no comment was made on their development. Opitz[6] coined the term "Golabi-Rosen" syndrome and added the reports of a further three males with similar features, but no prominent overgrowth. Shortly after this, Behmel *et al*[7] reported a five generation family with 13 affected males with features of X linked overgrowth and pointed out the similarities between these cases and the patients described initially by Simpson *et al.*[2] Neri *et al*[8] highlighted the similarities between the above authors' reports and proposed the designation "Simpson-Golabi-Behmel" syndrome to encompass this clinical entity. To date, at least 40 patients have been reported with the syndrome[1 3 9–12] and mutations in GPC3, a glypican gene, have recently been found to cause the condition. GPC3 is an extracellular proteoglycan and is inferred to play a major role in growth control of mesodermal tissues, possibly by modulating the actions of insulin-like growth factor 2.[13]

The two patients reported here both displayed marked neonatal macrosomia and the typical facial features of SGBS. The additional clinical findings of short, broad hands, pectus excavatum chest deformities, and supernumerary nipples, present in both boys, adds further credibility to the diagnosis. The congenital diaphragmatic hernia, reported in case 1, has been previously described in association with SGBS[11] and the inguinal hernias (case 1) are a well established feature of the condition.[9] Similarly, the macroglossia, submucous cleft palate, and single renal cyst, reported in case 2, are all features consistent with the diagnosis of SGBS.[9] The main differential diagnostic consideration in these children was Beckwith-Wiedemann syndrome, given the prenatal macrosomia and macrostomia seen in both boys and macroglossia, seen in case 2. This boy was initially considered to have BWS and the clinical overlap between SGBS and BWS is highlighted by similar examples of diagnostic confusion in published reports.[9] The particular constellation of clinical features in these two male sibs in addition to the presence of supernumerary nipples (a feature not reported in patients with BWS) clearly differentiate them as having SGBS. Another diagnostic possibility, given the coarse facies and behavioural problems present in these cases, was a mucopolysaccharide storage disorder. This diagnosis was excluded in both boys by appropriate urine testing.

Our two cases are distinct from all previously reported cases with SGBS because of their striking behavioural disturbances, not present in their unaffected 4 year old sister. There is relatively little mention of the behavioural phenotype in the published cases of SGBS. In the initial report by Simpson *et al*,[2] one of their cases (case 1, DG) was evaluated psychologically at 30 months of age and reported to have an "attention span of short duration". In the report of Behmel *et al*,[14] passing comment is made of the "severe emotional and behavioural troubles during adolescence" in one of their patients (II.3 in family 2) and "behavioural difficulties during school attendance" (that necessitated psychological treatment) in his nephew. These comments are not further elaborated and comprise the few references to behavioural phenotype in SGBS.

This is the first report of a specific behavioural pattern (ADHD) in patients with SGBS. It raises the question of whether other patients with SGBS are at risk of developing this (or other) neurobehavioural problems. The well recognised neurobehavioural patterns seen in patients with Prader-Willi[15] and velocardiofacial syndromes[16] serve as examples of other multisystemic disorders with clinically significant associated behavioural profiles. If SGBS is associated with a predisposition to specific behavioural disturbances, as indicated by the two cases reported here, it is important that the parents and clinicians who care for these children are aware of this. This knowledge will then allow the opportunity for anticipatory guidance to be provided for these families and for intervention and treatment strategies to be put in place.

The authors wish to thank Ms Lara Fitzgerald for her work in the preparation of this manuscript.

RAVI SAVARIRAYAN
AGNES BANKIER

Victorian Clinical Genetics Service, Royal Children's Hospital, Parkville, Victoria 3052, Australia

