hyperbilirubinemas in the neonatal period, and a family history that suggests an underlying genetic cause. An increasing number of studies have focused on the genetics of bilirubin, these studies have sought to detect genetic mutations that lead to elevated unconjugated serum bilirubin, such as those that affect the molecular structure of $\delta$-diphosphate glucuronosyltransferase (UGT) and those that result in glucose-6-phosphate dehydrogenase (G6PD) deficiency.  

G6PD deficiency is the most common genetic defect around the world, including Turkey. However, few studies have investigated the role of organic anion transporting polypeptide (OATP)-2 in neonatal hyperbilirubinemas. The human OATP family consists of 11 members: OATP1A2, -1B1, -1B3, -1C1, -1A1, -2B1, -3A1, -4A1, -4C1, -5A1, and -6A1. OATPs are encoded by genes of the SLC02 family (previously SLC21). The genes encoding human OATP1 family members are located in the short arm of chromosome 12 and consist of 2903 nucleotides and 14 exons, whereas genes encoding other OATPs are located in chromosomes 3, 5, 8, 11, 15, and 20. The human OATP2 (also known as LST-1 or OATP-C or OATP1B1; the symbol of gene: SLC21A6) showed uptake of monoglucuronosyl bilirubin, bisglucuronosyl bilirubin, and sulfoconjugates. Severe hyperbilirubinemas still occurs in Turkish neonates, and it is important to understand the genetic risk factors for the underlying causes of the disease.

In this study, the role of OATP-2 in neonatal hyperbilirubinemas with unknown etiology was investigated and compared in neonatal hyperbilirubinemas with known etiology. To the best of our knowledge, this is the first study to assess the OATP-2 gene in a case-control analysis of neonatal hyperbilirubinemas in Turkey.

METHODS

The study was approved by the Kocaeli University Faculty of Medicine ethics committee, and written informed consent was obtained from the parents of infants included in the study.

Selection of Study and Control Groups

A total of 207 healthy newborns with gestational ages $\geq 37$ weeks were delivered in the Kocaeli University Hospital during the study period. Sick infants who were transferred to the intensive care unit or sick baby nursery were excluded. Newborns with hyperbilirubinemas who had no problems other than hyperbilirubinemas were selected from either the nursery or the neonatal out-

\[ (5-9) \]

All newborns were observed for hyperbilirubinemas for first week according to the guidelines developed by the American Academy of Pediatrics until stabilization of hyperbilirubinemas. The patients were examined every 48 hours in the first 10 days of life, after which controls were performed weekly for prolonged hyperbilirubinemas until stabilization of the jaundice both as inpatients and outpatients. During follow-up visits, all infants received complete physical examinations, their medical histories were briefly recorded, and transcutaneous bilirubin measurements were taken using a Biliblue device (American Laubscher Corporation, Farmington, NY). If the transcutaneous bilirubin level was over 5 mg/dL, total serum bilirubin was rechecked for confirmation. Nonphysiological hyperbilirubinemas was defined as having $\geq 1$ mg/dL of serum total bilirubin above the 95th percentile value (high-risk zone).

All newborns with hyperbilirubinemas were investigated for the following reasons: congenital anomalies, increased hepatic enzyme levels, congenital hypothyroidism, sepsis, urinary tract infections, insufficient feeding ($> 10\%$ loss of birth weight), dehydration, blood extractions, a history of hypoxia, polycythemia or a metabolic disease, ABO or Rh incompatibility, G6PD enzyme deficiency, or a diabetic mother.

Healthy newborns followed for 4 weeks after birth for nonphysiological hyperbilirubinemas were enrolled as controls.

