2, 3, 4, and 5 respectively.

3.2. Clinical and biochemical features and treatment outcomes of individuals in the AsymX group (n = 9)

There were 9 individuals in the AsymX group: classic ($n = 8$) and clinical variant ($n = 1$) galactosemia. There were six females and three males in this group. Five individuals were identified by positive newborn screening and four individuals were identified by positive family history. Their average age at diagnosis was 6.5 ± 2.2 SD days. Their average current age was 26.4 ± 18.5 SD years (ranging from 6 months to 52 years). Jaundice was reported in two individuals (22%) in this group. The most common galactosemia-associated long-term complication was intellectual disability, which was identified in 37.5% of individuals (3/8), and all individuals had classic galactosemia. We excluded one individual (Gal014) in the AsymX group who has epileptic encephalopathy with neonatal onset seizures attributable to a porencephalic cyst in brain magnetic resonance imaging (MRI). We think that the severity of the phenotype was most likely associated with epileptic encephalopathy. For this reason, we have eight instead of nine individuals with intellectual disability as long-term complication associated with galactosemia type I in this group. Tremor was the second most common symptom and was reported in 22% of individuals (2/9) and both of those had classic galactosemia. All neuropsychology assessments, all bone mineral density results, all biochemical investigations during retrieval of positive newborn screening results, long-term follow-up investigation results of the individuals in the SymX group are summarized in Supplemental Tables 2, 3, 4, and 5 respectively.

Interestingly, one of the individuals (Gal011) was identified by

Table 1
Clinical, biochemical and molecular genetic features of SymX individuals with galactosemia are summarized in this table.

Patient#/study ID/sex/ diagnosis/age of diagnosis/ current age	Presenting symptom (age of onset)/other clinical features	Initial investigations (LE, direct bilirubin, INR, Alb, GALT)	Molecular genetic investigations	Outcome
1/GAL003/F/CG/3mo/34 yrs	Lethargy, jaundice, sepsis (group B streptococci)	↑/↑/↑/N/0%	HTZ c.563A>G p.Gln188Arg/ not investigated*	Cognitive dysfunction, tremor, ataxia, POI, low BMD, mild proteinuria
2/ GAL005/ F/CG/14 days/ 55 yrs	Vomiting, FTT	N/N/↑/N/0%	HTZ c.563A>G p.Gln188Arg/ not investigated*	POI
3/ GAL009/F/CG/ 11 days/ 28 yrs	Lethargy, poor feeding and *E. coli* meningitis	N/↑/N/N/16.25%) history of blood transfusion	Not carrying the common c.563A>G (p.Gln188Arg) variant*	Borderline ID, seizure, tremor, POI
4/GAL013/F/CG/ 21 days/ 56 yrs.	Jaundice, irritability, opisthotonos, hepatosplenomegaly	N/↑/N/N/NP	HMZ c.563A>G (p. Gln188Arg)	Mild ID, anxiety, depression, tremors, ataxia, osteoporosis
5/ GAL015/ F/CG/ 2 mo /4 yrs.	Jaundice, FTT, proteinuria, cataract	↑/↑/N/N/0%	CMP HTZ c.563A>G p. Gln188Arg / c.203A>C p.His68Pro	Cataract
6/ GAL022/ M/ CG/ 10 days/ 32 yrs.	Poor feeding and jaundice	N/↑/N/N/0%	CMP HTZ c.563A>G p. Gln188Arg/ c.855G>T p.Lys285Asn	Borderline ID (IQ 70), low BMD
7/ GAL023/ F/ CG/ 6 weeks/ deceased	Jaundice, lethargy, liver insufficiency, ascites	↑/↑/↑/↑/ NP	CMP HTZ c.502G>T p. Val168Leu/ c.347T>C p. Leu116Pro	Deceased
8/ GAL024/M/ CG/ 11 days/ 15 yrs.	Lethargy, vomiting, jaundice	↑/↑/↑/N/0%	NP	Normal (IQ 91)
9/ GAL025/M/ CG/ 6 days/ 34 yrs	Jaundice	N/↑/N/N/0%	NP	IQ 109, low BMD
10/ GAL007/ M/ CVG/ 10 days/ 31 yrs.	Jaundice, lethargy	↑/↑/↑/N/3.6%	CMP HTZ c.563A>G p. Gln188Arg/ c.425T>A p. Met142Lys	Cognitive dysfunction**, tremors, low BMD, mild proteinuria
11/ GAL016/ F/ CVG/ 5 mo/16 yrs.	FTT, poor feeding, *E. coli* UTI, Fanconi syndrome***, bilateral cataract, hepatomegaly	N/N/N/N/4.8%	CMP HTZ c.152G>T p. Arg51Leu/c.584T>C p. Leu195Pro	Cataract, borderline ID (IQ 76), tremors
12/ GAL017/F/ CVG/ 14 days/ 21 yrs.	Jaundice, FTT, hepatomegaly	↑/↑/N/N/6.3%	NP	Borderline ID (IQ 77), seizure, tremors