1 Hughes-Benzie RM, Hunter AGW, Allanson JE, Mackenzie AE. Simpson-Golabi-Behmel syndrome associated with renal dysplasia and embryonal tumour: localisation of the gene to Xqcen-q21. *Am J Med Genet* 1992;**43**:428-35.
2 Simpson JL, Landey S, New M, German J. A previously unrecognised X-linked syndrome of dysmorphia. *Birth Defects* 1975;**XI**:18-24.
3 Terespolsky D, Farrell SA, Siegel-Bartelt J, Weksberg R. Infantile lethal variant of Simpson-Golabi-Behmel syndrome associated with hydrops fetalis. *Am J Med Genet* 1995;**59**:329-33.
4 Golabi M, Rosen L. A new X-linked mental retardation-overgrowth syndrome. *Am J Med Genet* 1984;**17**:345-58.
5 American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington, DC: American Psychiatric Press, 1994.
6 Opitz JM. The Golabi-Rosen syndrome - report of a second family. *Am J Med Genet* 1984;**17**:359-66.
7 Behmel A, Plöchl E, Rosenkranz W. A new X-linked dysplasia gigantism syndrome: identical with the Simpson dysplasia syndrome? *Hum Genet* 1984;**67**:409-13.
8 Neri G, Marini R, Cappa M, Borrelli P, Opitz JM. Simpson-Golabi-Behmel syndrome: an X-linked encephalo-tropho-schisis syndrome. *Am J Med Genet* 1988;**30**:287-99.
9 Garganta CL, Bodurtha JN. Report of another family with Simpson-Golabi-Behmel syndrome and a review of the literature. *Am J Med Genet* 1992;**44**: 129-35.
10 Gurrieri F, Cappa M, Neri G. Further delineation of the Simpson-Golabi-Behmel (SGB) syndrome. *Am J Med Genet* 1992;**44**:136-7. 11 Chen E, Johnson JP, Cox VA, Golabi M. Simpson-Golabi-Behmel syndrome: congenital diaphragmatic hernia and radiologic findings in two patients and follow up of a previously reported case. *Am J Med Genet* 1993;**46**:574-8.
12 Lapunzina P, Badia I, Galoppo C, *et al.* A patient with Simpson-Golabi-Behmel syndrome and hepatocellular carcinoma. *J Med Genet* 1998;**35**: 153-6.
13 Pilia G, Hughes-Benzie RM, Mackenzie A, *et al.* Mutations in *GPC3*, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nat Genet* 1996;**12**:241-7.
14 Behmel A, Plöchl E, Rosenkranz W. A new X-linked dysplasia gigantism syndrome: follow up in the first family and report on a second Austrian family. *Am J Med Genet* 1988;**30**:275-85.
15 Dykens EM, Cassidy SB. Correlates of maladaptive behaviour in children and adults with Prader-Willi syndrome. *Am J Med Genet* 1995;**60**:546-9.
16 Chow EWC, Bassett AS, Weksberg R. Velo-cardio-facial syndrome and psychotic disorders: implications for psychiatric genetics. *Am J Med Genet* 1994;**54**:107-12.

J Med Genet 1999;**36**:576–578

Presence of a deletion in the 5' upstream region of the GALT gene in Duarte (D2) alleles

EDITOR—Galactosaemia is an autosomal recessively inherited metabolic disorder caused by a defect in the galactose-1-phosphate uridyltransferase (GALT) enzyme. Absence or severe reduction of GALT activity results in classical galactosaemia (G/G) while an approximately half reduction of enzyme activity leads to the Duarte variant of galactosaemia (D/D). Mutation Q188R was found to be the most common molecular defect among classical galactosaemia patients,[1] whereas N314D was predominantly detected in Duarte galactosaemia patients.[2] In recent studies, the Duarte (D2) allele with 50% of normal GALT activity and the Los Angeles (D1) allele with 110-130% of normal GALT activity were characterised as having

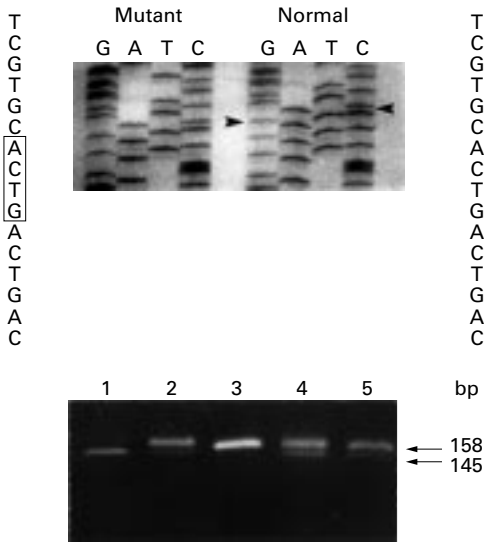

Figure 1 (Above) Sequence analysis of the 5' upstream region of the GALT gene. Partial sequence ladders from the –119del4 homozygote (left) and from a control (right). Arrows indicate mutation site within the sequence ladder. (Below) Restriction enzyme analysis of the –119del4 mutation. The DNA samples were amplified using the primers PROM del4: 5'-CAGGGCAGCCCAGTCACTCA-3' and PROM B: 5'-GCGTTGCTGAGGATCGGTTC-3' followed by digestion by DdeI. Lane 1: homozygous wild type. Lanes 2, 4, 5: heterozygous. Lane 3: homozygous mutant.

