Molecular Genetics and Metabolism xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?

K.P. Coss [a], E.P. Treacy [a,b,c], E.J. Cotter [a], I. Knerr [b], D.W. Murray [d], Y.S. Shin [e], P.P. Doran [a,*]

[a] University College Dublin, Clinical Research Centre, Mater Misericordiae University Hospital, Ireland
[b] National Centre for Inherited Metabolic Disorders, Ireland
[c] Trinity College Dublin, Ireland
[d] Royal College of Surgeons in Ireland, Department of Physiology and Medical Physics, Dublin 2, Ireland
[e] University Children's Hospital and Molecular Genetics and Metabolism Laboratory, Munich, Germany

ARTICLE INFO

Article history:
Received 6 July 2014
Received in revised form 6 August 2014
Accepted 6 August 2014
Available online xxxx

Keywords:
Galactosaemia
Biomarkers
Genomics
Glycosylation
Inflammation

ABSTRACT

Classical Galactosaemia is a rare disorder of carbohydrate metabolism caused by a deficiency of galactose-1-phosphate uridyltransferase (GALT). The disease is life-threatening in the neonate, and the only treatment option is life-long dietary restriction of galactose. However, long-term complications persist in treated patients including cognitive impairments, speech and language abnormalities and premature ovarian insufficiency in females. Microarray analysis of T-lymphocytes from treated adult patients identified systemic dysregulation of numerous gene pathways, including the glycosylation, inflammatory and inositol pathways. Analysis of gene expression in patient-derived dermal fibroblasts of patients exposed to toxic levels of galactose, with immunostaining, has further identified the susceptibility of the glycosylation gene alpha-1,2-mannosyltransferase (*ALG9*) and the inflammatory gene annexin A1 (*ANXA1*) to increased galactose concentrations. These data suggest that Galactosaemia is a multi-system disorder affecting numerous signalling pathways.

1. Introduction

Classical Galactosaemia (OMIM 230400) is a rare autosomal recessive disorder of carbohydrate metabolism, caused by deficiency of galactose-1-phosphate uridyltransferase (GALT E.C 2.7.7.12). The disease is fatal if untreated in the neonate. Currently, the only available treatment is a life-long galactose restricted diet. Although this treatment is life-saving in the neonate, long-term complications persist in treated patients including, but not limited to, cognitive impairments, speech and language abnormalities, ataxia and premature ovarian insufficiency (POI) [1–3]. The severity of these complications is not linked to genotype or age of therapeutic intervention [2–10].

The accumulation of galactose-1-phosphate (gal-1-p) and galactitol, and their toxic effects on a number of metabolic pathways including inhibition of GALT, UDP-glucose/galactose pyrophosphorylase, galactosyltransferases and competitive inhibition of inositol monophosphatase 1 (IMPase1), may be implicated in the pathophysiology [1,11]. In addition, over-restriction of galactose may also contribute to the disease phenotype by further depleting UDP-galactose and disrupting glycosylation [12]. In support of this, abnormalities of *N*-glycosylation have been shown in serum transferrin and whole serum from untreated, galactose intoxicated Galactosaemia neonates, [12–15]. We have also demonstrated on-going *N*-glycan processing defects in treated children and adults with Classical Galactosaemia [12,16]. There are numerous clinical similarities noted between Galactosaemia and congenital disorders of glycosylation (CDG) [15,17].

Galactosaemia, although described in 1908, remains a poorly treated rare disease. A key step in improving approaches to therapy to this, and related diseases, is the identification of accurate biomarkers which could predict current treatment (dietary intervention) effects, and new emerging modalities of treatment. To date, the measurement of RBC gal-1-p is effectively used to monitor the response to dietary galactose restriction in intoxicated Galactosaemia neonates (11,12). This biochemical marker has not however been shown to discriminate moderate galactose liberalisation in treated Galactosaemia patients or to be prognostic for long-term treated outcomes [5,12,18,19].

Earlier studies carried out on the exposure of patient-derived Galactosaemia dermal fibroblasts to intoxication levels of galactose

Abbreviations: CDG, congenital disorders of glycosylation; DAPI, 4′, 6-diamidino-2-phenylindole; DAVID-EASE, Database for Annotation, Visualisation and Integrated Discovery-Expression Analysis Systematic Explorer; FITC, fluorescein isothiocyanate; GHDF, Galactosaemia human dermal fibroblasts; Gal-1-p, galactose-1-phosphate; GALT, galactose-1-phosphate uridyltransferase; GPI, glycosylphosphatidylinositol; IMPase1, inositol monophosphatase 1; KEGG, Kyoto Encyclopaedia of Genes and Genomes; NHDF-Ad, normal human dermal fibroblast adult; PBS, phosphate buffer saline; POI, premature ovarian insufficiency; RBC, red blood cell; UPR, unfolded protein response.

* Corresponding author at: Mater Misericordiae University Hospital, Eccles St., Dublin 7, Ireland.
E-mail addresses: etreacy@mater.ie (E.P. Treacy), peter.doran@ucd.ie (P.P. Doran).

http://dx.doi.org/10.1016/j.ymgme.2014.08.004
1096-7192/

(0.1% galactose) indicated gross disruption of a number of genes, indicating a potential role for gene dysregulation in the early pathophysiology of Galactosaemia to include ER stress and up-regulation of the unfolded protein responses (UPR) [20].

We have previously described the T-lymphocyte expression pattern in four treated adult Galactosaemia patients and demonstrated dysregulation of numerous Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways [8]. A number of these genes were validated by qRT-PCR indicating ongoing systemic defects following treatment.

In this study, we now provide further validation of these genes and pathways by extending these studies to include twelve Galactosaemia patients with the development of an *in vitro* dermal fibroblast cellular model to further validate genes of interest; the effects of galactose intoxication (0.1% galactose) and a predicted cellular model of treatment (dietary intervention) (0.01% galactose) on mRNA and protein expression.

2. Materials and methods

2.1. Study patients

The clinical characteristics of the affected patients are detailed in Table 1. All adult patients were maintained on a monitored galactose restricted diet of up to 300 mg/gal per day. T-lymphocyte samples were taken from the twelve patients for DNA microarray analysis (six with FSIQ ≥ 80 and six with FSIQ < 80). Control samples were taken from three healthy adult patients.

The arrays from healthy controls were compared with the twelve treated Galactosaemia patients. Galactosaemia human dermal fibroblasts (GHDF) were derived from two patients (Q and M, Table 1). Control normal human dermal fibroblast adult (NHDF-Ad) primary cells were commercially obtained from Lonza.

2.2. T-lymphocyte collection and RNA extraction

T-lymphocyte extraction and DNA microarray analysis, using Affymetrix Human Genome U113 plus 2.0 arrays, was carried out as described in our previous study [8]. Only genes $p < 0.05$ were included for analysis. The Database for Annotation, Visualisation and Integrated Discovery-Expression Analysis Systematic Explorer (DAVID-EASE) programme was used to determine KEGG pathways affected in the groups [21,22].

2.3. Validation of microarray data

The expression profile of genes selected from the DNA microarrays were validated using qRT-PCR. In addition, *GRP78/BiP* was examined in fibroblasts as a marker of ER stress. Genes were validated in T-lymphocytes and dermal fibroblasts using qRT-PCR. Total RNA was extracted from cells using the TRI-reagent RNA extraction method (Ambion, AM9738) and was retro-transcribed using the avian reverse-transcriptase method (Sigma, A4464-1KU). qRT-PCR was performed on a rotor gene system (Corbett research) [8]. For each sample, genes were amplified in duplicate. *GAPDH* was used as the housekeeping gene for both cell types. There was no dysregulation of this gene noted in the 12 Galactosaemia microarray samples. Relative mRNA expression levels were determined using the $\Delta\Delta^{CT}$ method.

2.4. Cell culture conditions

The cells were maintained in Nutrient Mix F-10 HAM galactose free media (Sigma-Aldrich, Ireland N6908). Lonza NHDF-Ad cells were maintained in Fibroblast Growth Medium galactose free media (FGM; Lonza, supplied by Brennan and Company, Ireland CC-3132). Galactosaemia patient fibroblasts (GHDF) and NHDF-Ad were used between passages 3–4 (no cells were used over passage 5). Cells were grown in 6-well plates and each treatment done in triplicate. Cells were exposed to 1.5 ml of media per well. 0.1% galactose (Sigma-Aldrich, G5388) was used to mimic galactose intoxication in patients. 0.1% galactose in cell culture generates 1.3 mM gal-1-p which is equivalent to the gal-1-p level in galactose intoxicated patient erythrocytes (approximately 1000 to 2000 μmol/l) [20]. 0.01% galactose was used as an estimate of the RBC galactose concentration for diet-restricted Galactosaemia patients. Matched controls grown in galactose free

Table 1
Clinical characteristics of study subjects.