Abbreviations (listed alphabetically): Alb = albumin; BMD = bone mineral density; CG = classic galactosemia; CMP = compound heterozygous; CVG = clinical variant galactosemia; F = female; FTT = failure to thrive; GALT = galactose-1-phosphate uridyltransferase; HMZ = homozygous; HTZ = heterozygous; ID = intellectual disability; INR = international normalized ratio; LE = liver enzymes; M = male; N = normal; NP = not performed; POI = primary ovarian insufficiency; mo = month(s); yrs. = year(s).

* Full Sanger sequencing of *GALT* was not performed.

** secondary to birth asphyxia, cord around neck, spastic cerebral palsy, partial complex seizures.

*** presence of potassium, phosphate, protein and bicarbonate in urine.

positive newborn screening for galactosemia. The initial liver enzymes, prothrombin time (PT), direct bilirubin, urinalysis, urine amino acids, and ophthalmological exam were normal. This individual had GALT activity of 3.7% of normal during the initial retrieval investigations. Due to normal liver enzymes, bilirubin and PT, we repeated GALT activity and urine galactitol levels. Urine galactitol was always negative. The GALT activity ranged between 3.7 and 15.5% of normal during our monitoring investigations. All biochemical investigations of this individual are summarized in Supplemental Table 6. One of the GALT activity level was 15.5% of normal which was measured at the age of 3 months. We consider that there might be a laboratory error in the measurement of GALT activity in that sample. All other GALT activity levels were below 7% of normal and confirms clinical variant galactosemia type I. This individual received galactose-lactose-free diet for about 4 weeks. Since discontinuation of galactose-lactose-free diet, this individual has been clinically and biochemically stable. She has compound heterozygous *GALT* variants including p.Arg258Cys, and p. Ala303Ser.

3.3. Biochemical variant (Duarte) galactosemia (n = 4)

All individuals with biochemical variant (Duarte) galactosemia were identified by positive newborn screening. There were two females and two males in this group. Their average age at diagnosis was 8 ± 1.4 SD days (ranging 7–10 days). Their average current age was 3.75 ± 2.9 SD years (ranging 2–8 years). None of these individuals have been on galactose-lactose-free diet at the time of their last appointment. However, one individual (Gal019) was treated with galactose-lactose-free diet between the ages 1–8 years due to failure to thrive. Unfortunately, this diet did not improve the symptoms. Additionally, one individual (Gal020) had a 16p13.11 microdeletion syndrome and the speech-language delays were attributed to this copy number variant.

3.4. Comparison of individuals between the SymX and AsymX groups

The summary of symptom frequency within each group is summarized in Tables 4A, 4B. Two individuals were excluded from the comparisons due to intellectual disability and seizures, one from each group for the reasons described above.

