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Developmental, Genetic, Dietary, and Xenobiotic Influences on Neonatal Hyperbilirubinemia

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Running title: Advance and Prospect of Bilirubin-Induced Neurotoxicity

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Abbreviations:

UGT1A1: UDP-glucuronosyltransferase 1A1; BIND: bilirubin-induced neurologic dysfunction; TSB: total serum bilirubin; UCB: unconjugated bilirubin; CNS1: Crigler-Najjar syndrome type 1; CN syndrome: Crigler-Najjar syndrome; P-gp: P-glycoprotein; PXR: pregnane X receptor; CAR: constitutive androstane receptor; PPARα: peroxisome proliferator activated receptor alpha; PBREM: phenobarbital responsive element; aryl hydrocarbon receptor: Ah receptor; nuclear factor erythroid 2 related factor: Nrf2

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ABSTRACT

Hyperbilirubinemia, caused by the accumulation of unconjugated bilirubin, is one of the most common clinical diagnoses in both premature and term newborns. Owing to the fact that bilirubin is metabolized solely through glucuronidation by UDP-glucuronosyltransferase (UGT) 1A1, it is now known that immaturity of UGT1A1 in combination with overproduction of bilirubin during the developmental stage acts as a bottleneck to bilirubin elimination and predisposes the infant to high TBS levels. While neonatal jaundice is mostly benign, excessively high levels of serum bilirubin in a small percentage of newborns can cause bilirubin-induced neurologic dysfunction (BIND), potentially leading to permanent brain damage, a condition known as kernicterus. While a large portion of hyperbilirubinemia cases in newborns are associated with hemolytic diseases, we emphasize here the impaired ability of UGT1A1 to eliminate bilirubin that contributes to hyperbilirubinemia-induced neurotoxicity in the developmental stage. As a series of hereditary UGT1A1 mutations have been identified that are associated with UGT1A1 deficiency, new evidence has verified that delayed expression of UGT1A1 during early stages of neonatal development is a tightly controlled event, involving coordinated intrahepatic and extrahepatic regulation. This review recapitulates the progress that has been made in recent years in understanding etiology and physiopathology of severe hyperbilirubinemia, investigating molecular mechanisms underlying bilirubin-induced encephalopathy, and searching for potential therapies for treating pathologic hyperbilirubinemia. Several animal models have been developed to make it possible to examine bilirubin-induced neurotoxicity from multiple directions.

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Moreover, environmental factors that may alleviate or worsen the condition of hyperbilirubinemia are discussed.

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Introduction

Newborn hyperbilirubinemia, characterized by elevated levels of total serum bilirubin (TSB) manifesting as jaundice, is one of the most common clinical diagnoses in neonates, especially in preterm babies. Hyperbilirubinemia in neonates is caused by elevated levels of bilirubin resulting from increased erythrocyte turnover immediately after birth and a delayed expression of hepatic UDP-glucuronosyltransferase 1A1 (UGT1A1) (**Figure 1**), the sole enzyme responsible for bilirubin elimination through glucuronidation conjugation (Burchell et al. 1989; Fujiwara et al. 2015). More than 60% of otherwise healthy newborns develop hyperbilirubinemia during the first week of life with a majority of them experiencing temporary, physiological jaundice that has benign outcomes. A small portion of neonates, however, suffer dangerously high levels of unconjugated bilirubin (UCB) that are associated with bilirubin-induced neurologic dysfunction (BIND) (Bhutani and Johnson-Hamerman 2015). For example, newborns with Crigler-Najjar syndrome type I (CNS1) inheriting a severe form of UGT1A1 deficiency manifest with critical nonhemolytic icterus within the first few days of life. Phototherapy is the most common treatment to reduce bilirubin levels in these infants, otherwise they may succumb to kernicterus, an irreversible condition with an anatomic characteristic of yellow staining in certain regions of the brain, particularly the basal ganglia, hippocampus, cerebellum, and nuclei of the floor of the fourth ventricle (Shapiro, Bhutani, and Johnson 2006; Bhutani and Johnson-Hamerman 2015), exhibiting encephalopathy with symptoms of lethargy, ocular muscle paralysis, high-pitched crying, dystonia, seizures, mental retardation, and even death. (Bhutani et al. 2013) (Crigler and Najjar 1952). To date, liver transplantation is deemed the only long-lasting therapeutic

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alternative to cure severe forms of hyperbilirubinemia, including CNS1, before the occurrence of neurological damage (Schauer et al. 2003).

In preterm infants with less than 30 weeks of gestational age, the incidence of kernicterus is about 1.8 per 1000 births (Morioka et al. 2015), and it is estimated that the current risk of chronic kernicterus is about one in seven infants with TSB levels >30 mg/dL (513 μM) (Bhutani and Johnson 2009). However, in many parts of the world, especially in low-income countries, these ratios would underestimate the incidence of kernicterus because of major risk factors that induce hemolysis such as Rhesus disease and glucose-6-phosphate dehydrogenase deficiency. South Asia and Sub-Saharan Africa have the highest incidence with prevalence estimated at 3.9 per 1000 live births (Bhutani et al. 2013; Chime, Egenede, and Arute 2011), and recent global estimates found the prevalence of extreme hyperbilirubinemia (>25 mg/dL) being 4%, 32%, and 39% in Latin America, sub-Saharan Africa, and South Asia, respectively. At a country level, Nigeria was reported to have hyperbilirubinemia cases accounting for over 35% of all hospital admissions, with 9% of the newborns having developed kernicterus (Chime, Egenede, and Arute 2011). The inability to clear bilirubin in cases of rapid accumulation in the early phases of the neonatal window result from developmental delay or repression in expression of UGT1A1. We predict that improved understanding of the mechanisms behind this delay would become useful as a target to develop treatment approaches that would accelerate bilirubin clearance and eliminate the prospects of neurological toxicity.

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We document in this review that the UGT1A1 activity is influenced by genetic polymorphism and is regulated at the transcriptional level through a number of mechanisms during the developmental stage; in addition to genetic elements of the UGT1A1 gene, we focus on the role of environmental factors in modulating UGT1A1 bilirubin conjugation capacity. Emphasis is also placed on recent data obtained from novel animal models that delineate cellular and molecular events occurring in the brain in response to bilirubin neurotoxicity. Finally, new evidence suggesting that bilirubin metabolism is accomplished by both hepatic and extrahepatic UGT1A1 activities is presented.