1. Nonphysiological hyperbilirubinemas of unknown etiology (group I): This group included 65 newborn infants with gestational ages of $\geq 37$ weeks and ages of $< 10$ days. Patients were excluded for the following reasons: congenital anomalies, increased hepatic enzyme levels, congenital hypothyroidism, sepsis, urinary tract infections, insufficient feeding ($> 10\%$ loss of birth weight), dehydration, blood extractions, a history of hypoxia, polycythemia or a metabolic disease, ABO or Rh incompatibility, G6PD enzyme deficiency, prolonged hyperbilirubinemas or insufficient feeding ($> 10\%$ loss of birth weight), dehydration, or a diabetic mother.

2. Nonphysiological hyperbilirubinemas of known etiology with hemolytic or blood extractions (group II): This group included 37 newborns. In this group, the causes of the hyperbilirubinemas were identified as only ABO incompatibility ($n = 24$), only Rh incompatibility ($n = 7$), ABO and Rh incompatibility ($n = 2$), G6PD enzyme deficiency ($n = 2$), cephalhematoma ($n = 1$), or adrenal hemorhage ($n = 1$). Patients were excluded for the following reasons: congenital anomalies, increased hepatic enzyme levels, congenital hypothyroidism, sepsis, urinary tract infections, insufficient feeding ($> 10\%$ loss of birth weight), dehydration, a history of hypoxia, polycythemia or a metabolic disease, or a diabetic mother.
Neonatal Hyperbilirubinemia and Organic Anion Transporting Polypeptide-2 Gene Mutations

Gökhan Bıyıkkałe, M.D., Ph.D., Gülcen Turker, Ph.D., Murat Kasap, M.D., Gürler Akpinar, M.D., Engin Arsoy, M.D., Ayla Günlemez, M.D., Ph.D., and Ayse Gökalp, M.D.

ABSTRACT

The aim of this study was to investigate the genotypic distribution of organic anion transporting polypeptide 2 (OATP-2) gene mutations and the relationship with hyperbilirubinemia of unknown etiology. Polymerase chain reaction, restriction fragment length polymorphism, and agarose gel electrophoresis techniques were used for detection of OATP-2 gene mutations in 155 newborn infants: 37 with unexplained hyperbilirubinemia, 65 with explained hyperbilirubinemia, and 53 without hyperbilirubinemia. In the OATP-2 gene, we identified A→G transitions at nucleotide positions 388 and 411 and observed six polymorphic forms. The 388/411→A→G mutation was the most common form (43%) in subjects with hyperbilirubinemia of unknown etiology. Male sex (odds ratio [OR]: 3.08) and two polymorphic forms of the OATP-2 gene [the 388/411→A→G mutation (OR: 3.6) and the 388→411 mutation (OR: 2.4)] increased the risk of neonatal hyperbilirubinemia. In male infants with the 388→A→G mutation of the OATP-2 gene, the levels of unconjugated bilirubin in plasma were significantly increased compared with those observed in females. The polymorphic forms of 388 nucleotide of the OATP-2 gene were identified as risk factors for hyperbilirubinemia of unknown etiology.

KEYWORDS: Newborn, organic anion transporting polypeptide 2 gene, polymorphism, nonphysiological hyperbilirubinemia

Despite advanced therapeutic measures, the overall morbidity of neonatal jaundice remains high, especially in newborns in developing countries where acute bilirubin encephalopathy is a serious endemic problem. Hyperbilirubinemia is also a major health problem in Turkey, where the incidence of nonphysiological neonatal hyperbilirubinemia varies between 10.5 and 25.3%. However, in half of all cases, no risk factors associated with nonphysiological hyperbilirubinemia can be identified. As the field of molecular medicine progressed over the last decade, increasing attention was focused on the role of genetic factors in the development of severe hyperbilirubinemia and kernicterus. However, the relationship between hyperbilirubinemia and genetic factors remains unclear.