Code	Age	Genotype	Ethnicity	Complications	GALT[a]
Control group					
CA♀	29 years	*N/A*	Irish	N/A	N/A
CB♀	24 years	*N/A*	Irish	N/A	N/A
CC♀	25 years	*N/A*	Irish	N/A	N/A
Group A, FSIQ ≥ 80					
Q♂	25 years	*Q188R/Q188R*	Irish	FSIQ 80–89	<0.5
N♂	18 years	*Q188R/Q188R*	Irish	FSIQ 94, verbal dyspraxia	<0.5
O♂$^{\Omega}$	21 years	*Q188R/Q188R*	Irish	FSIQ 90	<0.5
P♂$^{\Omega}$	19 years	*Q188R/Q188R*	Irish	FSIQ 108	<0.5
A♀	26 years	*Q188R/Q188R*	Irish traveller	FSIQ 112, POI	<0.5
X♂$^{\psi}$	28 years	*Q188R/Q188R*	Irish	FSIQ 110, osteoporosis	<0.5
M♂	24 years	*Q188R/Q188R*	Irish Traveller	FSIQ 80–89, slight lens opacity	<0.5
JQ♂	16 years	*Q188R/Q188R*	Irish Traveller	FSIQ 81, speech disorder, osteopenia	<0.5
KP♂	18 years	*Q188R/Q188R*	Irish Traveller	FSIQ 80, speech disorder	<0.5
Group B, FSIQ < 80					
W♀$^{\psi}$	27 years	*Q188R/Q188R*	Irish	FSIQ 47, severe developmental delay, speech delay, ataxia, osteoporosis, POI	<0.5
U♂$^{\beta}$	16 years	*Q188R/Q188R*	Irish	FSIQ 47, severe developmental delay, ataxia, osteoporosis, dysmorphic facial features	<0.5
V♂$^{\beta}$	13 years	*Q188R/Q188R*	Irish	FSIQ 52, severe developmental delay, ataxia, osteoporosis, dysmorphic facial features	<0.5
H♀$^{\kappa}$	29 years	*Q188R/Q188R*	Irish	FSIQ 65, delayed speech, osteopenia, POI	<0.5
G♀$^{\kappa}$	27 years	*Q188R/Q188R*	Irish	FSIQ 57, stammering speech, ataxia, osteoporosis, POI	<0.5
F♀	22 years	*Q188R/Q188R*	Irish	FSIQ 70–79, osteopenia, POI	<0.5
CX♂	18 years	*Q188R/Q188R*	Irish Traveller	FSIQ 70–79	<0.5
BY♂	26 years	*Q188R/R333W*	Irish	FSIQ 70–79, bilateral non-progressive cataracts	<0.5
AZ♀$^{\lambda}$	20 years	*Q188R/R333W*	Irish	FSIQ 74	<0.5

POI: Premature Ovarian Insufficiency.
$^{\Omega, \lambda, \kappa, \beta, \psi}$Designate sibling pairs.
[a] GALT expressed as micromoles of Gal-1-p converted per hour per gramme.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

media were used. Cells were exposed overtime; for 30 min, 1 h, 2 h and 4 h. Gene expression was then analysed with qRT-PCR and immunofluorescence carried out.

2.5. Gal-1-p metabolite measurement assay

The gal-1-p assay was analysed using established protocols [23]. This allowed for the establishment of the cellular mimic of the post-treatment level of galactose exposure in Galactosaemia patients (0.01% galactose in culture media). 0.01% galactose is an estimation of the treatment level of galactose for Galactosaemia cells, based on the approximate 90% reduction in patient hemolysate gal-1-p levels following treatment with dietary intervention in the neonatal stage, where levels of gal-1-p drop from between 2.5 mM and 6.5 mM to 0.1 mM and 0.2 mM. Cells were exposed to these levels for 4 h to confirm the hypothesised increase and decrease in gal-1-p levels.

2.6. Time dependent alteration of gene expression in GHDF and NHDF cells

The gene analysis was carried out following exposure of Galactosaemia fibroblasts to either: galactose free media, 0.1% galactose containing media (inducing intoxication in Galactosaemia dermal fibroblasts [20]), or 0.01% galactose containing media. The cells were exposed to these levels of galactose for 4 h, after confirmation of increased gal-1-p levels after 4 h using the gal-1-p metabolite measurement assay.

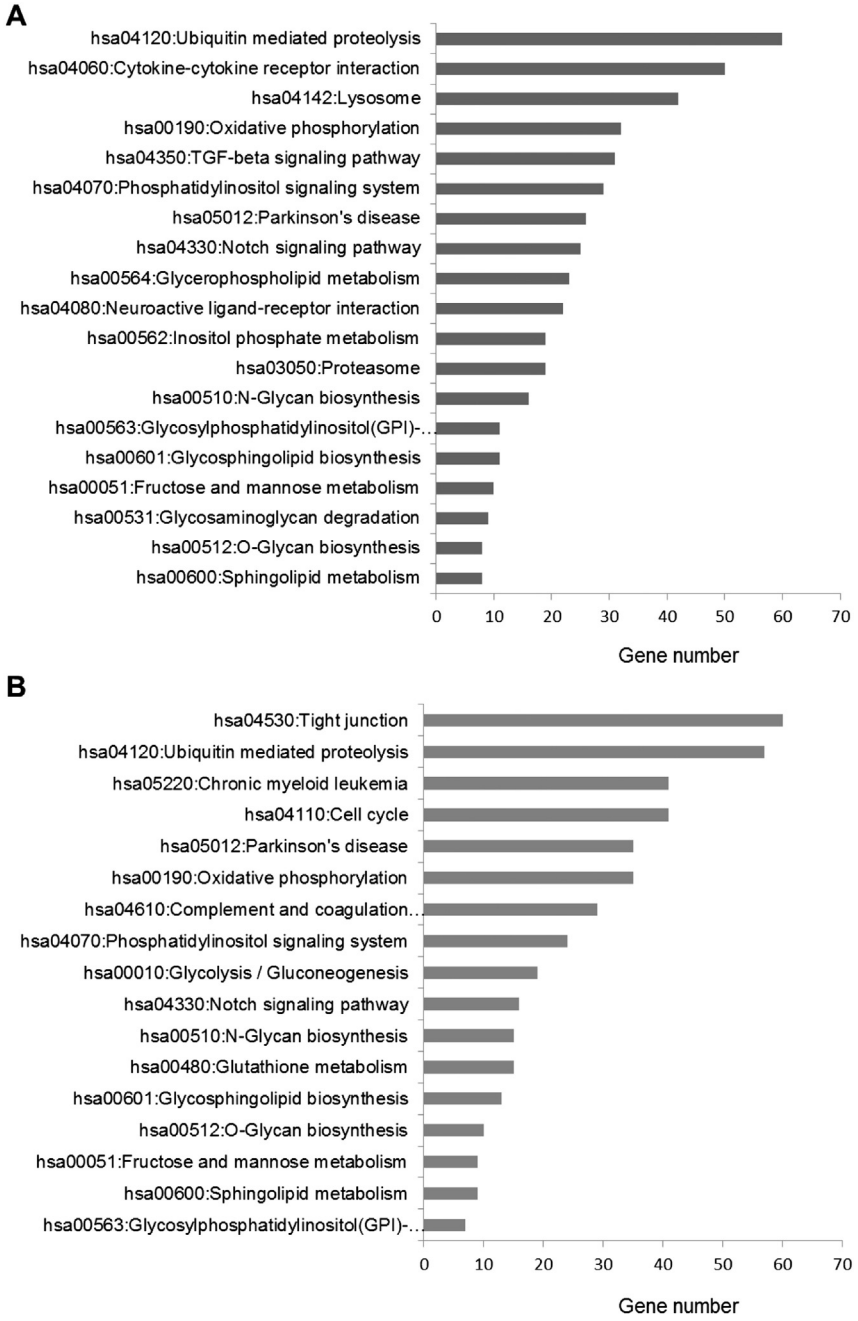

Fig. 1. A and B. Selection of KEGG pathways (A) up-regulated and (B) down-regulated in Galactosaemia *versus* control T-lymphocyte DNA microarray. The x axis illustrates the number of genes dysregulated in the pathway.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

Table 2
CDG *N*-glycan related genes dysregulated in Galactosaemia DNA Microarrays.