nucleotide alterations in addition to N314D.[3] A study of the GALT gene from 31 unrelated galactosaemia families and from 504 control subjects is reported.

Thirty three patients and their relatives from the Czech and Slovak Republics were investigated (100% of all known galactosaemia patients). All families were white. There is no galactosaemia newborn screening in our country. Clinical onset of classical galactosaemia began in all patients in the neonatal period. The patients were identified with typical symptoms of classical galactosaemia (vomiting, failure to thrive, icterus, sepsis, hepatosplenomegaly, cataracts) and diagnosed by erythrocyte GALT assay (residual GALT activity less than 3% of the control value).[4] A total of 504 non-galactosaemic subjects from the Czech Republic, including healthy donors and parents of α-1-antitrypsin deficiency patients, were used as a control group for population screening of the Duarte (D2) and Los Angeles (D1) alleles.

Mutation analysis was performed for the 5' upstream region and the whole coding region with flanking intronic sequences of the GALT gene using PCR/digestion, denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA), and sequencing methods. A total of 11 sequence variations in six mutated alleles was found. The two most common molecular defects were the mutations Q188R (45.2%) and K285N (27.5%). Two novel mutations in the coding region of the GALT gene, Y209S (3.2%) and 2142delGCC (1.6%), were detected; both were associated with severe reduction of enzyme activity. The previously described mutation L195P was found in exon 7 on three mutant alleles (4.8%). An unusual molecular genotype was observed in three classical galactosaemia alleles (4.8%), with six variations from the normal nucleotide sequence presented in cis (V151A, N314D, −119del4, 1105G→C, 1323G→A, and 1391G→A). A novel deletion of four GTCA nucleotides in the 5' promoter region, in a position 119 nucleotides upstream from the initiation codon (−119del4), was found in Duarte (D2) alleles (fig 1, above), in addition to N314D, 1105G→C, 1323G→A, and 1391G→A. The deletion abolished a *Dde*I restriction site in the amplification created restriction site (ACRS) detection system (fig 1, below). In addition, during analysis of the 5' promoter region, a discrepancy was found between published[5] and detected sequences (fig 2).

Using PCR/restriction digestion assay, we screened a sample of 1008 control alleles, obtained from 504 subjects, for N314D plus accompanying intron and exon variations. The control subjects had no clinical symptoms of galactosaemia (biochemically and electrophoretically untested). N314D was found on 82 of 1008 (8.2%) different control alleles examined. From these, 54 (5.4%) were Duarte (D2) alleles contained in cis N314D plus –119del4, 1105G→C, 1323G→A, and 1391G→A. Twenty eight (2.8%) were Los Angeles (D1) alleles carrying in cis mutation N314D plus the silent mutation L218L. From previously obtained results,[2 6] as well as the study presented here, it is evident that the Duarte (D2) and Los Angeles (D1) alleles are widespread among various populations. In both Czech galactosaemic V151A + Duarte (D2) and control Duarte (D2) alleles, the promoter deletion –119del4 and the intronic variations 1105G→C, 1323G→A, and 1391G→A were in linkage disequilibrium with N314D. Based on these results, we assume that the Duarte (D2) allele contains the promoter deletion –119del4 and the three intron substitutions, 1105G→C, 1323G→A, and 1391G→A, together with N314D. N314D appears to be an ancient genetic variant of the GALT gene, a background on which several other sequence variations were created.