UDP-glucuronosyltransferase 1A1 is the primary enzyme involving bilirubin encephalopathy

Under the normal physiological conditions, bilirubin is poorly water-soluble and is therefore required to be metabolized to allow its disposition and excretion. UGTs are a family of member-bound enzymes that catalyze the conjugation of a wide array of xenobiotics and endogenous substrates with glucuronic acid (Tukey and Strassburg 2000). Of all isoforms, only UGT1A1 has physiological relevance to metabolize bilirubin (Bosma et al. 1994), which is the rate limiting step for bilirubin biliary excretion and detoxification. Clinical data and animal experiments support the fact that regardless of the factors contributing to hyperbilirubinemia, bilirubin detoxification is predominantly determined by regulatory events that control expression of the *UGT1A1* gene.

(1). Inherited mutations of the *UGT1A1* gene and other contributing factors to hyperbilirubinemia: Congenital inborn errors of the *UGT1A1* gene are associated with

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altered UGT1A1 expression and thereby reduce or completely abolish bilirubin conjugating activity. Over 40 inherited mutations in the *UGT1A1* gene are associated with hyperbilirubinemia, and the degree of deficiency of UGT1A1 activity primarily determines the severity of hyperbilirubinemia and encephalopathy (Tukey and Strassburg 2000). Gilbert syndrome is a mild form of *UGT1A1* genetic polymorphism that results in a slight reduction in UGT1A1 activity (Kadakol et al. 2000) (Strassburg 2008), whereas Crigler-Najjar (CN) syndrome exhibits complete abolishment (type I) or severe reduction of UGT1A1 (type II) (Ciotti et al. 1997). A few key mutations in the coding region and the promoter region of the *UGT1A1* gene have been discovered in CN patients that are correlated with reduction or elimination of UGT1A1 activity (Kadakol et al. 2000; Fujiwara et al. 2015). Clinical data showed that untreated babies with CN type I rapidly develop high plasma levels of UCB (20-50 mg/dL), exposing them to the possibility of serious neurological damage.

Mild forms of UGT1A1 mutations result in benign jaundice; however, when coupling other genetically determined traits, severe hyperbilirubinemia may take place. For example, babies who have hemolytic conditions caused by glucose-6-phosphate dehydrogenase deficiency and Rhesus disease may be predisposed to severe hyperbilirubinemia (Huang et al. 2005) (Bhutani et al. 2013). Expression of P-glycoprotein (P-gp) in the brain has also been reported to be associated with bilirubin neurotoxicity. P-gp is expressed abundantly in brain capillary endothelial cells and astrocytes of the blood-brain barriers and has the ability to transport bilirubin out of the brain across the blood-brain-barrier by acting as a membrane efflux pump (Watchko, Daood, and Hansen 1998; Watchko et al. 2001).

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Compared with wild type mice, Mdr1a (P-gp encoding gene) null mice had a higher brain bilirubin content, possibly by enhanced brain bilirubin influx, implying that Pgp expression in the blood-barrier plays a role in protecting the CNS against bilirubin neurotoxicity (Watchko, Daood, and Hansen 1998; Watchko et al. 2001). In addition to the aforementioned genetic factors, prematurity, concurrent illness, and interventions that impede bilirubin-albumin binding are also considered to be risk factors for severe hyperbilirubinemia (Bhutani and Johnson 2009).

(2). Experimental models established for studying neonatal hyperbilirubinemia and regulation of UGT1A1: BIND is characterized by a wide range of neurological deficits, and the underlying molecular mechanisms are only starting to emerge as a number of animal models producing the neonatal hyperbilirubinemia condition have been developed in the past few years. The majority of hyperbilirubinemia animal models harbor *UGT1A1* mutations that occur naturally or are a result of genetic manipulations. **1) Gunn rats:** Gunn (Gunn 1938) discovered that mutant Wistar rats carrying a premature stop codon in the *Ugt1a1* gene and exhibiting a very low UGT1A1 activity developed jaundice. These spontaneously jaundiced rats, termed Gunn rats, are deemed to be the first hyperbilirubinemia animal model, which mimics the condition of CN syndrome type 1. Since then, many studies have employed Gunn rats in combination with administering sulfadimethoxine (displacing unconjugated bilirubin from albumin) or phenylhydrazine (inducing hemolysis) to examine acute bilirubin encephalopathy (Shapiro 1988; Rice and Shapiro 2008). **2) *hUGT1A1*28* mice**: To examine the role the human *UGT1A1* gene in bilirubin metabolism, mice were humanized with the *UGT1* locus encoding nine (-1A1, -

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1A3, -1A4, -1A5, -1A6, -1A7, -1A8, -1A9, and -1A10) functional UGT1A proteins. The generation of humanized *UGT1* mice was accomplished in a few steps: A transgenic mouse line carrying the entire human *UGT1* locus was first generated (Chen et al. 2005); the murine *Ugt1* locus was inactivated by inserting a mutation into exon 4 to inactivate the nine mouse UGT1A proteins (Nguyen et al. 2008); and eventually *Tg(UGT1) Ugt1*$^{-/-}$ mice were generated by crossing heterozygous *Ugt1*$^{+/-}$ mice with *TgUGT1* mice (Fujiwara et al. 2010). In conjunction with the fast breakdown of erythrocytes immediately after birth, these mice – carrying the genetic polymorphism of the $(TA)_7$ dinucleotide repeat in the TATAA box promoter element of the *UGT1A1* gene (termed h*UGT1A1*28* mice) – all developed neonatal hyperbilirubinemia with TSB peak levels exceeding 10 mg/dL. Approximately 10% of h*UGT1A1*28* newborns experienced acute encephalopathy with TSB levels >17 mg/dL at 7-12 days after birth, imitating clinical kernicterus in newborn infants (**Figure 2**). **3) *UGT1*$^{F/F}$ mice**: Cre-Lox recombination sites have been positioned around common exons 2 and 3 of the *Ugt1a1* gene. Breeding these mice with transgenic mice expressing tissue specific Cre recombinase allows investigators to examine the tissue specific impact of the *Ugt1a1* gene and the other genes encoded by the *Ugt1* locus. While the liver has been assumed to be the primary target of neonatal bilirubin clearance by Ugt1a1, direct deletion of the *Ugt1* locus in liver (*Ugt1*$^{\Delta Hep}$) led to only modest levels of hyperbilirubinemia (Chen et al. 2013) (**Figure 3**). Clearly, other tissues, such as the GI track, are capable of participating in the clearance of bilirubin during the neonatal window. When both the liver and intestinal epithelial cells of the GI track are targeted for deletion of the *Ugt1* locus, *Ugt1*$^{\Delta IE/Hep}$ mice develop high levels of TSB that are maintained in adult mice. **4) *UAC* mice**: Development of *Ugt1*$^{F/F}$ mice required the design of a targeting vector that