	Up-regulated		SD		*p*-Value
	Gene	Fold increase	Controls	Galactosaemia	
CDG-I	*ALG9*	7.9	0.36	0.54	$<7 \times 10^{-7}$
	ALG2	3.9	0.07	0.80	$<1 \times 10^{-3}$
	DHDDS	2.9	0.46	0.59	$<1 \times 10^{-3}$
	ALG8	1.9	0.63	0.49	$<2 \times 10^{-2}$
	DPAGT1	4.7	0.26	0.45	$<2 \times 10^{-6}$
	RFT1	4.1	0.40	0.86	$<1 \times 10^{-3}$
	ALG1	2.5	0.40	0.67	$<6 \times 10^{-3}$
	Down-regulated		SD		*p*-Value
	Gene	Fold decrease	Controls	Galactosaemia	
CDG-I	*DPM1*	1.7	0.11	0.29	$<1 \times 10^{-3}$
	DDOST	3.2	0.04	0.69	$<1 \times 10^{-3}$
	RPN2	2.4	0.30	0.40	$<2 \times 10^{-4}$
CDG-II	*B4GALT1*	1.9	0.51	0.52	$<1 \times 10^{-2}$
	MGAT2	2.6	0.16	0.20	$<3 \times 10^{-4}$

2.7. Immunofluorescence

Both NHDF and GHDF cells were cultured on Nunc Lab-Tek II Chamber Slides (Sigma-Aldrich, Ireland). All cell-lines were treated with 0.1% galactose (1 h, 2 h and 4 h exposure times, depending on the time taken for significant changes in gene expression to be seen). Matched controls (grown in galactose free media) were included for all treatments or time-points. A no primary antibody control was also included to check for non-specific binding of the secondary antibodies. Post-treatment cells were fixed using formaldehyde (4% v/v in PBS) and permeabilised with Triton X (0.1% v/v in phosphate buffer saline (PBS)), before being incubated with blocking solution (10% BSA w/v in PBS) for 30 min. Cells were incubated overnight with either 1:150 rabbit anti-ANXA1 (Biosciences, Ireland, 71-3400), or 1:25 goat anti-ALG9 (Santa Cruz, USA sc-109496), before being detected with 1:200 goat anti-rabbit Alexa Fluor© 488 (Invitrogen, Ireland A-11008) or 1:200 mouse anti-goat fluorescein isothiocyanate (FITC) (Santa Cruz, USA sc-2356) respectively, incubated for 1 h and 30 min. All antibody dilutions were prepared in BSA solutions (10% in PBS) to prevent non-specific binding, and secondary antibody-only controls were included. A wash buffer (0.1% Tween in PBS) was also used (4×200 μl washes, after each antibody incubation). All wells were counterstained with the nuclear specific 4′, 6-diamidino-2-phenylindole (DAPI) stain to allow visualisation of cell nuclei. After staining, cells were mounted and photographed using an Olympus fluorescent microscope equipped with appropriate fluorescent filters.

2.8. Statistical analysis

Standard deviations and statistical analysis, using student *t*-tests, were carried out using GraphPad prism 5, with statistical significance determined as $p < 0.05$.

2.9. Ethics

Ethical approval for the study was obtained from the Ethics Committee of the Children's University Hospital, Dublin.

3. Results

3.1. Analysis of KEGG pathways dysregulated in Galactosaemia

The DAVID-EASE programme was used to display the DNA microarray results as KEGG pathways affected [21,22]. A selection of these pathways is shown in Figs. 1A and B. These affected pathways correspond with many of the proposed mechanisms for the systemic effects in Galactosaemia. For example, the ubiquitin-mediated proteolysis pathway was shown to be grossly dysregulated with sixty known genes up-regulated and fifty-seven genes down-regulated. The ubiquitin-

Table 3
Top ten genes (A) up- and (B) down-regulated in Galactosaemia, with the proposed gene function.

Gene title	Gene symbol	Protein function	Fold increase	Control SD	Galactosaemia SD	*p*-Value
Ribosomal protein, large, P2	*RPLP2*	Role in the elongation step of protein synthesis	273.0	0.53	0.20	$<1 \times 10^{-15}$
Ribosomal protein L14	*RPL14*	RPL14 interacts with PHLDA1, which has a role in the anti-apoptotic effects of insulin-like growth factor-1	125.8	0.57	0.09	$<1 \times 10^{-15}$
Regulator of G-protein signalling 10	*RGS10*	Inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits.	122.2	0.50	0.45	$<5 \times 10^{-12}$
ATPase, H + transporting, lysosomal 9 kDa, V0 subunit e1	*ATP6V0E1*	Acidifies a variety of intracellular compartments in eukaryotic cells	91.6	0.64	0.46	$<3 \times 10^{-11}$
S100 calcium binding protein A8	*S100A8*	Calcium-binding protein. An inflammatory marker.	88.8	0.09	2.66	$<1 \times 10^{-3}$
Ribosomal protein S11	*RPS11*	Ribosomal protein that is a component of the 40S subunit	86.1	0.66	0.13	$<3 \times 10^{-14}$
Amyloid beta (A4) precursor-like protein 2	*APLP2*	Regulation of haemostasis.	81.3	0.35	0.49	$<2 \times 10^{-11}$
Spectrin, beta, non-erythrocytic 1	*SPTBN1*	Functions in the determination of cell shape/organization of organelles	80.3	1.99	0.28	$<2 \times 10^{-8}$
Transmembrane protein with EGF-like and two follistatin-like domains 2	*TMEFF2*	Survival factor for hippocampal and mesencephalic neurons	78.6	1.01	0.27	$<2 \times 10^{-11}$
BolA homolog 2 (E. coli)	*BOLA2*	Involved in cell proliferation/cell-cycle regulation	76.9	0.73	0.22	$<5 \times 10^{-13}$
Annexin A1	*ANXA1*	Glycoprotein with anti-inflammatory activity.	92.3	0.46	0.54	$<6 \times 10^{-11}$
Zinc finger protein 36, C3H type-like 2	*ZFP36L2*	Probable regulatory protein in response to growth factors	52.6	0.74	0.65	$<5 \times 10^{-9}$
Interleukin 23, Alpha Subunit P19	*IL23A*	Involved in autoimmune inflammation.	48.8	0.31	0.49	$<8 \times 10^{-11}$
ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	*ST6GAL1*	Transfers sialic acid from the donor of substrate CMP-sialic acid to galactose containing acceptor substrates	48.0	0.85	0.40	$<2 \times 10^{-10}$
Folate receptor 1 (adult)	*FOLR1*	Binds to folate and reduced folic acid derivatives	37.2	0.35	0.97	$<6 \times 10^{-7}$
Myeloid cell leukaemia sequence 1 (BCL2-related)	*MCL1*	Regulation of apoptosis *versus* cell survival.	26.4	0.45	0.51	$<2 \times 10^{-9}$
Trophoblast-derived noncoding RNA	*TncRNA*	Novel noncoding RNA; may have an important structural role in nuclear paraspeckles	23.5	0.43	0.55	$<6 \times 10^{-9}$
Family with sequence similarity 132, member A	*FAM132A*	Regulation of glucose metabolism	21.1	0.15	0.75	$<2 \times 10^{-7}$
GTPase, IMAP family member 1	*GIMAP1*	May regulate lymphocyte survival	20.5	0.18	1.24	$<5 \times 10^{-5}$
prostaglandin E receptor 4 (subtype EP4)	*PTGER4*	Member of G-protein coupled receptor family, receptor identified for prostaglandin E2 (PGE2)	19.8	0.18	0.49	$<2 \times 10^{-9}$

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

mediated proteolysis pathway may relate to aberrant glycosylation, degradation of proteins and the unfolded protein response (UPR) pathway. In addition, over 60 genes involving the oxidative phosphorylation pathway were either upregulated or down-regulated.

The phosphatidylinositol signalling system, which has been reported as a potential pathway in the pathophysiology of Galactosaemia [24–26], was also dysregulated with twenty-nine genes up-regulated and twenty-four down-regulated. A number of glycosylphosphatidylinositol (GPI)-anchor biosynthesis genes (involved in the synthesis or attachment of proteins to a GPI-anchor, linking proteins to membranes) were also dysregulated. There are eleven genes up-regulated and thirteen down-regulated in the glycosphingolipid biosynthesis pathway [27].

Of relevance to our earlier studies was the significant dysregulation of the *N*- and *O*-glycan biosynthesis pathways. Twelve *N*- and six *O*-glycan biosynthesis genes were up-regulated in Galactosaemia, while another ten *N*- and eight *O*-glycan biosynthesis genes were down-regulated. Of the genes involved in *N*-glycan biosynthesis, ten genes (seven up-regulated, three down-regulated) were linked to CDG-I and two genes (both down-regulated) to CDG-II (see Table 2). A number of genes in the fructose and mannose metabolism