Risk factors for neonatal hyperbilirubinemia include East Asian descent (particularly Japanese, Korean, or Chinese descent), a sibling who was affected by...
3. Control group (group III): This group included 53 healthy full-term newborns. Control subjects had maximum value of total bilirubin levels < 40th percentile (low-risk zone) during the study period. If infants with prolonged or nonphysiological hyperbilirubinemia for 1 month were excluded from the control group. Prolonged hyperbilirubinemia was identified in infants who had serum bilirubin levels of 150 μmol/L (8.8 mg/dL) or more for 30 days. Newborns were observed for jaundice, and concentrations were measured when visible jaundice was noticed, both as inpatients and outpatients, until stabilization of jaundice. Forty-five newborns were excluded from the study and control groups because of prolonged jaundice or other characteristics. Eleven infants with insufficient feeding jaundice were excluded. The following information was recorded for each subject: name, gestational age, birth weight, blood group, direct Coombs test result, reticulocyte count, hemoglobin and peripheral smear results, hepatic function test results, family history, and therapeutic methods (i.e., phototherapy, exchange transfusion, and phenobarbital use).

Total serum bilirubin levels were measured by a spectrophotometric method (B-105 Digital bilirubinometer, Enka, Inc., Japan). G6PD enzyme levels were analyzed quantitatively by a UV kinetic measurement method with Trinity Biotech test kits (NY+5) in the Cobas Mira chemical analyzer. Newborns with G6PD levels less than 4.6 U/g/Hb were considered to have a G6PD deficiency. ABO incompatibility was defined if the mother's blood type was O and her infant's blood type was A or B. Direct Coombs tests were performed by gel centrifugation, a sensitive technique for identifying immunoglobulin G-coated red blood cells.

Genetic Analysis
Blood samples (2 to 3 mL) were collected in tubes containing ethylenediaminetetraacetic acid to investigate the etiology of the hyperbilirubinemia. Genomic DNA was isolated using the ammonium acetate method. DNA samples were kept at 4°C for short-term storage. The 25-μL polymerase chain reaction (PCR) mixtures consisted of 1 × PCR buffer, 0.2 mmol/L of each dNTP, 1.0 mmol/L of each primer, 1.25 mmol/L MgCl2, 1.0 U of Taq polymerase, and 100 ng of genomic DNA. An initial 5-minute denaturation at 94°C was followed by 35 cycles consisting of 30 seconds of denaturation at 94°C, 1 minute of annealing at 55°C, and 1 minute of elongation at 72°C. PCR reactions ended with a 10-minute final elongation at 72°C. PCR products were analyzed with agarose or polyacrylamide gel electrophoresis. PCR products were cleaned with a PCR purification kit (Qiagen+5) and sequenced when necessary (Iontek Inc., Istanbul, Turkey).

For OATP2, the sense and antisense primers used were 5’-ATAATGGTGCAATAAAGGCGG-3’ and 5’-ACTATCTTGATGCTCTA-3’, respectively. The OATP2 PCR products were cleaved with TaqI under recommended conditions ( Fermentas Inc+8). The cleavage products were analyzed by polyacrylamide gel electrophoresis, and the bands were visualized with silver nitrate staining. The final fragment size was 214 bp.

RESULTS
The descriptive characteristics of the three groups are summarized in Table 1. Differences in birth weight and gestational age among the three groups were not statistically significant. Turkey’s post hoc statistical analysis showed that there were significantly more males than females in group I (p < 0.0001; Table 1). The total serum bilirubin levels in group II were statistically higher than those in group I (p < 0.023). There was not a statistically significant difference in the use of phototherapy treatments between group I and group II (p = 0.4). Exchange transfusion was not performed on any members of group

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptive Characteristics of Subjects with Nonphysiological Hyperbilirubinemia</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group I (n = 65)</td>
</tr>
<tr>
<td>Sex</td>
<td>.crypto-</td>
</tr>
<tr>
<td></td>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Birth weight (g) (mean ± standard deviation)</td>
<td>3156 ± 569</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38 ± 1.7</td>
</tr>
<tr>
<td>Peak total bilirubin level (mg/dL)</td>
<td>21 ± 4.1</td>
</tr>
<tr>
<td>G6PD</td>
<td>0</td>
</tr>
<tr>
<td>Phototherapy, n (%)</td>
<td>60 (92)</td>
</tr>
<tr>
<td>Exchange transfusion, n (%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Chi-square tests were used for statistical analysis.
†Cusak-Wallis.
‡Kruskal-Wallis.
Group I, subjects with nonphysiological hyperbilirubinemia of unknown etiology; group II, subjects with known etiology; group III, control group.
Table 2: The Distribution of OATP-2 Gene Mutations in the Three Groups of Newborns