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contained the neomycin phosphotransferase (*neo*^{r}) gene to allow antibiotic selection following heterologous recombination in ES cells. The *neo*^{r} gene was flanked by Flp recombinase sites for easy removal of the *neo*^{r} gene following identification of the *Ugt1*$^{LoxP/FRTneoFRT/LoxP}$ mice. Deletion of the FRTneoFRT sequence was accomplished by crossing these mice with FLP recombinase transgenic mice, generating *Ugt1*$^{F/F}$ mice. However, if the *Ugt1*$^{LoxP/FRTneoFRT/LoxP}$ mice (*UFP* mice) are bred to homozygosity, the insertion of the targeting construct along with the *neo*^{r} gene causes the mice to be hypomorphic for the *Ugt1a1* gene, with all of the newborns and adults displaying hyperbilirubinemia (**Figure 4**). This results from poor expression of the *Ugt1a1* gene in all tissues. By targeting the deletion of the *Ugt1a1* gene in liver tissue using Cre/loxP recombination technology, *UFP*/albumin-*C*re (*UAC* mice) mice are created. Because of the preexisting hyperbilirubinemia in *UFP* mice, a robust kernicterus mouse model was developed in *UAC* mice with no mature hepatic *Ugt1a1* gene or protein expression detected at 14 days after birth. The inability of the *UAC* mice to metabolize bilirubin results in TSB accumulation in the brain causing severe CNS damage and leading to a 95% lethality rate (Barateiro et al. 2016). These mice serve as an excellent model to study the developmental impact of severe hyperbilirubinemia towards the onset of bilirubin induced CNS toxicity. **5) *Ugt1* mutant mice:** The mouse *Ugt1* locus was targeted and a single base deletion was introduced in exon 4 of the *Ugt1* gene leading to a premature stop codon for generating *Ugt1* mutant mice. These mice suffered severe neonatal hyperbilirubinemia within 5 days after birth (Bortolussi et al. 2015), very similar to the original *Ugt1*$^{-/-}$ mice (Nguyen et al. 2008). The resulting bilirubin neurotoxicity is irreversible and mice die shortly after birth.

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(3). Developmental regulation of UGT1A1: It is clear that UGT1A1 expression is a highly regulated event during development. By using *hUGT1A1*28* mice, studies have shown that reduction of liver *UGT1A1* gene expression in the developmental stage, which corresponds to the onset of hyperbilirubinemia and high levels of TSB, is actively regulated by pregnane X receptor (PXR). Reverse genetic experiments using PXR deficient mice with the humanized *UGT1* background demonstrated that in the absence of PXR, mice expressed significantly higher levels of UGT1A1 with a decrease in TSB levels, avoiding severe neonatal hyperbilirubinemia (Chen et al. 2012). These findings strongly indicate that PXR acts as a transcriptional repressor of the UGT1A1 gene during the neonatal period. This is regulated as a developmental event, since activation of the liver *UGT1A1* gene in adult hUGT1/Pxr$^{-/-}$ mice is not observed.

Bilirubin-induced neurotoxicity

In the normal state, over 99.9% of UCB in blood is bound to plasma albumin and only a small fraction of unbound bilirubin may diffuse into the brain and cerebrospinal fluid. With blood bilirubin exceeding a certain range (≥ ~20 mg/dL) that saturates serum protein binding capacity, unbound UCB would dramatically rise and enter brain tissue (Ostrow, Mukerjee, and Tiribelli 1994). By comparing homozygous j/j Gun rats with non-jaundiced littermate heterozygous J/j controls with or without sulfadimethoxine exposure, studies have demonstrated that the levels of CNS bilirubin calculated correlate well with brain physiological abnormalities and signs of neurotoxicity, attributable to the albumin-bilirubin binding constant and albumin concentrations in the brain (Daood, McDonagh, and Watchko 2009; Daood and Watchko 2006). The calculated neurotoxic bilirubin levels for

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Gunn rat pups are compatible with those in human neonates with extremely high levels of TSB (35 mg/dL) (Daood, McDonagh, and Watchko 2009). Results derived from the Gunn rat model along with in vitro brain cell line studies provide evidence of the involvement of multiple mechanisms in bilirubin neurotoxicity, including central auditory processing abnormalities, mitochondrial impairment, prolonged hippocampal synaptic plasticity, and neuronal excitotoxicity, triggering downstream events, such as activation of apoptotic and necrotic cell death (Shapiro 1988; Rice and Shapiro 2008; Chang et al. 2009). High levels of UCB in the brain also resulted in chronic learning and memory deficits as evidenced by the results of the Morris water maze test and place navigation ability test in hyperbilirubinemia rats (Song et al. 2014). In *hUGT1A1*28* mice that develop signs of kernicterus, clear cognitive impairment can be observed as the mice have obvious balance issues, followed by grand mall seizures and dystonia (Fujiwara et al. 2010).