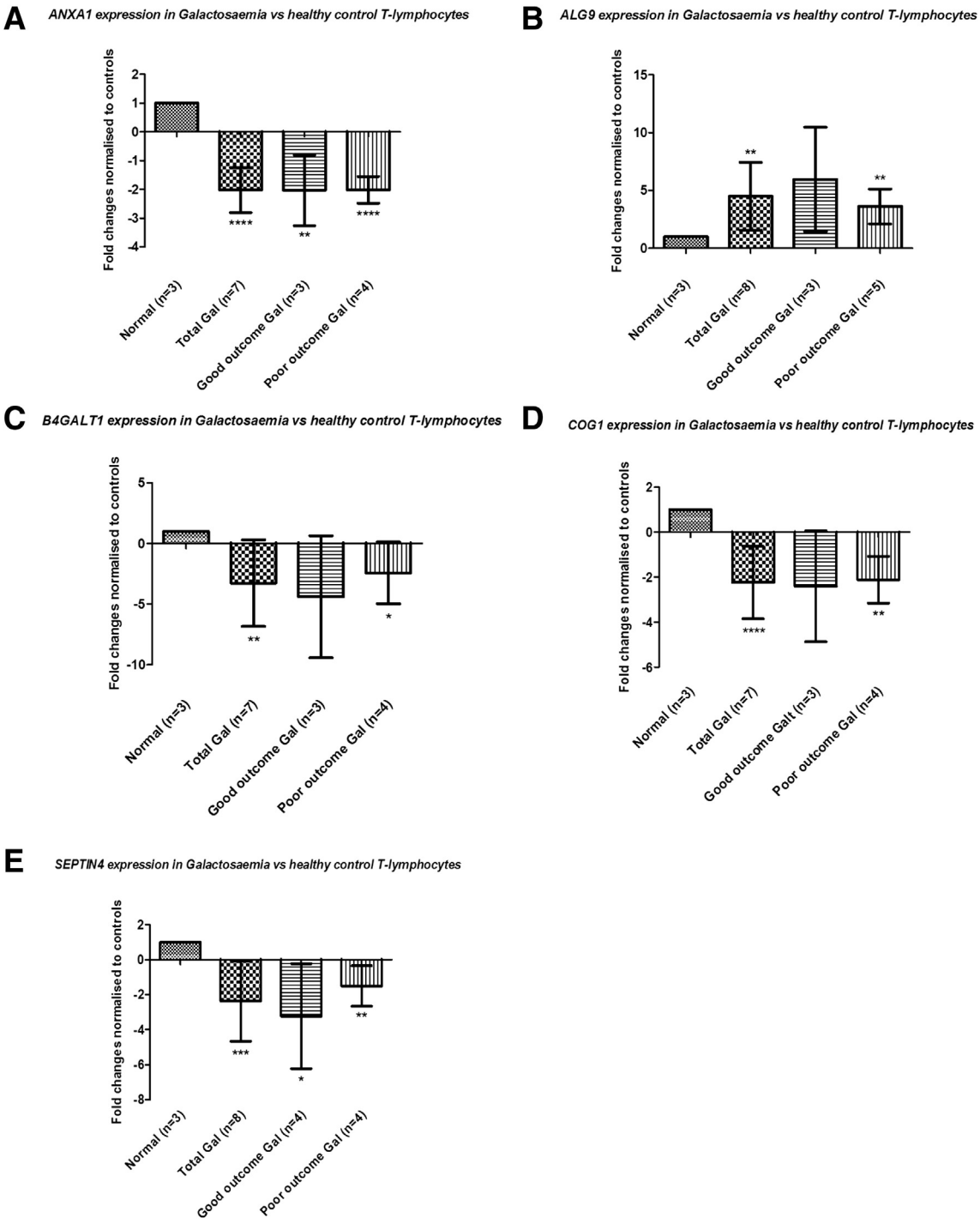

Fig. 2. A–E. Gene expression validation using qRT-PCR. The fold change is presented, normalised to healthy controls, ±SD. Statistical significance was determined using Students *t*-test, with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ $^{****}p < 0.0001$.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

pathway, of direct relevance to glycosylation, were also observed to be dysregulated (ten genes up-regulated and nine genes down-regulated).

3.2. Dysregulated gene expression in Galactosaemia

Significant dysregulation of mRNA expression was identified in 16,877 genes. 8087 genes were significantly up-regulated and 8790 down-regulated. Genes with significance level of $p < 0.05$ were retained for analysis.

The top 10 most up- and down-regulated genes are shown in Table 3. Of the most significantly affected genes, a number have functions that potentially relate to Galactosaemia pathophysiology, with biological plausibility. For example, as illustrated in Table 3, these include up-regulation of regulator of G-protein signalling 10 (*RGS10*) gene linked to the activation of microglia, indicating a role in inflammatory responses in the brain [28], S100 calcium binding protein A8 (*S100A8* a pro-inflammatory agent [29] which may be involved in synapse formation and function [30]) and secreted protein, acidic, cysteine-rich (osteonectin) (*SPARC*). These genes were also found to be dysregulated in our earlier study [8]. In this study we also observed the overexpression of spectrin, beta, non-erythrocytic 1 (*SPTBN1*), which has been shown to increase neurite branching in human dopaminergic neuron cells [31].

The down-regulated genes of significance include the most significantly down-regulated gene, annexin A1 (*ANXA1*), a glycoprotein [32]. *ANXA1* is a pro-apoptotic marker and potent anti-inflammatory agent, with a role in microglia cells in the central nervous system (CNS) [33]. The truncated form of the ANXA1 protein can act as a pro-inflammatory, released from neutrophils, this activity contrasts the anti-inflammatory role of the full-length ANXA1 protein [34].

ST6 beta-galactosamide alpha-2,6-sialyltransferase 1 (*ST6GAL1*) is a sialyltransferase involved in the addition of the sialic acid residue to galactose on the *N*-glycan chain. The decreased expression of this gene further supports the dysregulation of glycosylation in Galactosaemia, potentially linked to the decreased bioavailability of UDP-galactose which consequently may affect glycan sialylation.

We noted that the leptin receptor gene (*LEPR*) was up-regulated in Galactosaemia *versus* healthy controls. Leptin signalling has a well characterised role in fertility and the hypothalamic–pituitary–ovarian axis [35,36]. It has also been reported to have a potential neuroprotective role and disruption of leptin signalling may have a role in cognitive defects [37–40]. Of note, we have recently described altered leptin levels and hypoleptinaemia in patients with Galactosaemia [41].

3.3. Validation of genes of interest from DNA microarrays using qRT-PCR

Genes were selected for further analysis based on their potential involvement in the pathophysiology of Galactosaemia. The five genes chosen for qRT-PCR evaluation are shown in Figs. 2A–E. Three of these genes were related to glycosylation pathways; *B4GALT1*, *ALG9* and *COG1*.

The expression of *ANXA1* (Fig. 2A) was 1.5 fold decreased in Galactosaemia *versus* healthy controls in qRT-PCR ($p = 0.0000003$). The expression of *ALG9* (Fig. 2B), encoding a mannosyltransferase involved in *N*-glycan assembly, and mutated in *ALG9*-CDG (CDG-Il), was increased by 4.3 fold in Galactosaemia *versus* healthy controls, in qRT-PCR ($p = 0.005$). The expression of *B4GALT1* (Fig. 2C), encoding a galactosyltransferase involved in glycan processing, and mutated in *B4GALT1*-CDG (CDG-IId), was decreased by 2.9 fold in Galactosaemia *versus* healthy controls, ($p = 0.01$).

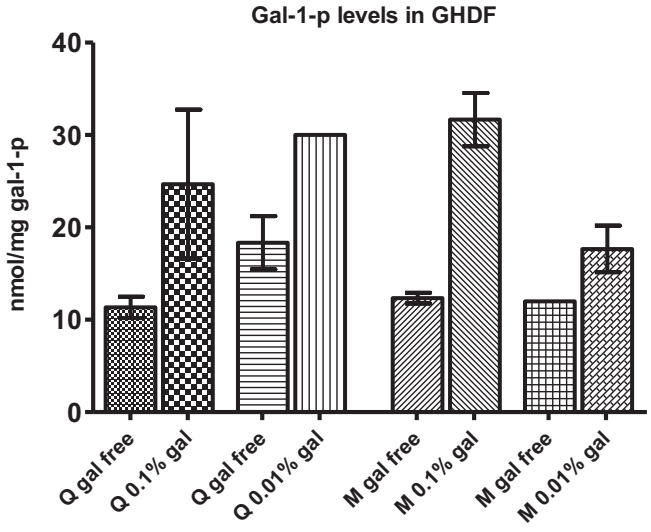

Fig. 3. Gal-1-p levels in 2 GHDF cell-lines (Q and M) grown in galactose-free and galactose containing media for 4 h.

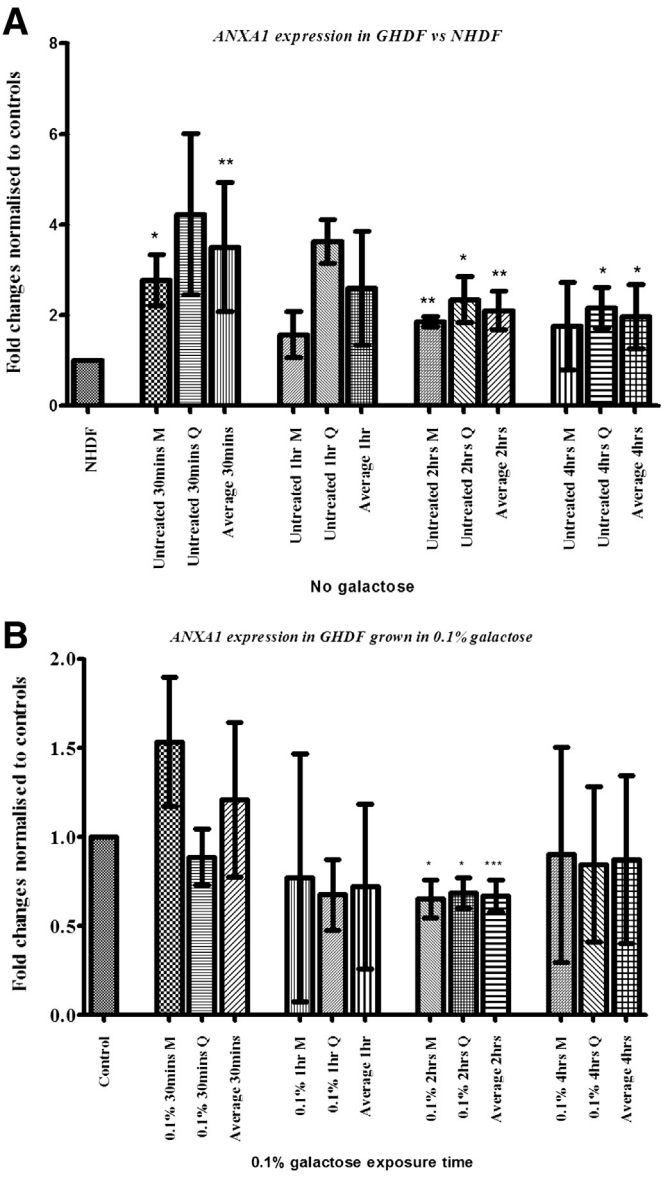

Fig. 4. A and B. *ANXA1* expression in (A) GHDF *versus* NHDF, grown in galactose free media and (B) GHDF cells grown in 0.1% galactose. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ $^{****}p < 0.0001$.