<table>
<thead>
<tr>
<th>OATP-2 Gene AG Mutations</th>
<th>388–388 Mutation, n (%)</th>
<th>388–388/411 Mutation, n (%)</th>
<th>388–411 Mutation, n (%)</th>
<th>388–388/411 Mutation, n (%)</th>
<th>388–411 Mutation, n (%)</th>
<th>411–411 AG Mutation, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1 (1.5)</td>
<td>3 (4.6)</td>
<td>15 (23.0)</td>
<td>4 (6.1)</td>
<td>28 (43.0)</td>
<td>14 (21.5)</td>
</tr>
<tr>
<td>Group II</td>
<td>1 (2.7)</td>
<td>3 (8.1)</td>
<td>5 (13.5)</td>
<td>4 (10.8)</td>
<td>8 (21.6)</td>
<td>16 (43.2)</td>
</tr>
<tr>
<td>Group III</td>
<td>2 (3.7)</td>
<td>4 (7.5)</td>
<td>10 (18.8)</td>
<td>6 (11.3)</td>
<td>14 (26.4)</td>
<td>17 (32.0)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (2.5)</td>
<td>10 (6.4)</td>
<td>30 (19.3)</td>
<td>14 (6.0)</td>
<td>50 (32.2)</td>
<td>47 (30.3)</td>
</tr>
</tbody>
</table>

Group I, subjects with nonphysiological hyperbilirubinemia of unknown etiology; group II, subjects with nonphysiological hyperbilirubinemia of known etiology; group III, control group. OATP-2, organic anion transporter 2.

I, although three female newborns in group II received exchange transfusion. These three newborns who received exchange transfusion were diagnosed with ABO incompatibility. Exchange transfusion was performed because their total bilirubin levels were 2 mg/dL above the threshold for exchange transfusion. The difference in the exchange transfusion rates of groups I and II was not significant (p = 0.13; Table 2).

OATP-2 Polymorphisms

A—G single nucleotide transitions were observed at nucleotide positions 388 and 411 of the OATP-2 gene; these changes created cut sites for TagI (Fig. 1). If a transition occurred at nucleotide position 388 but not at nucleotide position 411, two fragments of 128 and 86 bp were expected to be generated. If a transition occurred at nucleotide position 411 but not at nucleotide position 388, two fragments of 151 and 63 bp were expected to be generated. If transitions occurred at nucleotide positions 388 and 411, three fragments of 128, 86, and 63 bp were expected to be generated (Fig. 1A). Analysis of the polycrylamide gels confirmed these different DNA fragmentation patterns for the OATP-2 gene as six polymorphic forms (Fig. 1B).

The six different DNA fragmentation patterns were as follows: only homozygous 388 (388–388) A—G mutation; homozygous 388 and heterozygous 411 (388–388/411) A—G mutation; homozygous 388 and 411 (388–388/411–411) A—G mutation; heterozygous 388 and 411 (388/411) A—G mutation; heterozygous 388 and homozygous 411 (388/411–411) A—G mutation; and homozygous 411 (411–411) A—G mutations (Figs. 1A and 1B). In group I, DNA fragmentation patterns showed that the 388–411–411 mutation of the OATP-2 gene was most common; in groups II and III, the homozygous 411 (411–411) A—G mutation of the OATP-2 gene was most common (Table 2). The 388 A—G mutation of the OATP-2 gene significantly increased both female and male infants (approximately threefold) but in infants with this mutation, total bilirubin levels significantly increased in male compared with female (as median; female: 16.1 mg/dL, male: 19.1 mg/dL, p = 0.02).