The involvement of innate immunity signaling in palliating bilirubin-induced neurotoxicity was illustrated by the study of kernicterus neonates using *UGT1A1*28* mice (Yueh et al. 2014). The authors showed that despite the antioxidant property of bilirubin, excessively high levels of TSB produced systematic oxidative stress as indicated by the decreased ratio of GSH/GSSG and activation of the NADPH oxidase complex as well as anti-oxidant response genes (e.g., heme-oxygenase 1) in the brain (Yueh et al. 2014). While bilirubin escapes metabolism by UGT1A1 and continues to increase, accelerating TSB levels lead to cellular neuroinflammation, manifested as activation of microglia and astrocytes in the brain. It appears that the TLR2-medicated signaling pathway is critical in regulating gliosis, proinflammatory mediators, and oxidative stress and serves as an overall

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protective mechanism under the condition of severe hyperbilirubinemia as *hUGT1A1*28*/*Tlr2*$^{-/-}$ mice failed to activate glial cells, proinflammatory cytokines, and stress response genes, resulting in a significant drop in survival rates of hyperbilirubinemia neonates. One hypothesis that still needs to be proven is that TLR2 signaling may have originated from ligands produced by the gut microbiota. That is, disturbed gut bacteria (dysbiosis) owing to high levels of serum bilirubin damage the intestinal barrier, leading to activation of innate immunity through TLR signaling.

To evaluate the role of oxidative stress in BIND, sulfadimethoxine-induced jaundiced Gunn rat neonates were administered antioxidants to alter their redox status, resulting in reduced lipid peroxidation. However, the inhibition of oxidative stress did not lead to blockage of neurotoxicity as these pups still exhibited neurobehavioral abnormalities and bilirubin encephalopathy, resembling what was observed in control Gunn rat pups in the absence of antioxidants (Daood, Hoyson, and Watchko 2012). One possible explanation of these results is that oxidative stress may occur in later rather than in initial stages of the disease, and ROS are secondary manifestations of neuronal degeneration resulting from early events, such as inflammatory responses.

Results of experiments with *Ugt1* mutant mice harboring a mutation in the *Ugt1a1* gene showed that accumulated bilirubin in the brain leading to neurotoxicity is manifested as cerebellar abnormalities, hypoplasia, and neuronal cell death. Histological studies further demonstrated that neonates' cerebellar architecture was significantly affected, together with reductions in Purkinje cell number and dendritic arborization (Bortolussi et al. 2015).

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Another mechanism of action that is linked to clinical features of kernicterus shown in *UAC* mice is axonal damage and myelin degeneration. These neonatal mice exhibited axonopathy as an early sign of BIND characterized by deficits in the myelin sheath formation in different brain regions, especially in the cerebellum (**Figure 5**) (Barateiro et al. 2016). The connection between astroglial and microglial reactivity and myelination impairment in hyperbilirubinemia neonates has also been established based on histology and immunostaining studies, suggesting the occurrence of these events in the same brain regions. These results are consistent with previous findings using ganglia-oligodendrocyte cultures indicating that unconjugated bilirubin impairs oligodendrocyte differentiation and subsequent myelination (Barateiro et al. 2013). Indeed, clinical studies have also observed a decrease in the density of myelinated fibers and a reduction in cerebellar axons of a preterm infant with kernicterus (Brites 2012).

Contribution of environmental factors to hyperbilirubinemia

(1) Breast milk jaundice and the role of extrahepatic UGT1A1: The connection between breast milk and hyperbilirubinemia was first described by Arias and colleagues in 1964 (Arias et al. 1964). Early studies of breast milk jaundice showed a metabolite of progesterone, pregnane-3-20-dio, was present in breast milk and was implied in the development of jaundice (Hargreaves and Piper 1971) although eventually no scientific consensus was reached. To date, no specific component or combinations of components have been demonstrated to definitely contribute to breast milk jaundice.

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In keeping with the concept that TSB levels are higher and last longer in infants fed on breast milk, experiments using the humanized *UGT1A1*28* mouse model (Fujiwara et al. 2012) reiterated that neonatal hyperbilirubinemia that occurred following breast milk feeding disappeared when mice were fed formula. In contrast to conventional knowledge, however, this study further revealed that expression of extrahepatic UGT1A1, particularly intestinal UGT1A1, is subject to induction by formula feeding and is crucial for bilirubin metabolism and clearance during postnatal transition of hepatic UGT1A1 activity, which is present only later at the end of the suckling period (Fujiwara et al. 2012; Chen et al. 2012). Conversely, breast milk contributes to the development of hyperbilirubinemia by suppressing UGT1A1 expression in the small intestine. Breast milk was found to suppress intestinal IκB kinase α and β, resulting in inactivation of nuclear receptor NF-κB and nearly complete abolishment of intestinal UGT1A1 expression (Fujiwara et al. 2012).

(2) UGT1A1 activation by xenobiotics through nuclear receptors. Since UGT1A1 levels and activities determine bilirubin conjugation capacity, efforts have been made in seeking endogenous and exogenous compounds that have the ability to induce UGT1A1 expression. Several studies have demonstrated that UGT1A1 expression is modulated by a number of nuclear receptors, including PXR, constitutive androstane receptor (CAR), and peroxisome proliferator activated receptor alpha (PPARα), in a ligand-dependent fashion. Upon ligand binding, the nuclear receptor binds to the corresponding consensus response element in the *UGT1A1* promoter region and activates *UGT1A1* gene expression (Yueh et al. 2003; Xie et al. 2003; Chen et al. 2005; Senekeo-Effenberger et al. 2007; Yueh and Tukey 2007). For example, phenobarbital, a CAR agonist inducing

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UGT1A1 expression through interacting with a phenobarbital responsive element (PBREM), has been used clinically in conjunction with phototherapy for the treatment of severe jaundice in infants to enhance bilirubin metabolism, thus reducing the need for exchange transfusion (Valaes et al. 1980; Murki et al. 2005). PBREM involvement in controlling UGT1A1 expression is also supported by a study linking polymorphism of the UGT1A1 PBREM (T-3279G) to an increased risk of hyperbilirubinemia (Sugatani et al. 2002). Glucocorticoids have also been used to treat hyperbilirubinemia: Dexamethasone-treated infants experienced lower incidence of hyperbilirubinemia than the untreated controls (Madarek and Najati 2003). Studies in mice with neonatal hyperbilirubinemia indicated that PXR serves as a key regulator following glucocorticoid treatment by inducing liver UGT1A1 expression and reducing TSB levels (Chen et al. 2012). In addition to being regulated by the aforementioned NRs belonging to the nuclear hormone receptor superfamily, UGT1A1 is also subject to regulation by the nuclear receptor aryl hydrocarbon (Ah) receptor and nuclear factor erythroid 2 related factor (Nrf2) – activation of which can transcriptionally induce UGT1A1, resulting in higher levels of bilirubin excretion (Fujiwara et al. 2010; Yueh et al. 2003; Yueh and Tukey 2007). It is worth noting that bilirubin itself has been shown to activate Ah receptor and induce UGT1A1 through a ligand-dependent manner (Togawa, Shinkai, and Mizutani 2008).