The expression of *COG1* (Fig. 2D), encoding a subunit of the COG complex involved in protein trafficking in the Golgi, particularly the localisation of glycosyltransferases to Golgi subunits [42], and mutated in *COG1*-CDG, was decreased by 1.8 fold in Galactosaemia *versus* healthy controls, ($p = 0.0002$). As evidenced in our earlier study, *SEPTIN4*, a cytoskeletal protein involved in sperm motility was again significantly decreased in Galactosaemia *versus* healthy controls, 1.7 fold ($p = 0.001$) [8].

3.4. Gal-1-p levels in patient-derived dermal fibroblasts

The gal-1-p levels (measured as nmol/mg gal-1-p) in both cell-lines are shown in Fig. 3. In cell-line Q an approximate 14.3% decrease in gal-1-p level was observed in 0.01% galactose cells *versus* galactose intoxicated cells. There was no substantial increase in the levels of gal-1-p in this patient, during 0.1% or 0.01% galactose exposure. This could indicate that this subject may have altered accessory galactose metabolism pathways mitigating the effects of galactose intoxication. In contrast, for cell-line M there is an approximate 70% decrease in gal-1-p levels between 0.01% galactose cells and galactose intoxicated cells, similar to the approximate 90% decrease in gal-1-p levels observed in Galactosaemia patients during treatment with dietary intervention.

3.5. Time dependent alteration of gene expression in GHDF and NHDF cells

There were five genes selected for further evaluation in GHDF (cell-line Q and M) and NHDF cells based on validation of these cells in T-lymphocytes (*COG1*, *B4GALT1*, *SEPT4*, *ANXA1* and *ALG9*). *ANXA1* and *ALG9* were the only genes that showed statistical significant alterations in expression in both GDHF cell-lines compared to NHDF cells grown in galactose free media.

Expression of *ANXA1*, grown in galactose free media, was increased significantly in both GHDF cell-lines at 2 h compared with NHDF (M $p = 0.005$, Q $p = 0.045$ and average $p = 0.001$) (Fig. 4A). *ANXA1* expression was also significantly increased in cell-line M at 30 min ($p = 0.03$) and Q at 4 h ($p = 0.02$). While on average expression is significantly increased at 30 min ($p = 0.008$) and 4 h ($p = 0.02$).

When GHDF cells were exposed to 0.1% galactose and compared to untreated matched cells from each donor, the expression of *ANXA1* (Fig. 4B) decreased significantly at 2 h in both cell-lines (M $p = 0.03$ and Q $p = 0.02$, average $p = 0.0003$).

Expression of *ALG9* grown in galactose free media was decreased significantly in both GHDF cell-lines at 30 min, compared with NHDF (M $p = 0.004$, Q $p = 0.001$ and average $p = 0.00002$) (Fig. 5A). Cell-line M also shows significantly decreased expression at 2 h ($p = 0.04$); on average expression is also significantly decreased at 2 h ($p = 0.01$).

When GHDF cells were exposed to 0.1% galactose and compared to untreated matched cells from each donor, the expression of *ALG9* (Fig. 5B) decreased significantly at 1 h in both cell-lines (M $p = 0.002$ and Q $p = 0.03$, average $p = 0.0001$). The expression was significantly decreased at 4 h in cell-line M ($p = 0.01$) and on average ($p = 0.0008$). The cell-line M is also significantly decreased at 30 min ($p = 0.0002$), showing possible increased susceptibility of this patient during galactose exposure.

3.6. Immunofluorescence of ANXA1 and ALG9 expression in GHDF versus NHDF exposed to 0.1% galactose

We selected the genes *ANXA1* and *ALG9* for further validation at a protein level using immunocytochemistry.

Staining for the ANXA1 protein showed little change with 0.1% galactose treatment in all cell-lines after 2 h, with a slight reduction in staining visible in GHDF cell-line M (Fig. 6A, indicated with arrows).

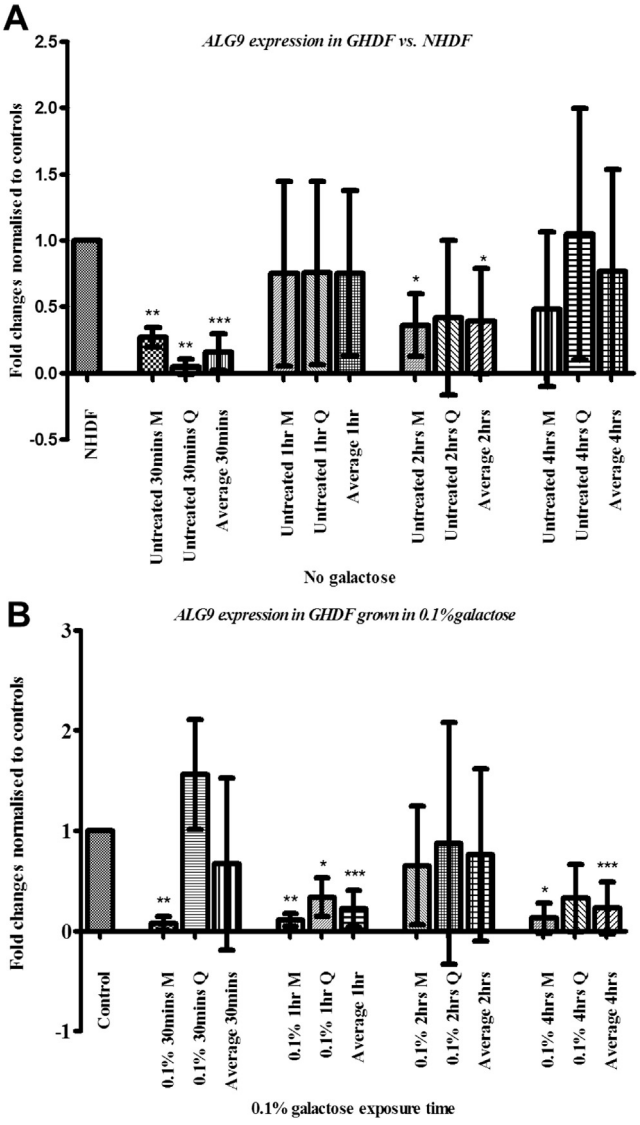

Fig. 5. A and B. *ALG9* expression in (A) GHDF *versus* NHDF, grown in galactose free media and (B) GHDF cells grown in 0.1% galactose. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ $^{****}p < 0.0001$.

After 4 h exposure to 0.1% galactose, there is a minor decrease in GDHF cell-line M (Fig. 6B, indicated with arrows). There is also a slight decrease in staining levels in NDHF visible after 0.1% galactose exposure. There were no changes in the localisation of the protein during 0.1% galactose exposure in any cell-line. However there are some changes in protein localisation in untreated (galactose free media) GDHF cell-lines *versus* untreated NHDF, at 2 h and 4 h, these changes being more pronounced in cells derived from patient M (Figs. 6A and B). The no primary antibody control is shown in Fig. 6C. There are also some decreases in ALG9 protein in cell-line Q, and no specific changes in cell-line M at 1 hour exposure to 0.1% galactose (Fig. 7A, indicated with arrows). There are no visible changes in the localisation of the protein following exposure to 0.1% galactose (Fig. 7A).