Interestingly, two siblings with classic galactosemia (Gal012 and Gal013), one diagnosed symptomatically at 21 days of age and the other one diagnosed at 7 days of age due to the positive family history, had similar phenotypes including intellectual disability, tremor, osteoporosis and anxiety disorder.

There was a statistically significant difference between SymX and AsymX group for the frequency of jaundice ($p = 0.0092$), lethargy ($p = 0.0451$), conjugated hyperbilirubinemia ($p = 0.0092$), coagulation abnormalities ($p = 0.0451$), elevated liver enzymes ($p = 0.0186$) at the time of the diagnosis. It seemed that intellectual disability was more common in the SymX group (54%) compared to ASymX group (37.5%) as a long-term complication, however there was no statistically significant difference between both groups ($p = 0.645$). It seemed that movement disorders were more common in the SymX group (50%) (6/12) compared to ASymX group (22%) as a long-term complication. However, there was no statistically significant difference ($p = 0.367$) between both groups. The premature ovarian failure was present in both groups: SymX (37.5%) and AsymX (16.7). There was no statistically significant difference between both groups for the frequency of the premature ovarian failure ($p = 0.580$). It seemed that anxiety and depression were more common in the AsymX group compared to SymX group as a long-term complication, however there was no statistically significant difference ($p = 0.553$).

Additionally, we compared the frequency of intellectual disability between classic (44%; 7/16) and clinical variant (67%; 2/3) galactosemia in our study (Supplemental Table 7). There was no statistically

Table 2
Clinical, biochemical and molecular genetic features of AsymX individuals are summarized in this table.

Patient#/study ID/sex/ diagnosis/age of diagnosis/ current age	Presenting symptom (age of onset)/other clinical features	Initial investigations (LE, direct bilirubin, INR, Alb, GALT %)	Molecular genetic investigations	Outcome
1/ GAL004/ F/CG/7 days/ 33 yrs	No/ positive family history	N/N/N/N/0%	HTZ c.563A>G p. Gln188Arg/ not investigated*	Cognitive dysfunction, POI, low BMD
2/ GAL006/F/CG/7 days/ 13 yrs	No	N/N/N/N/0%	HMZ c.563A>G p.Gln188Arg	Normal IQ anxiety, tremors, ataxia
3/ GAL008/ M/CG/7 days/ 24 yrs	No/ positive family history	N/N/N/N/ NP	CMP HTZ c.563A>G p. Gln188Arg/ c.892A>G p. Met298Val	Cognitive dysfunction, ADHD, tremors, fathered a child
4/GAL010/ M/CG/ 7 days/ 36 yrs	No	N/N/N/N/0%	HMZ c.563A>G p.Gln188Arg	Adult-onset idiopathic epilepsy**, low sperm, non-motile
5/ GAL012/F/CG/7 days/ 52 yrs.	Jaundice, opisthotonos, irritability/ positive family history	N/↑/N/N/ NP	HMZ c.563A>G p.Gln188Arg	Mild ID, tremors, ataxia, depression, osteoporosis, mild proteinuria
6/ GAL014/F/CG/ 1 day/ 46 yrs.	Seizure on day one due to left porencephalic cyst and right posterior calcification/ positive family history	N/N/N/N/ NP	HMZ c.563A>G p.Gln188Arg	Severe ID, seizure, low BMD
7/ GAL018/ M/CG/ 7 days/ 11 mo	No	N/N/N/N/0%	CMP HTZ c.442C>T p. Arg148Trp/ c.563A>G p. Gln188Arg	Normal
8/ GAL021/ F/CG /7 days/ 32 yrs.	No	N/N/N/N/0%	CMP HTZ c.563A>G p. Gln188Arg/c.584T>C p. Leu195Pro	Proteinuria
9/ GAL011/ F/CVG/ 9 days/6 mo	No	N/N/N/N/3.7%	CMD HTZ c.772C>T p. Arg258Cys/c.907G>T p. Ala303Ser	Normal

Abbreviations (listed alphabetically): ADHD = attention deficit hyperactivity disorder; Alb = albumin; BMD = bone mineral density; CG = classic galactosemia; CMP = compound heterozygous; CVG = clinical variant galactosemia; F = female; GALT = galactose-1-phosphate uridyltransferase; HMZ = homozygous; HTZ = heterozygous; ID = intellectual disability; INR = international normalized ratio; IQ = intelligence quotient; LE = liver enzymes; M = male; N = normal; NP = not performed; POI = primary ovarian insufficiency; mo = month(s); yrs. = year(s).