(3) Modulation of UGT1A1 expression by environmental chemicals: When hyperbilirubinemia neonatal mice were exposed to the environmental chemicals arsenic and cadmium, their TSB levels unexpectedly decreased, correlated with elevated levels of intestinal UGT1A1 expression with no detectable changes in expression of hepatic UGT1A1. Gene expression profiling data and biochemical studies revealed that, as potent inducers

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of oxidative stress, arsenic and cadmium alter the redox state of the intestines, leading to induction of UGT1A1 and a dramatic reduction in TSB levels (Liu et al. 2016). These results suggest that modulation of intestinal UGT1A1 activity by initiating the oxidative stress signaling pathway may be an unconventional alternative to lower TSB and improve hyperbilirubinemia.

Alternative approaches to treat hyperbilirubinemia

(1). Hepatocyte transplantation and gene transfer therapy: For CNS1 patients, phototherapy is often the first-line therapy, but it may transiently lower the serum bilirubin concentrations and gradually become ineffective beyond infancy. At present, liver transplantation is the curative treatment to prevent neurological sequelae and kernicterus but often requires continuous immunosuppression with substantial risks (Schauer et al. 2003).

As evidence indicated that only ~5% of normal UGT1A1 activity is adequate to significantly lower the plasma bilirubin concentration and eliminate the risk of kernicterus (Fox et al. 1998), alternative therapies intending to alleviate hyperbilirubinemia with persistent expression of the UGT1A1 enzyme are underway in the experimental stage. A recent study illustrated that the advantage of neonatal hepatocytes over adult hepatocytes lies in the fact that neonatal hepatocytes exhibit better engraftment and repopulation capacity after transplantation, thus resulting in better bilirubin clearance in icteric Gunn rats (Tolosa et al. 2015). Significant progress has also been made by means of gene therapy in past decades using adenovirus-based or similar techniques or the

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correction of *UGT1A1* gene defects with the site-directed gene repair approach to treat hyperbilirubinemia animals (Li et al. 1998; Kren et al. 1999; Roy-Chowdhury et al. 2001; Bellodi-Privato et al. 2005). A gene therapy study showed that a single injection of a helper-dependent adenoviral vector expressing UGT1A1 that specifically targets the liver tissue can completely correct hereditary hyperbilirubinemia in Gunn rats with long-lasting effects and low-chronic toxicity (Toietta et al. 2005).

(2). Administration of albumin: Due to the high affinity of albumin to bilirubin, in a normal state, UCB is bound to albumin following transportation through the circulation to the liver (Ostrow, Mukerjee, and Tiribelli 1994). When UCB levels exceed the capacity of albumin, free bilirubin is capable of crossing the blood-brain-barrier and accumulating in the brain. Therefore, a potential approach to prevent bilirubin accumulation in the brain is to increase bilirubin binding capacity by albumin supplementation. When hyperbilirubinemia neonatal mice carrying inherited mutations of *Ugt1a1* were subject to daily albumin infusion, they were rescued from neurological damage and lethality. By increasing plasma bilirubin-binding capacity, albumin mobilizes bilirubin from tissues to plasma and results in reduced systemic plasma bilirubin levels (Vodret et al. 2015).

Regardless of efficacy of these alternative treatments, they are still in the experimental stage and clinical trials are apparently needed to evaluate the acute toxicity, immunogenic responses, and long-term safety profile before they can be applied in the market to humans.

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(3). Develop therapeutics targeted to induce *UGT1A1* gene expression. The use of animal models, such as the humanized *UGT1A1*28* mice, helps define the mechanisms that control neonatal hyperbilirubinemia and provides an important venue to exploit the impact of safe and therapeutic chemicals to regulate the *UGT1A1* gene and lower TSB levels. These non-invasive approaches could take advantage of drug delivery directly to newborns or alternatively by lactation following drug administration to nursing mothers. In vivo studies with humanized *UGT1A1*28* mice can directly exploit tissue specific contributions, such as the liver and gastrointestinal tract, that direct bilirubin clearance, while also being able to examine pharmacokinetics parameters of the inducing agents.

Summary

Severe neonatal hyperbilirubinemia and prevention of bilirubin encephalopathy remain clinical concerns. Clinical data indicated that while physiologic jaundice is a common benign condition observed in newborns, additional sources of hemolysis resulting in increased heme catabolism and severe UGT1A1 deficiency may lead to reversible or irreversible neuropathological conditions. Studies in animals have revealed that UGT1A1 levels can be regulated by environmental and dietary compounds through activation of nuclear receptors or alteration of oxidative-stress status. While the complex cascade of molecular and cellular events leading to bilirubin-induced neurotoxicity and kernicterus remains incompletely delineated, emerging evidence indicates that high levels of TSB activate innate immunity and cause myelination impairment. As we have learned more about bilirubin metabolism and neurologic injury with the advent of novel toxicology models, some of the conventional knowledge regarding hyperbilirubinemia is

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now being challenged: Intestinal UGT1A1 is subject to the regulation of breast milk and environmental compounds and plays a critical role during the developmental stage when expression of hepatic UGT1A1 is delayed.

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Reference List

Arias, I.M., L.M. Gartner, S Seifter, and M Furman. 1964. 'Prolonged Neonatal Unconjugated Hyperbilirubinemia associated with Breast Feeding and a Steriod, Pregnane-3(alpha), 20(beta)-Diol, in Maternal Milk that Inhibits Glucuronide Formation in vitro', *J Clin. Invest*, 43: 2037-47.