At 4 hour exposure to 0.1% galactose (Fig. 7B, indicated with arrows) there is a slight increase in staining for ALG9 in both NHDF and GHDF cell-line Q, while staining levels in cell line M are largely unchanged. Interestingly at both time-points (Figs. 7A and B) the GDHF cell-lines grown in galactose free conditions display altered protein localisation compared to NDHF cells grown in galactose free conditions, with more intense staining present at the cell boundaries. The no primary antibody control is shown in Fig. 7C.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

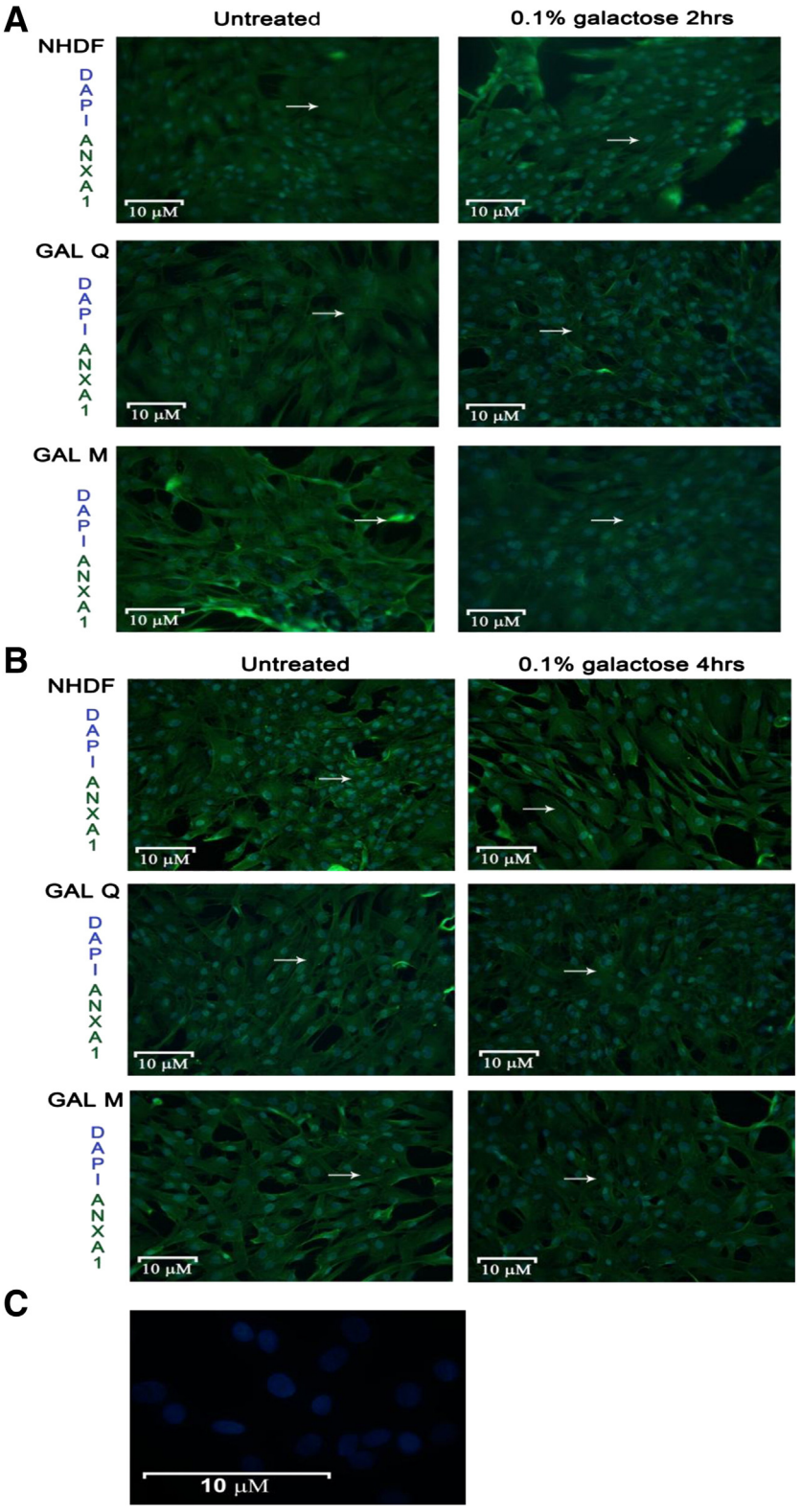

Fig. 6. A–C. ANXA1 protein expression at (A) 2 hour exposure 60× and (B) 4 hour exposure 60×, in NHDF and GHDF grown in galactose-free and 0.1% galactose media; (C) represents NHDF no primary antibody control at 60×. (A) At 2 hour incubation at 60× magnification no change was observed in the localisation of the ANXA1 protein in NHDF and cell-line Q. Reduced staining was observed in cell-line M (indicated with arrows). (B) At 4 hour incubation there was no change in expression of ANXA1 in NHDF or cell-line Q exposed to 0.1% galactose; there is a minor decrease in cell-line M. There was no change in the localisation of the ANXA1 protein in cell-lines exposed to 0.1% galactose (indicated with arrows). There are some changes in localisation in GHDF compared to NHDF, in galactose free media. (C) Shows the no primary antibody control, using representative NHDF cells at 60× magnification. There is no staining for ANXA1 antibody, indicating there is no non-specific binding of the secondary antibody.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

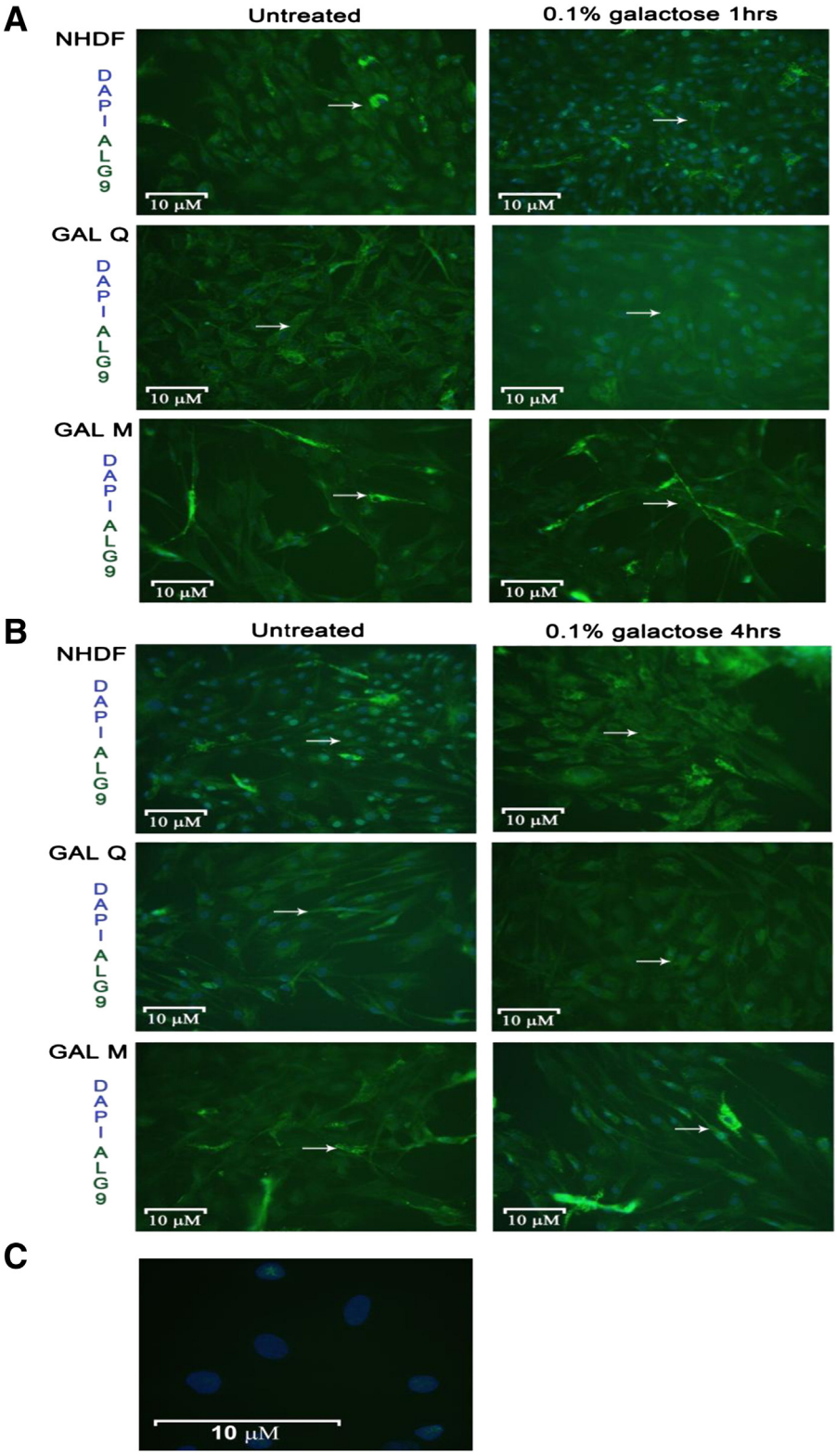

Fig. 7. A–C. ALG9 protein expression at (A) 1 hour exposure 60× and (B) 4 hour exposure 60×, in NHDF and GHDF grown in galactose-free and 0.1% galactose media; (C) represents NHDF no primary antibody control at 60×. (A) At 1 hour incubation decreased expression of ALG9 in NHDF and cell-line Q can be observed during 0.1% galactose exposure with no expression changes observed in cell-line M (indicated with arrows). There are no changes in the localisation of the ALG9 protein in any of the cell-lines *versus* matched cells. (B) At 4 h some increased expression of ALG9 in NHDF and cell-line Q can be seen, while staining is largely unchanged in cell-line M. There is widespread localisation of the ALG9 protein in NHDF cells (0.1% galactose) while localisation of the protein is altered in both GHDF cell-lines (galactose free and 0.1% galactose) with more intense staining present at the cell boundaries. (C) Shows the no primary antibody control, using representative NHDF cells at 60x magnification with absence of staining for ALG9 antibody.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

4. Discussion

The genomic analysis described in this study has demonstrated the dysregulation of numerous systemic and glycosylation related genes in classical Galactosaemia treated individuals. The dysregulation of the genes linked to CDG-I and II disorders suggests that there may be more extensive dysregulation to the *N*-glycosylation pathway in Galactosaemia. It is also possible that pre-natal and neonatal galactose intoxication may result in alteration of epigenetic control on glycosylation pathways, as this pathway is now emerging as a significant modifier [43,44].