* Full Sanger sequencing of *GALT* was not performed.

** Not associated with galactosemia.

Table 3
Clinical, biochemical, and molecular genetic features of individuals with biochemical variant (Duarte) galactosemia are summarized in this table.

Patient#/ study ID/ sex/ diagnosis/ age of diagnosis/ current age	Presenting symptom (age of onset)/ other clinical features	Initial investigations (LE, direct bilirubin, INR, Alb, GALT %)	Molecular genetic investigations	Outcome
1/ GAL001/ F/ DG/ 1 week/ 3 yrs.	No	N/N/N/N/ 17%	CMP HTZ c.-119_-116del/ c.563A>G p. Gln188Arg	Normal
2/ GAL002/ F/DG/ 10 days/ 2 yrs.	No	N/N/N/N/ 42%	HMZ c.-119_-116del	Normal
3/ GAL019/ M/ DG/ 7 days/8 yrs	No	N/N/N/N/ 23.8%	c.-119_-116del (non coding) c.940A>G p. Asn314Asp c.563A>G p. Gln188Arg	FTT*
4/ GAL020/ M/ DG/ 8 days/ 2 yrs	No	N/N/N/N/ 16%	CMP HTZ c.563A>G p. Gln188Arg/ c.940A>G p. Asn314Asp	Speech delay due to 16p13.11 microdeletion syndrome

Abbreviations (listed alphabetically): Alb = albumin; CMP = compound heterozygous; DG = variant (Duarte) galactosemia; F = female; FTT = failure to thrive; GALT = galactose-1-phosphate uridyltransferase; HMZ = homozygous; HTZ = heterozygous; INR = international normalized ratio; LE = liver enzymes; M = male; N = normal; mo = month(s); yrs. = year(s).
* Unrelated as did not respond to galactose-lactose-free diet.

significant difference between groups ($p = 0.582$). There was also no statistically significant difference in the frequency of tremor between classic (41%; 7/17) and clinical variant galactosemia (75%; 3/4) ($p = 0.310$).

4. Discussion

We report 25 individuals with galactosemia type I from a single metabolic genetics center in Alberta followed over 38 years. Forty-eight percent of individuals presented symptomatically. Biochemical variant (Duarte) galactosemia was identified by positive newborn screening since 2019 [31]. The newborn screening and positive family history of galactosemia significantly decreased the frequency of jaundice, elevated liver enzymes and coagulation abnormalities. However, long-term complications associated with galactosemia type I seem to occur in SympX and ASymX group similarly. Interestingly, in the SymX group tremor seems to be more common, but it was not statistically significantly different between the two groups. When we compared classic and clinical variant galactosemia for tremors, there was no statistically significant difference between both groups. We report one novel variant in *GALT* (p.Ala303Ser) in an individual with clinical variant galactosemia type I. To the best of our knowledge, we report for the first time an individual with clinical variant galactosemia who has normal liver enzymes, coagulation studies, normal renal tubular functions, no cataract who had average GALT activity of 7.3% of normal, mildly elevated average galactose-1-phosphate level (although still below the reference range of therapeutic levels) and normal urine galactitol levels on a normal galactose-lactose diet.

Rubio-Gozalbo et al. reported data from the GalNet registry, which included 509 individuals with classic galactosemia from 32 different centers [32]. Neonatal cataract was reported in 25.8% of individuals and

Table 4A
Comparison of clinical features of SymX and AsymX individuals with galactosemia are summarized in this table.