Barateiro, A., S. Chen, M.F. Yueh, A. Fernandes, H.S. Domingues, J. Relvas, O. Barbier, N. Nguyen, R.H. Tukey, and D. Brites. 2016. 'Reduced Myelination and Increased Glia Reactivity Resulting from Severe Neonatal Hyperbilirubinemia', *Mol. Pharmacol*, 89: 84-93.

Barateiro, A., V.E. Miron, S.D. Santos, J.B. Relvas, A. Fernandes, C. Ffrench-Constant, and D. Brites. 2013. 'Unconjugated bilirubin restricts oligodendrocyte differentiation and axonal myelination', *Mol. Neurobiol*, 47: 632-44.

Bellodi-Privato, M., D. Aubert, V. Pichard, A. Myara, F. Trivin, and N. Ferry. 2005. 'Successful gene therapy of the Gunn rat by in vivo neonatal hepatic gene transfer using murine oncoretroviral vectors', *Hepatology*, 42: 431-8.

Bhutani, V. K., and L. Johnson-Hamerman. 2015. 'The clinical syndrome of bilirubin-induced neurologic dysfunction', *Semin Fetal Neonatal Med*, 20: 6-13.

Bhutani, V. K., and L. Johnson. 2009. 'Kernicterus in the 21st century: frequently asked questions', *J Perinatol*, 29 Suppl 1: S20-4.

Bhutani, V. K., A. Zipursky, H. Blencowe, R. Khanna, M. Sgro, F. Ebbesen, J. Bell, R. Mori, T. M. Slusher, N. Fahmy, V. K. Paul, L. Du, A. A. Okolo, M. F. de Almeida, B. O. Olusanya, P. Kumar, S. Cousens, and J. E. Lawn. 2013. 'Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels', *Pediatr Res*, 74 Suppl 1: 86-100.

Bortolussi, G., E. Codarin, G. Antoniali, C. Vascotto, S. Vodret, S. Arena, L. Cesaratto, A. Scaloni, G. Tell, and A.F. Muro. 2015. 'Impairment of enzymatic antioxidant defenses is associated with bilirubin-induced neuronal cell death in the cerebellum of Ugt1 KO mice', *Cell Death. Dis*, 6: e1739.

Bosma, P.J., J. Seppen, B. Goldhoorn, C. Bakker, Elferink RP Oude, J.R. Chowdhury, N.R. Chowdhury, and P.L. Jansen. 1994. 'Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man.', *J. Biol. Chem*, 269: 17960-64.

Brites, D. 2012. 'The evolving landscape of neurotoxicity by unconjugated bilirubin: role of glial cells and inflammation', *Front Pharmacol*, 3: 88.

Burchell, B., M. Coughtrie, M. Jackson, D. Harding, S. Fournel-Gigleux, J. Leakey, and R. Hume. 1989. 'Development of human liver UDP-glucuronosyltransferases', *Dev Pharmacol Ther*, 13: 70-7.

Chang, F.Y., C.C. Lee, C.C. Huang, and K.S. Hsu. 2009. 'Unconjugated bilirubin exposure impairs hippocampal long-term synaptic plasticity', *PLoS. One*, 4: e5876.

Chen, S., D. Beaton, N. Nguyen, K. Senekeo-Effenberger, E. Brace-Sinnokrak, U. Argikar, R.P. Remmel, J. Trottier, O. Barbier, J.K. Ritter, and R.H. Tukey. 2005. 'Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (*UGT1*) locus', *J. Biol. Chem*, 280: 37547-57.

Chen, S., M.F. Yueh, C. Bigo, O. Barbier, K. Wang, M. Karin, N. Nguyen, and R.H. Tukey. 2013. 'Intestinal glucuronidation protects against chemotherapy-induced toxicity by irinotecan (CPT-11)', *Proc. Natl. Acad. Sci. U. S. A*, 110: 19143-48.

Chen, S., M.F. Yueh, R.M. Evans, and R.H. Tukey. 2012. 'The Pregnane-X-receptor controls hepatic glucuronidation during pregnancy and neonatal development in humanized UGT1 Mice', *Hepatology*, 56: 658-67.

Chime, H. E. , J. A. Egenede, and J.E. Arute. 2011. 'Prevalence of Neonatal Jaundice on Central Hospital, Warri, Delta State, Nigeria', *International Journal of Health Research*: 123-26.

Ciotti, M., R. Obaray, M.G. Martin, and I.S. Owens. 1997. 'Genetic defects at the *UGT1* locus associated with Crigler-Najjar type I disease, including a prenatal diagnosis', *Am. J. Med. Genet*, 68: 173-78.

Crigler, J.F., Jr., and V.A. Najjar. 1952. 'Congenital familial nonhemolytic jaundice with kernicterus; a new clinical entity', *AMA. Am. J. Dis. Child*, 83: 259-60.

Daood, M. J., M. Hoyson, and J. F. Watchko. 2012. 'Lipid peroxidation is not the primary mechanism of bilirubin-induced neurologic dysfunction in jaundiced Gunn rat pups', *Pediatr Res*, 72: 455-9.

Daood, M. J., A. F. McDonagh, and J. F. Watchko. 2009. 'Calculated free bilirubin levels and neurotoxicity', *J Perinatol*, 29 Suppl 1: S14-9.

Daood, M. J., and J. F. Watchko. 2006. 'Calculated in vivo free bilirubin levels in the central nervous system of Gunn rat pups', *Pediatr Res*, 60: 44-9.

Fox, I.J., J.R. Chowdhury, S.S. Kaufman, T.C. Goertzen, N.R. Chowdhury, P.I. Warkentin, K. Dorko, B.V. Sauter, and S.C. Strom. 1998. 'Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation', *N. Engl. J. Med*, 338: 1422-26.

Fujiwara, R., S. Chen, M. Karin, and R.H. Tukey. 2012. 'Reduced expression of UGT1A1 in intestines of humanized UGT1 mice via inactivation of NF-kappaB leads to hyperbilirubinemia', *Gastroenterology*, 142: 109-18.

Fujiwara, R., Y. Maruo, S. Chen, and R.H. Tukey. 2015. 'Role of extrahepatic UDP-glucuronosyltransferase 1A1: advances in understanding breast milk-induced neonatal hyperbilirubinemia', *Toxicol. Appl. Pharmacol*.