The mechanism of partial GALT activity impairment in the Duarte alleles still remains to be fully understood. Two possible explanations have been described. Lai *et al*[7] showed that the N314D mutation reduces the biological stability of the GALT dimeric protein in human lymphoblastoid cell lines; they stated, however, that no nucleotide changes other than N314D had been found in the GALT genes they studied. This finding is, though, in conflict with results obtained from the yeast expression system, in which the N314D subunit dimerises well both with wild type GALT and with itself. The reduced stability was not seen when N314D containing GALT protein was overexpressed via exogenous promoters in yeast.[8] On the other hand, Podskarbi *et al*[9] reported that, besides N314D, Duarte

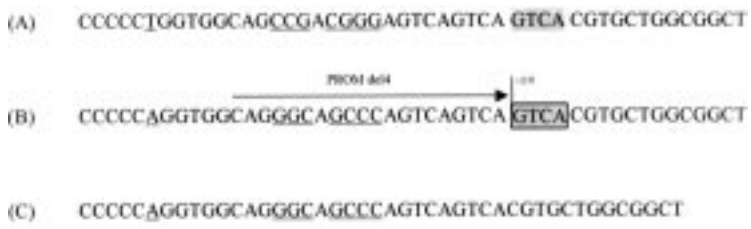

Figure 2 (A-C) Diagram of part of the GALT promoter sequence. Underlined nucleotides were incorrect in originally published sequence.[5] The arrow over the nucleotides in (B) denotes the position of the PROM del4 primer. The shaded box denotes the position of the nucleotides deleted in the Duarte (D2) allele. The number over the grey box denotes the nucleotide position upstream from the initiation codon. (A) Originally published sequence, Leslie et al.[5] (B) Corrected sequence of normal allele. (C) Duarte (D2) allele sequence.

alleles (D2) carry two "intronic mutations", 1105G→C and 1391G→A. They suggested that these intron alterations might be "regulatory mutations" involved in regulation of GALT gene expression.

In the present work, we have described a new DNA alteration on Duarte alleles, the deletion of four nucleotides (GTCA) in the 5' promoter region of the GALT gene (−119del4). There is a high probability that this deletion is located in the transcription factor binding region. For this reason a computer search for potential regulatory DNA elements in the area of the deletion was performed.[10] Two *Homo sapiens* binding factors (activator proteins AP1 Q2 and AP1 Q4), which lose their binding motifs in Duarte (D2) alleles, were found. We conclude that the –119del4 promoter mutation is perhaps the main factor in Duarte allele enzyme activity reduction caused by a decrease in the synthesis of mRNA. This hypothesis will be tested further; however, Shin *et al*[6] reported that in competitive RT-PCR, the RNA level from homozygous Duarte (D2) cultured human lymphocytes was lower than that obtained from control cultured human lymphocytes.

We thank Drs M Andrejková, V Bryšová, V Bzdúch, R Gaillyová, I Grochová, K Hálová, Z Kalina, V Kozich, V Smolka, Šaligová, A Šantavá, M Vasil, and M Vojtíšková for providing clinical data and blood samples from the patients and Erik Piper for critical reading of the manuscript. This study was supported by grant Interní grantová agentura (IGA) MZ No 4376-3 from the Ministry of Health, Czech Republic.

LIBOR KOZÁK
HANA FRANCOVÁ
Research Institute of Child Health, Department of Biochemical and Molecular Genetics, Cernopolní 9, CZ-662 62 Brno, Czech Republic

ANNA PIJÁCKOVÁ
JINDRISKA MACKU
Research Institute of Child Health, Clinical Department, Brno, Czech Republic

SYLVIE STASTNÁ
KAROLÍNA PESKOVOVÁ
OLGA MARTINCOVÁ
JAKUB KRIJT
Institute for Inherited Metabolic Disorders, Prague, Czech Republic