A number of key signalling pathways are affected in this disease, findings which we identified in our earlier studies, a number of which may relate to abnormal glycosylation including the UPR pathway and also the involvement of inflammatory pathways and oxidative phosphorylation. Dysregulation of the oxidative phosphorylation pathway has been reported as having a role in the severity of outcome in the *Drosophila melanogaster* Galactosaemia model [45].

Dysregulation of the inositol metabolism pathway has previously been reported in a number of neurological conditions in addition to Galactosaemia [46]. Depletion of inositol can also induce the UPR pathway [47]. While decreased expression of *SLC5A3* was no longer statistically significant following qRT-PCR validation in the current study, the systemic dysregulation of the inositol pathway suggests a potential ongoing role for the long-term disruption of this pathway in the pathophysiology of Galactosaemia.

The dysregulation of inflammatory pathways as presented in these studies is a novel finding in Galactosaemia which may relate to the neurological and reproductive complications. The validated decreased expression of the anti-inflammatory, glycoprotein *ANXA1*, is of interest due to its involvement as a neuroprotective agent in the central nervous system [33] and as it is a potential marker of bovine oocyte maturation [48]. *ANXA1* dysregulation has been linked to a number of cancers, including lung [49], breast [50] and oral cancers [51]. The dysregulation of *ANXA1* in Galactosaemia may indicate a potential risk factor that warrants further investigation.

Decreased expression of *SEPT4* (described in our earlier study [8]) has possible clinical application. The septin family of proteins are involved in interacting with the cytoskeleton to recruit proteins and interacting with the membrane to form discrete domains in the cell [52]. *SEPT4* (along with other septins) is also expressed in the annulus of sperm. *SEPT4* knockout mice have non-motile, structurally defective sperm [53,54]. The expression of septin 12 is localised to the testis and men suffering from hypospermatogenesis were shown to have decreased expression of septin 12 [55]. While offspring generated from murine stem cells that contained a septin 12 mutant gene were infertile with decreased sperm numbers, these findings suggest a role for septin in spermatogenesis [55].

The decreased expression of the galactosyltransferase *B4GALT1* also has a link to male fertility, where it is expressed on sperm cells and involved in capacitation of sperm [56]. The *B4GALT1* knockout mouse is fertile but exhibits an inability to undergo acrosome reaction upon binding the zona pellucida 3 receptors on oocytes; the knockout mouse exhibited a ~7% efficacy for fertilization when compared to wild type sperm owing to poor penetration of the oocyte and these mutant mice had an 85% mortality rate, suffering from endocrine insufficiency [57].

Validation of altered gene expression in patient derived cell-lines treated with 0.1% galactose did not show up-regulation of key ER stress marker *GRP78/BiP* or other ER stress proteins as noted earlier by Slepak et al. [20]. However, this lack of induction of ER stress response could be patient specific as both cell line studies were obtained from patients who did not exhibit complications of Galactosaemia and who were noted to tolerate increased galactose intake during a glycomic study of diet relaxation suggesting the upregulation of accessory galactose metabolism pathways [12].

ALG9 and *ANXA1* were the only genes that were significantly altered in both cell-lines at specific time points during galactose exposure and when grown in galactose-free media and compared to NHDF. There was also altered gene expression patterns in the genes of interest in fibroblasts *versus* T-lymphocytes suggesting that some of the gene expression alterations observed may be cell or tissue dependent.

The changes in ALG9 protein levels and localisation upon exposure to 0.1% galactose in cells may have consequences for glycosylation during galactose intoxication of cells. Localisation of glycosyltransferases is as important during glycosylation as the bioavailability of the required sugar substrates [58]. If galactose intoxication disrupts the localisation of ALG9, even to a minor degree, this in combination with decreased UDP-galactose levels, and the dysregulation of other glycosyltransferases, could have a significant role to play in systemic glycosylation defects. Severe galactose intoxication during the neonatal stage results in gross assembly defects in patients [8,12–15,59], which supports our findings here of a susceptibility of the assembly stage of glycosylation during exposure to galactose.

The abnormal expression of *ALG9* gene, and potential defects in protein level and localisation in cells, may be a marker for individuals at risk of aberrant glycosylation during increased galactose intake. The variation in the protein expression of ALG9 in the two GHDF cell-lines suggests that ALG9 expression may be susceptible to increased galactose exposure in some affected Galactosaemia individuals. The interaction of ALG9 with ALG12 is required for the ultimate formation of the disaccharide glycan, which influences downstream processing of glycans, indicating a potential regulatory role for ALG9 in glycosylation [60]. Our date suggest that *ALG9* may also influence the *N*-glycan processing defects observed in Galactosaemia.

Also of interest is the observation that the ALG9 and ANXA1 proteins in untreated cells show differences in cellular localisation between NHDF and GHDF cells; suggesting fundamental dysregulation in Galactosaemia, independent of the effects of acute galactose toxicity.

As over half of all known eukaryotic proteins are *N*-glycosylated [61], the dysregulation of the glycosylation pathways observed in Galactosaemia likely predicts abnormalities in many gene pathways.

This study presents novel findings demonstrating the dysregulation of the glycosylation and inflammatory pathways at a gene and protein level in Galactosaemia; suggestive of their importance in the enigmatic pathophysiology of this condition. While clearly further studies are required to fully elucidate the exact interplay of gene expression and metabolic factors at play, further extended population gene validation analysis may lead to the discovery of biomarkers for monitoring patients and the development of novel therapeutics.

Conflict of interest

The authors declare that there has been no conflict of interest.

5. Grant support

CFFH, Temple St Childrens University Hospital and the Medical Research Charities Group-Health Research Board (MRCG/2012/2) are acknowledged for the funding to perform these studies.

References

[1] J.L. Fridovich-Keil, J.H. Walter, Galactosaemia chapter 72. The online metabolic and molecular bases of inherited disease, OMMBID, in: D. Valle, B. Vogelstein, K.W. Kinzler, S.E. Antonarakis, A. Ballabio, et al., (Eds.), Part 7: Carbohydrates, McGraw Hill, New York, 2008.
[2] D.D. Waggoner, N.R. Buist, G.N. Donnell, Long-term prognosis in galactosaemia: results of a survey of 350 cases, J. Inherit. Metab. Dis. 13 (6) (1990) 802–818.
[3] S. Schweitzer, et al., Long-term outcome in 134 patients with galactosaemia, Eur. J. Pediatr. 152 (1) (1993) 36–43.
[4] K.P. Coss, et al., Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment, J. Inherit. Metab. Dis. 36 (1) (2013) 21–27.