Fujiwara, R., N. Nguyen, S. Chen, and R.H. Tukey. 2010. 'Developmental hyperbilirubinemia and CNS toxicity in mice humanized with the UDP glucuronosyltransferase 1 (*UGT1*) locus', *Proc. Natl. Acad. Sci. U. S. A*, 107: 5024-29.

Gunn, C.H. 1938. 'Hereditary acholuric jaundice in a new mutant strain of rats.', *J Hered*, 29: 137-39.

Hargreaves, T., and R. F. Piper. 1971. 'Breast milk jaundice. Effect of inhibitory breast milk and 3 alpha, 20 abeta-pregnanediol on glucuronyl transferase', *Arch Dis Child*, 46: 195-8.

Huang, C. S., M. J. Huang, M. S. Lin, S. S. Yang, H. C. Teng, and K. S. Tang. 2005. 'Genetic factors related to unconjugated hyperbilirubinemia amongst adults', *Pharmacogenet Genomics*, 15: 43-50.

Kadakol, A., S.S. Ghosh, B.S. Sappal, G. Sharma, J.R. Chowdhury, and N.R. Chowdhury. 2000. 'Genetic lesions of bilirubin uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype', *Hum. Mutat*, 16: 297-306.

Kren, B.T., B. Parashar, P. Bandyopadhyay, N.R. Chowdhury, J.R. Chowdhury, and C.J. Steer. 1999. 'Correction of the UDP-glucuronosyltransferase gene defect in the gunn rat model of crigler-najjar syndrome type I with a chimeric oligonucleotide', *Proc. Natl. Acad. Sci. U. S. A*, 96: 10349-54.

Li, Q., S.S. Murphree, S.S. Willer, R. Bolli, and B.A. French. 1998. 'Gene therapy with bilirubin-UDP-glucuronosyltransferase in the Gunn rat model of Crigler-Najjar syndrome type 1', *Hum. Gene Ther*, 9: 497-505.

Liu, M., S. Chen, M. F. Yueh, R. Fujiwara, C. Konopnicki, H. Hao, and R. H. Tukey. 2016. 'Cadmium and arsenic override NF-kappaB developmental regulation of the intestinal UGT1A1 gene and control of hyperbilirubinemia', *Biochem Pharmacol*, 110-111: 37-46.

Madarek, E.O., and N. Najati. 2003. 'The effect of glucocorticoid therapy in preventing early neonatal complications in preterm delivery', *J Perinat. Med*, 31: 441-43.

Morioka, I., H. Nakamura, T. Koda, T. Yokota, H. Okada, Y. Katayama, T. Kunikata, M. Kondo, M. Nakamura, S. Hosono, S. Yasuda, N. Yokoyama, H. Wada, S. Itoh, M. Funato, Y. Yamauchi, Y. K. Lee, and M. Yonetani. 2015. 'Current incidence of clinical kernicterus in preterm infants in Japan', *Pediatr Int*, 57: 494-7.

Murki, S., S. Dutta, A. Narang, U. Sarkar, and G. Garewal. 2005. 'A randomized, triple-blind, placebo-controlled trial of prophylactic oral phenobarbital to reduce the need for phototherapy in G6PD-deficient neonates', *J Perinatol*, 25: 325-30.

Nguyen, N., J.A. Bonzo, S. Chen, S. Chouinard, M. Kelner, G. Hardiman, A. Belanger, and R.H. Tukey. 2008. 'Disruption of the *Ugt1* locus in mice resembles human Crigler-Najjar type I disease', *J. Biol. Chem*, 283: 7901-11.

Ostrow, J. D., P. Mukerjee, and C. Tiribelli. 1994. 'Structure and binding of unconjugated bilirubin: relevance for physiological and pathophysiological function', *J Lipid Res*, 35: 1715-37.

Rice, A.C., and S.M. Shapiro. 2008. 'A new animal model of hemolytic hyperbilirubinemia-induced bilirubin encephalopathy (kernicterus)', *Pediatr. Res*, 64: 265-69.

Roy-Chowdhury, N., A. Kadakol, B. S. Sappal, N. R. Thummala, S. S. Ghosh, S. W. Lee, and J. Roy-Chowdhury. 2001. 'Gene therapy for inherited hyperbilirubinemias', *J Perinatol*, 21 Suppl 1: S114-8; discussion S25-7.

Schauer, R., M. Stangl, T. Lang, A. Zimmermann, A. Chouker, A. L. Gerbes, F. W. Schildberg, and H. G. Rau. 2003. 'Treatment of Crigler-Najjar type 1 disease: relevance of early liver transplantation', *J Pediatr Surg*, 38: 1227-31.

Senekeo-Effenberger, Kathy, Shujuan Chen, Erin Brace-Sinnokrak, Jessica A. Bonzo, Mei Fei Yueh, Upendra Argikar, Jenny Kaeding, Jocelyn Trottier, Rory P. Remmel, Joseph K. Ritter, Olivier Barbier, and Robert H. Tukey. 2007. 'Expression of the Human UGT1 Locus in Transgenic Mice by 4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic Acid (WY-14643) and Implications on Drug Metabolism through Peroxisome Proliferator-Activated Receptor α Activation', *Drug Metabolism and Disposition*, 35: 419-27.

Shapiro, S. M. 1988. 'Acute brainstem auditory evoked potential abnormalities in jaundiced Gunn rats given sulfonamide', *Pediatr Res*, 23: 306-10.

Shapiro, S.M., V.K. Bhutani, and L. Johnson. 2006. 'Hyperbilirubinemia and kernicterus', *Clin. Perinatol*, 33: 387-410.

Song, S., Y. Hu, X. Gu, F. Si, and Z. Hua. 2014. 'A novel newborn rat kernicterus model created by injecting a bilirubin solution into the cisterna magna', *PLoS One*, 9: e96171.

Strassburg, C.P. 2008. 'Pharmacogenetics of Gilbert's syndrome', *Pharmacogenomics*, 9: 703-15.

Sugatani, J., K. Yamakawa, K. Yoshinari, T. Machida, H. Takagi, M. Mori, S. Kakizaki, T. Sueyoshi, M. Negishi, and M. Miwa. 2002. 'Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia', *Biochem Biophys Res Commun*, 292: 492-7.