Abnormal clinical features	SymX group ($n = 12$) M = 4, F = 8	AsymX group($n = 9$) M = 3, F = 6	Statistical analysis (Fisher's Exact Test)
GDD/ID	54.5% ($n = 6/11$)	37.5% ($n = 3/8$)	0.645
Seizure	9% ($n = 1/11$)	12.5% ($n = 1/8$)	1
Hepatomegaly	25% ($n = 3/12$)	0% ($n = 0/9$)	0.229
Jaundice	83.3% ($n = 10/12$)	22.2% ($n = 2/9$)	0.0092*
FTT	33.3% ($n = 4/12$)	0% ($n = 0/9$)	0.104
Vomiting	16.7% ($n = 2/12$)	0% ($n = 0/9$)	0.486
Poor feeding	25% ($n = 3/12$)	0% ($n = 0/9$)	0.229
Lethargy	41.7% ($n = 5/12$)	0% ($n = 0/9$)	0.0451
Irritability	8.3% ($n = 1/12$)	11% ($n = 1/9$)	1
Tremors	50% ($n = 6/12$)	22.2% ($n = 2/9$)	0.367
Ataxia	25% ($n = 3/12$)	11% ($n = 1/9$)	0.603
POI	50% (3 out of 6 females)	20% (1 out of 5 females)	0.242
Cataract	16.7% ($n = 2/12$)	0% ($n = 0/9$)	0.486
Sepsis prior to diagnosis, E Coli ($n = 2$); group B streptococci ($n = 1$)	25% ($n = 3/12$)	0% ($n = 0/9$)	0.229
Anxiety/ depression	8.3% ($n = 1/12$)	22.2% ($n = 2/9$)	0.553

Abbreviations (listed alphabetically): F = female; FTT = failure to thrive; GDD = global developemental delay; ID = intellectual disability; M = male; n = number; POI = primary ovarian insufficiency.
* Significant at alpha = 0.05.

in half of them, cataract was resolved on treatment in that registry. Sometimes, despite galactose-lactose-free diet, cataract may not resolve and require surgical removal [7,33,34]. In our study cohort, cataract was reported in 17% of individuals in the SymX group which required surgical removal.

Renal tubular dysfunction characterized by galactosuria, phosphaturia, aminoaciduria, and albuminuria, also called Fanconi syndrome, is a known complication of galactosemia [35]. Galactose supplementation in adult male rats resulted in proteinuria, blebbing and ballooning of the glomerular epithelial cells and increased glycation of the glomerular basement membrane. The authors suggested that protein glycation may affect the renal glomerular permselectivity which leads to the renal pathogenesis [36]. Proteomics analysis of urinary exovesicles in individuals with classic galactosemia was performed. There was a significant increase in serum glycoproteins such as fetuin, immunoglobulins, prostaglandin, H2 D-isomerase, albumin, leucine-rich α-2-glycoprotein and α-1-microglobulin protein (AMBP). The findings were suggestive of subclinical failure of kidney filter function. The authors hypothesized that kidney failure was the result of dysglocosylation of proteins leading to disturbed basal membrane assembly [37]. Thus, even though renal tubular dysfunction has been reported in galactosemia type I, neither its prevalence nor its pathogenesis is well known. In our study cohort, we identified two individuals with Fanconi syndrome: 1) one with classic galactosemia and liver dysfunction and 2) one with clinical variant galactosemia with normal cellular and synthetic liver functions at the time of the diagnosis. The prevalence of Fanconi syndrome was 9.5% in our study cohort. We also had four individuals (three with classic and one with clinical variant galactosemia) who had proteinuria during their follow-up visits despite being compliant with their galactose-free diet.

Table 4B
Comparison of abnormal investigations of SymX and AsymX individuals with galactosemia are summarized in this table.