We used forward stepwise logistic regression analysis to determine the risk factors for nonphysiological hyperbilirubinemia of unknown etiology. We entered birth weight, the six different polymorphic forms of the OATP-2 gene, sex, and G6PD enzyme deficiency as risk factors into the logistic regression program. Only the 388/411–411 and the 388–411 A—G mutations of the OATP-2 gene as well as sex were identified as risk factors for hyperbilirubinemia of unknown etiology (Table 3). Birth weight and other observed polymorphic forms of OATP-2 were not identified as risk factors for neonatal hyperbilirubinemia of unknown etiology (Table 3).

Male sex increased the risk of neonatal nonphysiological hyperbilirubinemia of unknown etiology by a factor of 3.08 (odds ratio [OR]), the 388/411–411 A—G mutation of the OATP-2 gene increased the risk by a factor of 3.6 (OR), and the 388–411 A—G mutation of the gene increased the risk by a factor of 2.4 (OR).

DISCUSSION

Despite the extensive use of phototherapy, exchange transfusion, and maternal anti-D immunoglobulin (Rhogam®), kernicterus still occurs; these cases highlight the need for continued study of the etiology of hyperbilirubinemia. Neither hyperbilirubinemia nor kernicterus are reportable diseases, and there are no reliable sources of information providing national annual estimates. The primary risk factors include blood group incompatibility, maternal factors (such as diabetes or drug use), birth trauma, breast-feeding, weight loss, premature birth, polycythemia, and ethnicity. However, these risk factors are present in only ~50% newborns with nonphysiological hyperbilirubinemia, thus further investigations are required to establish the causes of this condition. Nonphysiological hyperbilirubinemia is more evident and frequent in children from Eastern Mediterranean and Asian groups than in those from black and white ethnic groups. Nonphysiological hyperbilirubinemia with unknown etiology among Turkish newborns is prevalent, suggestive of genetic risk in this population. In our country, G6PD
Figure 1 The bands for the polymorphic forms of the organic anion transporting polypeptide 2 (OATP-2) gene after TaqI digestion were detected on a polyacrylamide gel. (A) In silico analysis of expected fragmentation patterns for the polymorphic forms of OATP-2 gene after TaqI digestion. (B) A silver-stained polyacrylamide gel showing the observed polymorphic band patterns. The polymorphic form 1/1 is a homozygotic form in which the OATP-2 gene was cut at base number 388. The polymorphic form 1/2 is a heterozygotic form with a cut site at base 388 on both alleles and at base 411 on one allele. The polymorphic form 1/3 is a heterozygotic form with a cut site at base 388 on one allele and at base 411 on the other allele. The polymorphic form 2/2 is a homozygotic form with cut sites at bases 388 and 411 on both alleles. The polymorphic form 2/3 is a heterozygotic form with cut sites at bases 388 and 411 on one allele and at base 411 on the other allele. The polymorphic form 3/3 is a homozygotic form with a cut site at base 411 on both alleles. The 23-bp band was not within the resolution limits of the polyacrylamide gel used in this study.

Table 3 Risk Factors for Nonphysiological Hyperbilirubinemia of Unknown Etiology

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP-2 388/411-411 mutation</td>
<td>1.28</td>
<td>3.8 (1.63–7.96)</td>
<td>0.0014</td>
</tr>
<tr>
<td>OATP-2 388-411 mutation</td>
<td>0.00</td>
<td>2.4 (1.009–6.16)</td>
<td>0.048</td>
</tr>
<tr>
<td>Gender</td>
<td>1.1</td>
<td>3.08 (1.53–6.10)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Constant</td>
<td>-2.64</td>
<td>0.0006</td>
<td></td>
</tr>
</tbody>
</table>