1 Reichardt JKV, Packman S, Woo SLC. Molecular characterization of two galactosemia mutations: correlation of mutations with highly conserved domains in galactose-1-phosphate uridyl transferase. *Am J Hum Genet* 1991;**49**:860-7.
2 Elsas LJ, Dembure PP, Langley S, Paulk EM, Hjelm LN, Fridovich-Keil JL. A common mutation associated with the Duarte galactosemia allele. *Am J Hum Genet* 1994;**54**:1030-6.
3 Greber S, Guldberg P, Scheibenreiter S, Strobl W. Mutations in classical and Duarte 2 galactosemia. *Pediatr Res* 1995;**38**:434.
4 Beutler E, Baluda MC. Improved method for measuring galactose-1-phosphate uridyl transferase activity of erythrocytes. *Clin Chim Acta* 1966; **13**:369-79.
5 Leslie ND, Immerman EB, Flach JE, Florez M, Fridovich-Keil JL, Elsas LJ. The human galactose-1-phosphate uridyl transferase gene. *Genomics* 1992; **14**:474-80.
6 Shin YS, Koch HG, Köhler M, Hoffmann G, Patsoura A, Podskarbi T. Duarte-1 (Los Angeles) and Duarte-2 (Duarte) variants in Germany: two new mutations in the GALT gene which cause a GALT activity decrease by 40-50% of normal in red cells. *J Inherit Metab Dis* 1998;**21**:232-5.
7 Lai K, Sharon D, Langley S, Dembure PP, Hjelm LN, Elsas LJ. Duarte allele impairs biostability of galactose-1-phosphate uridyltransferase in human lymphoblasts. *Hum Mutat* 1998;**11**:28-38.
8 Fridovich-Keil JL, Quimby BB, Wells L, Mazur LA, Elsevier PJ. Characterization of the N314D allele of human galactose-1-phosphate uridyltransferase using a yeast expression system. *Biochem Mol Med* 1996;**56**:121-30.
9 Podskarbi T, Kohlmetz T, Gathof BS, *et al*. Molecular characterization of Duarte-1 and Duarte-2 variants of galactose-1-phosphate uridyltransferase. *J Inherit Metab Dis* 1996;**19**:638-44.
10 Heinemeyer T, Wingender E, Reuter I, *et al.* Databases on transcriptional regulation: TRANSFAC, TRRD, and COMPEL. *Nucleic Acids Res* 1998;**26**:362-7.

J Med Genet 1999;**36**:578–579

Familial congenital diaphragmatic hernia: is an imprinting mechanism involved?

EDITOR—Isolated congenital diaphragmatic hernia (ICDH) may be sporadic or familial. The mode of inheritance in familial cases (IFCDH) is a matter for debate and different patterns have been proposed. For example, multifactorial inheritance was suggested by Wolff[1] and by Norio *et al*.[2] However, autosomal recessive inheritance has been suggested in clinical studies[3–6] and also in animal studies.[7 8] Also, there have been various families reported to date in which other patterns of inheritance are possible (table 1).

If all the published pedigrees with familial CDH are analysed, autosomal dominant, autosomal recessive, and X linked inheritance patterns can be seen. We propose a hypothesis which unifies these various mechanisms, which is to consider imprinting as involved in the inheritance pattern of this condition.

In 1994, two non-consanguineous girls with isolated CDH and balanced translocations involving 8q22.3 were reported by Temple *et al*.[14] More recently, Tokuhara *et al*[15] have cloned the gene HFZ6 and localised it to 8q22.3-q23.1. HFZ6 corresponds to the human frizzled-6 gene and is a member of the family of frizzled genes that encode receptors for Wnt proteins, which are secreted proteins involved in cell-cell interaction during embryonic development and tumorigenesis.[16] Moreover, these frizzled genes are homologous to the *Drosophila frizzled* gene family, a group of homeobox genes determining morphogenesis and planar polarity phenotypes both in *Drosophila* and vertebrates.[17–19]

Frizzled genes have been thought to be related to spatial control during the embryological development of various structures, in particular the heart.[20] Other authors[21–24] and ourselves[25] have found that when CDH is accompanied by other anomalies, these usually involve the heart and CNS, suggesting a developmental association. Frizzled gene deletions have already been associated with other genetic disorders, such as Williams syndrome,[26] in which they could have a role in early brain developmental anomalies.

Table 1 Published isolated familial congenital diaphragmatic hernia (IFCDH) cases

Affected family members	*Possible mendelian inheritance mechanism involved*	*No assigned to the family in our study*	*No in prototype pedigree figures*	*References*
Two brothers and a maternal uncle	Sex linked recessive, autosomal dominant with incomplete penetrance	1,2	1a	9, 10
Two half brothers from the same mother	Sex linked recessive or dominant, autosomal dominant	3	1b	11
Father and daughter	Sex linked dominant, autosomal dominant	4	1c	12
Mother and son	Sex linked dominant or recessive, autosomal dominant	5	1d	13
Various affected family members in only one generation of a consanguineous kindred	Autosomal recessive	6	1e	5