Abnormal investigations	SymX group (*n* = 12) M = 4, F = 8	AsymX group (*n* = 9) M = 3, F = 6	Statistical analysis (Fisher's Exact Test)	SymX group (*n* = 12) M = 4, F = 8	AsymX group (*n* = 9) M = 3, F = 6	Statistical analysis (Fisher's Exact Test)
	At presentation			At last appointment		
Elevated LE	50% (*n* = 6/12)	0% (*n* = 0/9)	0.0186*	0% (*n* = 0/12)	0% (*n* = 0/9)	1
Conjugated hyperbilirubinemia	83.3% (*n* = 10/12)	22.2% (*n* = 2/9)	0.0092*	0% (*n* = 0/12)	0% (*n* = 0/9)	1
Coagulation abnormalities**	41.7% (*n* = 5/12)	0% (*n* = 0/9)	0.0451*	0% (*n* = 0/12)	0% (*n* = 0/9)	1
Proteinuria	16.7% (*n* = 2/12)	0% (*n* = 0/9)	0.486	16.7% (*n* = 2/12)	22.2% (*n* = 2/9)	1
Low BMD	0% (*n* = 0/12)	0% (*n* = 0/9)	1	41.7% (*n* = 5/12)	33.3% (*n* = 3/9)	1
Abnormal liver ultrasound	8.3% (*n* = 1/12)	0% (*n* = 0/9)	1	16.7% (*n* = 2/12)	0% (*n* = 0/9)	0.486

Abbreviations (listed alphabetically): BMD = bone mineral density; F = female; INR = international normalized ratio; LE = liver enzymes; M = male; *n* = number; PT = prothrombin time; PC = prothrombin concentration.
* Significant at alpha = 0.05.
** Prolonged INR, PTT or PC.

The prevalence of proteinuria was 19% as a long-term complication of classic and clinical variant galactosemia in our study. We report the prevalence of renal tubular dysfunction in galactosemia type I in a small cohort of individuals from our center in our current study to increase the knowledge regarding rarely reported complications of galactosemia type I.

Rubio-Gozalbo et al. reported decreased bone mineral density in 26.5% of individuals with classic galactosemia [32]. In a recent metanalysis, bone mineral density of individuals with classic galactosemia was lower than the general population [7,38–40]. In our study cohort, 38% of the individuals with galactosemia (seven with classic and one with clinical variant galactosemia) had decreased bone mineral density in spine, hip and femur. Decreased bone mineral density seems to be more common in our study cohort despite good compliance with galactose-lactose-free diet and vitamin D and calcium supplements.

Gonadal complications including hypergonadotropic hypogonadism, primary ovarian insufficiency, and low sperm count are long-term complications of galactosemia type I in adult females and males. Rubio-Gozalbo et al. reported primary ovarian insufficiency in 79.7% of the individuals with classic galactosemia [32]. In our study, 36% of the post-pubertal women with classic galactosemia had primary ovarian insufficiency. It seems that this long-term complication was not so common compared to GalNet registry data [32]. Delayed puberty, and cryptorchidism were reported in <10% of males with classic galactosemia in the GalNet registry [32]. There are limited number of reports regarding male infertility in individuals with galactosemia [1,7,32,41]. The prevalence of males who were able to conceive a child, was 7.8% in the GalNet registry [32]. In our study, we had five adult males (four with classic and one with clinical variant galactosemia) and only one male with classic galactosemia had a child. It seems that we have a higher prevalence of male infertility in our study cohort, although it might be due to their choice of reproduction decisions at a young age between 24 and 36 years at the time of this study.