Constant: nonphysiological hyperbilirubinemia of unknown etiology. Other variables were glucuronosyl transferase enzyme deficiency, other 388-388 A→G mutation, 388-388/411-411 A→G mutation, 388/411 A→G mutation, and 411-411 A→G mutation. OATP-2, organic anion transporter 2; B, estimate of the change in the dependent variable that can be attributed to a change of one unit in the independent variable; CI, confidence interval; OR, odds ratio.

deficiency and UGT1A1 gene polymorphisms have been investigated in many studies. To date, however, no study has investigated mutations in OATP-2. This study also investigated the role of OATP-2 gene polymorphisms in neonatal hyperbilirubinemia.

G6PD deficiency and mutation is another genetic risk factor causing neonatal hyperbilirubinemia. In this study, G6PD deficiency was present in 2% of newborns with hyperbilirubinemia. There has been no more information about the ratio of G6PD deficiency in Kocaeli region. But alternatively, several studies have examined these mutations in another region of Turkey. Atay et al. found that the frequency of G6PD deficiency in subjects with increased indirect bilirubin levels was 3.8%, and Kocabay et al. found that this frequency varied from 1
to 5%. Keskin et al reported the frequency of G6PD deficiency as 1.2% in their subjects, and the frequency of the 563 C-T mutations in the G6PD Mediterranean gene was 79% in their patients. False-negative results with biochemical methods may have been obtained by these investigators due to their methods, which were designed for diagnosis of G6PD deficiency. Or the cutoff point can be set by following the appropriate instructions and by trial and error in the individual diagnostic laboratory. Luzatto and Poggio reported that ideally, every patient found to be G6PD deficient by screening should be confirmed by the spectroscopic assay. But we did not confirm by the spectroscopic assay. Neonatal hyperbilirubinemia is more common in male infants. Our results confirm the findings of previous studies in Turkey, which indicated that 61.1% of newborns with nonphysiological hyperbilirubinemia of unknown etiology and 51.3% of newborns with nonphysiological hyperbilirubinemia of known etiology are male. Heterozygotes have been shown to have a high incidence of hyperbilirubinemia and even kernicterus. Male gender may be considered a risk factor for neonatal hyperbilirubinemia due to the false-negativity of G6PD deficiency. We believe that during the hyperbilirubinemia period, enzyme levels studied with biochemical methods may give false-negative results, and tests should thus be repeated. Based on these results, we conclude that G6PD deficiency mutations must be studied in the Turkish population. Polymorphisms in the UGT1A1 gene may also be involved in neonatal hyperbilirubinemia. The UGT1A1 gene, located on chromosome 2 (2q37), is composed of 1,599 nucleotides. The A[T]A:TAA variant, resulting from a mutation in the promoter region of the UGT1A1 gene, is less common in Asians than in whites. The A[T]A:TAA variant is observed at a rate between 10% and 16.8% in Japanese, 15.2% in Chinese, 18.8% in Malaysian, and 14.3% in Taiwanese subjects; its rate is higher in whites (between 35.7% and 41.5%) and Indians (35.1%). In Turkey, Kılıc et al reported a homozygotic A[T]A:TAA frequency of 24.3% in the UGT1A1 gene and a heterozygotic A[T]A:TAA frequency of 10%. Other Turkish researchers have reported values of other polymorphisms in the UGT1A1 gene between 5% and 56%. But we did not study any mutation in the UGT1A1 gene; therefore, we have no knowledge about the role of this gene in our population. Mutations have been identified at nucleotides 388, 463, 521, and 1463 of the OATP2 (SLC21A6) gene located in the short arm of chromosome 12. In previous studies, only the G-A base variation (D131N) at nucleotide 388 was shown to significantly increase the risk of nonphysiological unconjugated hyperbilirubinemia. But Wong et al found that variants of OATP2 gene at nucleotide 388 were not significant risk factors associated with severe unconjugated hyperbilirubinemia in Malaysian Chinese infants. High-affinity uptake of unconjugated bilirubin by OATP2 occurred in the presence of albumin. In vitro, OATP2 has been shown to transport both unconjugated and conjugated bilirubin. Also Cui et al showed that bilirubin bound to albumin is taken up across the basolateral membrane by OATP2 and conjugated in the hepatocyte by the UGT1A1, resulting in monoglucuronosyl bilirubin and diglucuronosyl bilirubin. Bilirubin glucuronides are finally excreted into bile by the apical conjugate export pump multidrug resistance protein 2 localized to the hepatocyte canalicular (apical) membrane. The differentiation between carrier-mediated and diffusion bilirubin uptake into the liver will be supported by the identification of mutations in the OATP2 (SLC21A6) gene leading to the loss or functional impairment of OATP2 in the basolateral membrane of hepatocytes. Van de Steeg et al found marked conjugated hyperbilirubinemia in mice with the mutation of OATP genes. OATP transporters play an essential role in hepatic reuptake of conjugated bilirubin and uptake of unconjugated bile acids and drugs. Therefore, van de Steeg et al hypothesized that substantial sinusoidal secretion and subsequent OATP transporter-mediated reuptake of glucuronidated compounds could occur in hepatocytes under physiological conditions. However, in mice, only the OATP2 and OATP8 subfamilies are controlled by this gene, but in humans, only one member of OATP2 subfamily is controlled by this gene. Therefore, we may suggest that the cause of marked conjugated bilirubin may be due to structural abnormality in all five members of OATP2 and OATP8 subfamily in mice. Also we found that in mice with the 388 A-G mutation of OATP2, unconjugated bilirubin in plasma was significantly increased compared with females who have the same mutation. van de Steeg et al found that in male mice with OATP mutation, unconjugated bilirubin in plasma was significantly increased, compared with female mice as in our study. Therefore, we may suggest that this mutation may affect unconjugated bilirubin levels in male. Maybe the reason for increased the risk of neonatal hyperbilirubinemia in males is the clinical presentation of OATP2 gene mutation in males as the clinical presentation of G6PD gene mutation. Moreover, in view of the fact that current knowledge of the human OATP family is not complete, additional transport proteins may further contribute to the selective uptake of bilirubin from the blood circulation into liver as suggested by Cui et al and Van de Steeg et al.