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004

[5] K. Krabbi, et al., Long-term complications in Estonian galactosemia patients with a less strict lactose-free diet and metabolic control, Mol. Genet. Metab. 103 (3) (2011) 249–253.
[6] J. Hughes, et al., Outcomes of siblings with classical galactosemia, J. Pediatr. 154 (5) (2009) 721–726.
[7] G.M. Komrower, D.H. Lee, Long-term follow-up of galactosaemia, Arch. Dis. Child. 45 (241) (1970) 367–373.
[8] D.J. Coman, et al., Galactosemia, a single gene disorder with epigenetic consequences, Pediatr. Res. 67 (2010) 286–292.
[9] P.P. Jumbo-Lucioni, et al., Diversity of approaches to classic galactosemia around the world: a comparison of diagnosis, intervention, and outcomes, J. Inherit. Metab. Dis. 35 (6) (2012) 1037–1049.
[10] S. Waisbren, et al., The adult galactosemic phenotype, J. Inherit. Metab. Dis. 35 (2) (2012) 279–286.
[11] K. Lai, et al., GALT deficiency causes UDP-hexose deficit in human galactosemic cells, Glycobiology 13 (4) (2003) 285–294.
[12] K.P. Coss, et al., IgG N-glycans as potential biomarkers for determining galactose tolerance in Classical Galactosaemia, Mol. Genet. Metab. 105 (2) (2012) 212–220.
[13] J. Charlwood, et al., Defective galactosylation of serum transferrin in galactosemia, Glycobiology 8 (4) (1998) 351–357.
[14] L. Sturiale, et al., Hypoglycosylation with increased fucosylation and branching of serum transferrin N-glycans in untreated galactosemia, Glycobiology 15 (12) (2005) 1268–1276.
[15] E. Quintana, et al., Screening for congenital disorders of glycosylation (CDG): transferrin HPLC versus isoelectric focusing (IEF), Clin. Biochem. 42 (4–5) (2009) 408–415.
[16] K.P. Coss, C.P. Hawkes, B. Adamczyk, H. Stockmann, E. Crushell, R. Saldova, I. Knerr, M.E. Rubio-Gozalbo, A.A. Monavari, P.M. Rudd, E.P. Treacy, N-glycan abnormalities in children with galactosaemia, J. Proteome Res. 13 (2) (2014) 385–394.
[17] H. Stibler, et al., Carbohydrate-deficient transferrin in galactosaemia, Acta Paediatr. 86 (12) (1997) 1377–1378.
[18] B. Panis, et al., Untreated classical galactosemia patient with mild phenotype, Mol. Genet. Metab. 89 (3) (2006) 277–279.
[19] P.J. Lee, et al., A woman with untreated galactosaemia, Lancet 362 (9382) (2003) 446.
[20] T.I. Slepak, et al., Involvement of endoplasmic reticulum stress in a novel Classic Galactosemia model, Mol. Genet. Metab. 92 (1–2) (2007) 78–87.
[21] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (1) (2009) 44–57.
[22] W. Huang da, W. Huang da, B.T. Sherman, R.A. Lempicki, Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists, Nucleic Acids Res. 37 (1) (2009) 1–13.
[23] Y.S. Shin, Galactose metabolites and disorders of galactose metabolism, in: F.A. Hommes (Ed.), Techniques in Diagnostic Human Biochemical Genetics, Wiley-Liss, New York, 1990, pp. 267–284.
[24] G.T. Berry, Is prenatal myo-inositol deficiency a mechanism of CNS injury in galactosemia? J. Inherit. Metab. Dis. 34 (2) (2011) 345–355.
[25] R. Buccafusca, et al., Characterization of the null murine sodium/myo-inositol cotransporter 1 (Smit1 or Slc5a3) phenotype: myo-inositol rescue is independent of expression of its cognate mitochondrial ribosomal protein subunit 6 (Mrps6) gene and of phosphatidylinositol levels in neonatal brain, Mol. Genet. Metab. 95 (1–2) (2008) 81–95.
[26] C. Nishimura, M.F. Lou, J.H. Kinoshita, Depletion of myo-inositol and amino acids in galactosemic neuropathy, J. Neurochem. 49 (1) (1987) 290–295.
[27] T.K. Koch, et al., Neurologic complications in galactosemia, Pediatr. Neurol. 8 (3) (1992) 217–220.
[28] J.K. Lee, et al., Regulator of G-protein signaling 10 promotes dopaminergic neuron survival via regulation of the microglial inflammatory response, J. Neurosci. 28 (34) (2008) 8517–8528.
[29] C. Lood, et al., Protein synthesis of the pro-inflammatory S100A8/A9 complex in plasmacytoid dendritic cells and cell surface S100A8/A9 on leukocyte subpopulations in systemic lupus erythematosus, Arthritis Res. Ther. 13 (2) (2011) R60.
[30] D. Aydin, S.W. Weyer, U.C. Muller, Functions of the APP gene family in the nervous system: insights from mouse models, Exp. Brain Res. 217 (3–4) (2012) 423–434.
[31] H.J. Lee, K. Lee, H. Im, α-Synuclein modulates neurite outgrowth by interacting with SPTBN1, Biochem. Biophys. Res. Commun. 424 (3) (2012) 497–502.
[32] Z. Dai, et al., Identification and analysis of altered alpha1,6-fucosylated glycoproteins associated with hepatocellular carcinoma metastasis, Proteomics 6 (21) (2006) 5857–5867.
[33] E. Solito, et al., Annexin A1 in the brain—undiscovered roles? Trends Pharmacol. Sci. 29 (3) (2008) 135–142.
[34] S.L. Williams, et al., A proinflammatory role for proteolytically cleaved annexin A1 in neutrophil transendothelial migration, J. Immunol. 185 (5) (2010) 3057–3063.
[35] A.G. Goumenou, et al., The role of leptin in fertility, Eur. J. Obstet. Gynecol. Reprod. Biol. 106 (2) (2003) 118–124.
[36] M.G. Myers Jr., Leptin receptor signaling and the regulation of mammalian physiology, Recent Prog. Horm. Res. 59 (2004) 287–304.
[37] J.A. Matochik, et al., Effect of leptin replacement on brain structure in genetically leptin-deficient adults, J. Clin. Endocrinol. Metab. 90 (5) (2005) 2851–2854.
[38] G.J. Paz-Filho, et al., Leptin replacement improves cognitive development, PLoS One 3 (8) (2008) e3098.
[39] S.J. Greco, et al., Leptin reduces Alzheimer's disease-related tau phosphorylation in neuronal cells, Biochem. Biophys. Res. Commun. 376 (3) (2008) 536–541.
[40] K.F. Holden, et al., Serum leptin level and cognition in the elderly: findings from the Health ABC Study, Neurobiol. Aging 30 (9) (2009) 1483–1489.
[41] I. Knerr, et al., Leptin levels in children and adults with classic galactosaemia, JIMD Rep. 9 (2013) 125–131.
[42] I.D. Pokrovskaya, et al., Conserved oligomeric Golgi complex specifically regulates the maintenance of Golgi glycosylation machinery, Glycobiology 21 (12) (2011) 1554–1569.
[43] V. Zoldos, S. Grgurevie, G. Lauc, Epigenetic regulation of protein glycosylation, BioMolecular Concepts, 2010, p. 253.
[44] V. Zoldos, et al., Genomics and epigenomics of the human glycome, Glycoconj. J. 30 (1) (2013) 41–50.
[45] P.P. Jumbo-Lucioni, et al., Oxidative stress contributes to outcome severity in a *Drosophila melanogaster* model of classic galactosemia, Dis. Model. Mech. 6 (1) (2013) 84–94.
[46] G.T. Berry, et al., In vivo brain myo-inositol levels in children with Down syndrome, J. Pediatr. 135 (1) (1999) 94–97.
[47] R.M. Deranieh, M.L. Greenberg, Cellular consequences of inositol depletion, Biochem. Soc. Trans. 37 (Pt 5) (2009) 1099–1103.
[48] S. Mamo, et al., Sequential analysis of global gene expression profiles in immature and in vitro matured bovine oocytes: potential molecular markers of oocyte maturation, BMC Genomics 12 (2011) 151.
[49] R. Biaoxue, et al., Upregulation of Hsp90-beta and annexin A1 correlates with poor survival and lymphatic metastasis in lung cancer patients, J. Exp. Clin. Cancer Res. 31 (2012) 70.
[50] H. Kang, J. Ko, S.W. Jang, The role of annexin A1 in expression of matrix metalloproteinase-9 and invasion of breast cancer cells, Biochem. Biophys. Res. Commun. 423 (1) (2012) 188–194.
[51] P.C. Faria, et al., Expression of annexin A1 mRNA in peripheral blood from oral squamous cell carcinoma patients, Oral Oncol. 46 (1) (2010) 25–30.
[52] C.S. Weirich, J.P. Erzberger, Y. Barral, The septin family of GTPases: architecture and dynamics, Nat. Rev. Mol. Cell Biol. 9 (6) (2008) 478–489.
[53] M. Ihara, et al., Cortical organization by the septin cytoskeleton is essential for structural and mechanical integrity of mammalian spermatozoa, Dev. Cell 8 (3) (2005) 343–352.
[54] H. Kissel, et al., The Sept4 septin locus is required for sperm terminal differentiation in mice, Dev. Cell 8 (3) (2005) 353–364.
[55] Y.H. Lin, et al., The expression level of septin12 is critical for spermiogenesis, Am. J. Pathol. 174 (5) (2009) 1857–1868.
[56] C. Rodeheffer, B.D. Shur, Sperm from beta1,4-galactosyltransferase I-null mice exhibit precocious capacitation, Development 131 (3) (2004) 491–501.
[57] Q. Lu, B.D. Shur, Sperm from beta 1,4-galactosyltransferase-null mice are refractory to ZP3-induced acrosome reactions and penetrate the zona pellucida poorly, Development 124 (20) (1997) 4121–4131.
[58] G. Lauc, V. Zoldos, Epigenetic regulation of glycosylation could be a mechanism used by complex organisms to compete with microbes on an evolutionary scale, Med. Hypotheses 73 (4) (2009) 510–512.
[59] K. Petry, et al., Characterization of a novel biochemical abnormality in galactosemia: deficiency of glycolipids containing galactose or N-acetylgalactosamine and accumulation of precursors in brain and lymphocytes, Biochem. Med. Metab. Biol. 46 (1) (1991) 93–104.
[60] J.F. Cipollo, R.B. Trimble, The Saccharomyces cerevisiae alg12delta mutant reveals a role for the middle-arm alpha1,2Man- and upper-arm alpha1,2Manalpha1,6Man-residues of Glc3Man9GlcNAc2-PP-Dol in regulating glycoprotein glycan processing in the endoplasmic reticulum and Golgi apparatus, Glycobiology 12 (11) (2002) 749–762.
[61] N. Pivac, et al., Human plasma glycome in attention-deficit hyperactivity disorder and autism spectrum disorders, Mol. Cell. Proteomics 10 (1) (2011) (p. M110 004200).

Please cite this article as: K.P. Coss, et al., Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism?, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.08.004