The clinical variant galactosemia is due to hypomorphic alleles in *GALT* resulting in a higher residual GALT activity (1–10% of normal) [1]. The p.Ser135Leu *GALT* variant is the most commonly reported hypomorphic *GALT* variant associated with the clinical variant galactosemia [1,42]. Katler et al. reported that individuals who are homozygous or compound heterozygous for the p.Ser135Leu *GALT* variant exhibited initial presentations similar to classic galactosemia in the neonatal period. Their long-term complications were similar to classic galactosemia regarding cognitive and motor dysfunctions [42]. Welsink-Karssies et al. reported tremors in two individuals with clinical variant galactosemia who were homozygous for the p.Ser135Leu *GALT* variant. However, none of the individuals with clinical variant galactosemia and with the other genotypes had tremor [34]. Katler et al. reported motor outcomes including hypotonia, spasticity, ataxia, intention tremors, gait instability, problems with fine and gross motor skills in individuals with galactosemia type I and with the p.Ser135Leu *GALT* variant (either homozygous or compound heterozygous). Motor abnormalities were reported in 40% of individuals with GALT-null, 28% of individuals with compound heterozygous p.Ser135Leu *GALT* variant, and 27% of the individuals with homozygous p.Ser135Leu *GALT* variant [42]. Adverse ovarian outcomes were reported in 25% of individuals with homozygous p.Ser135Leu *GALT* variant whereas it was reported in 98% of individuals with *GALT*-null variants [42]. Welsink-Karssies et al. reported additional hypomorphic *GALT* variants resulting in clinical variant galactosemia including p.Val128lle, p.Met219Lys, p.Arg201His and c.-96T>G [34]. Interestingly, in our study cohort, three individuals with clinical variant galactosemia had tremors and none of them had p.Ser135Leu *GALT* variant. We report one novel hypomorphic *GALT* variant (p.Ala303Ser) in our study. Interestingly two other individuals with clinical variant galactosemia had compound heterozygous variants and one of their alleles was reported previously in individuals with classic galactosemia including p.Gln188Arg; p.Leu195Pro. We think that for the first time we report other genotypes associated with clinical variant galactosemia and tremors which expands the phenotypic spectrum of clinical variant galactosemia based on the genotypes.

Our study had several limitations including: 1) This is a retrospective cohort study. 2) There was a lack of a uniform evaluation pathway for monitoring of the outcomes of individuals with galactosemia in our center. 3) Some of the individuals were too young as the long-term complications may take several years to be present. Despite these limitations, the data obtained from a single metabolic center and the outcomes observed in these individuals provide valuable insights and contribute to the knowledge of galactosemia type I.

In conclusion, we report 25 individuals with galactosemia type I from a single metabolic genetic center diagnosed and followed over 38 years. We report for the first time an individual with clinical variant galactosemia who is clinically asymptomatic on normal diet since being identified by positive newborn screening for galactosemia type I. To the best of our knowledge, all individuals with clinical variant galactosemia have galactose-lactose free or restricted diet. Our case with clinical variant galactosemia raises questions if there could be future clinical studies to investigate use of galactose-lactose restricted diet. There were statistically significant differences between SymX and AsymX groups for early symptoms associated with galactosemia type I, however we did not find any statistical significance for the long-term complications of galactosemia between both groups. It is timely to find new therapeutic strategies that can reduce the frequency of late-onset complications in

galactosemia type I.

Author statement

We state that all data used to generate this manuscript is included into the supplemental tables.

We state that we did not use generative AI and AI-assisted technologies in the writing process. For this reason, we did not include this information into the manuscript.

We state that all procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the Institutional Research Ethics Board, Approval number Pro00117378, (informed consent was waived for retrospective chart review study). The study was approved by The Northern Alberta Clinical Trials and Research Centre (NACTRC) and Alberta Health Services provided operational and administrative approval (PRJ38569). The first approval date 20/01/2022. The recent re-approval date December 5, 2023 (expiry December 4, 2024).

CRediT authorship contribution statement

Nihal Almenabawy: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Shalini Bahl:** Data curation, formal analysis, review editing. **Alyssa-Lyn Ostlund:** Data curation, review editing. **Shailly Ghai-Jain:** Data curation, review editing. **Iveta Sosova:** Data curation, Formal analysis, Investigation, review editing. **Alicia Chan:** Data curation, review editing. **Saadet Mercimek-Andrews:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

All authors declare no conflict of interest.

Data availability

All data included in the manuscript and supplementary document

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2024.101055.

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