In the present study, we used the TaqI restriction enzyme to obtain three PCR fragments and six polymorphic forms of the OATP2 gene (Fig. 1). Although Huang et al showed that the 411 A-G restriction site was not polymorphic, we have demonstrated that this
site carries polymorphic properties. The 388/411–411 mutation of the OATP-2 gene was most common among newborns with hyperbilirubinemia of unknown etiology. The homozygous 411 A→G mutation of the OATP-2 gene occurred in 43.2% of newborns with jaundice of unknown etiology. In addition to male sex, the 388/411–411 and 388–411 mutation forms of the OATP-2 gene were statistically identified as risk factors for hyperbilirubinemia of unknown etiology. Male sex increased the risk of neonatal hyperbilirubinemia by a factor of 3.08, the 388/411–411 mutation of the OATP-2 gene increased the risk by a factor of 3.6, and the 388–411 mutation of the OATP-2 gene increased the risk by a factor of 2.4.

CONCLUSION
Male sex and two polymorphic forms of the OATP-2 gene (388/411–411 and 388–411) increased the risk of neonatal nonphysiological hyperbilirubinemia. In males with the 388 A→G mutation of OATP-2 gene, unconjugated bilirubin in plasma was significantly increased compared with females. Therefore, we suggest that this mutation may affect unconjugated bilirubin levels in males. Further studies are needed to better understand the genetics of jaundice and how to avoid the sequel associated with neonatal nonphysiological hyperbilirubinemia.

REFERENCES


Neonatal Hyperbilirubinemia and Organic Anion Transporting Polypeptide-2 Gene Mutations

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