Togawa, H., S. Shinkai, and T. Mizutani. 2008. 'Induction of human UGT1A1 by bilirubin through AhR dependent pathway', *Drug Metab Lett*, 2: 231-7.

Toietta, G., V. P. Mane, W. S. Norona, M. J. Finegold, P. Ng, A. F. McDonagh, A. L. Beaudet, and B. Lee. 2005. 'Lifelong elimination of hyperbilirubinemia in the Gunn rat with a single injection of helper-dependent adenoviral vector', *Proc Natl Acad Sci U S A*, 102: 3930-5.

Tolosa, L., S. Lopez, E. Pareja, M. T. Donato, A. Myara, T. H. Nguyen, J. V. Castell, and M. J. Gomez-Lechon. 2015. 'Human neonatal hepatocyte transplantation induces long-term rescue of unconjugated hyperbilirubinemia in the Gunn rat', *Liver Transpl*, 21: 801-11.

Tukey, R.H., and C.P. Strassburg. 2000. 'Human UDP-Glucuronosyltransferases: Metabolism, Expression, and Disease', *Annu Rev Pharmacol Toxicol*, 40: 581-616.

Valaes, T., K. Kipouros, S. Petmezaki, M. Solman, and S.A. Doxiadis. 1980. 'Effectiveness and safety of prenatal phenobarbital for the prevention of neonatal jaundice', *Pediatr. Res*, 14: 947-52.

Vodret, S., G. Bortolussi, A. B. Schreuder, J. Jasprova, L. Vitek, H. J. Verkade, and A. F. Muro. 2015. 'Albumin administration prevents neurological damage and death in a mouse model of severe neonatal hyperbilirubinemia', *Sci Rep*, 5: 16203.

Watchko, J. F., M. J. Daood, and T. W. Hansen. 1998. 'Brain bilirubin content is increased in P-glycoprotein-deficient transgenic null mutant mice', *Pediatr Res*, 44: 763-6.

Watchko, J.F., M.J. Daood, B. Mahmood, K. Vats, C. Hart, and M. Ahdab-Barmada. 2001. 'P-glycoprotein and bilirubin disposition', *J. Perinatol*, 21 Suppl 1: S43-S47.

Xie, W., M.F. Yeuh, A. Radominska-Pandya, S.P.S. Saini, Y. Negishi, B.S. Bottroff, G.Y. Cabrera, R.H. Tukey, and R.M. Evans. 2003. 'Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor', *Proc Natl Acad Sci USA*, 100: 4150-55.

Yueh, M.F., S. Chen, N. Nguyen, and R.H. Tukey. 2014. 'Developmental Onset of Bilirubin-induced Neurotoxicity Involves Toll-like Receptor 2-dependent Signaling in Humanized UDP-glucuronosyltransferase1 Mice', *J. Biol. Chem*, 289: 4699-709.

Yueh, M.F., Y.H. Huang, A. Hiller, S.J. Chen, N. Nguyen, and R.H. Tukey. 2003. 'Involvement of the xenobiotic response element (XRE) in ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1', *Journal of Biological Chemistry*, 278: 15001-06.

Yueh, M.F., and R.H. Tukey. 2007. 'Nrf2-Keap1 Signaling Pathway Regulates Human *UGT1A1* Expression in Vitro and in Transgenic UGT1 Mice', *J. Biol. Chem*, 282: 8749-58.

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FOOTNOTES

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FIGURE LEGENDS:

Figure 1. Release of bilirubin into blood and transport to the liver. The rapid increase in oxygen after birth stimulates red blood cell production and senescence, resulting in the release of heme from hemoglobin by the reticuloendothelial system. Heme undergoes metabolism by heme oxygenase and biliverdin reductase resulting in the production of bilirubin, which is released into blood and bound to serum proteins. Following its uptake into the liver, bilirubin undergoes glucuronidation by UDP-glucuronosyltransferase 1A1 located in the endoplasmic reticulum. Bilirubin-glucuronide exits the hepatocyte assisted by the multidrug resistance-associated protein 2 (MRP2) where it works its way through the biliary canaliculi into the lumen of the intestines.

Figure 2. The upper panel shows the genetic background of h*UGT1A1*28* mice. The top diagram is a representation of the human *UGT1A* locus, which was inserted into the mouse genome (*Tg-UGT1*), and the bottom diagram shows the targeted disruption of the mouse *Ugt1* locus with an insertion of the neo resistant gene into exon 4. Middle panel (left): The $Ugt1^{-/-}$ neonate exhibits the phenotypic trait of jaundice with a yellow skin color compared with in $Ugt1^{+/-}$ mice. The majority of the $Ugt1^{-/-}$ mice die before day 7. Middle panel (right): Expression of UGT1A1 in neonatal liver and small intestine tissues in h*UGT1A1*28* mice. The bottom diagram shows the comparisons of total serum bilirubin between the $Ugt1^{-/-}$ and h*UGT1A1*28* mice during the developmental period.

Figure 3. Top: By using the Cre-loxP recombination technology, the hepatocyte- or intestinal enterocyte-specific deletion of the *Ugt1a1* gene ($Ugt1^{\Delta Hep}$ or $Ugt1^{\Delta IE}$) was

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achieved. The bottom diagram shows the impact of the tissue-specific deletion of the *Ugt1a1* gene on serum bilirubin levels.

Figure 4. Top: UFP mice carrying the target construct Ugt1a1loxP[FRTneoFRT]loxP were bred into transgenic Albumin-Cre mice to generate UGT1a1F/F/Albumin-Cre mice (UAC mice). Middle (left): The comparisons of total serum bilirubin levels between UFP and UAC mice during the developmental period. Middle (right): The Kaplan-Meier survival curves analyze the survival rates of UFP and UAC mice. Bottom: Excessively high levels of bilirubin penetrate to the brain of the 15-day-old UAC mouse.

Figure 5. Images from cerebellum, medulla, pons, and corpus callosum indicate reduced myelination, as shown by the reduction of the presence of myelin basic protein (MBP, green) in neurons (neurofilament, red).

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Figure 1

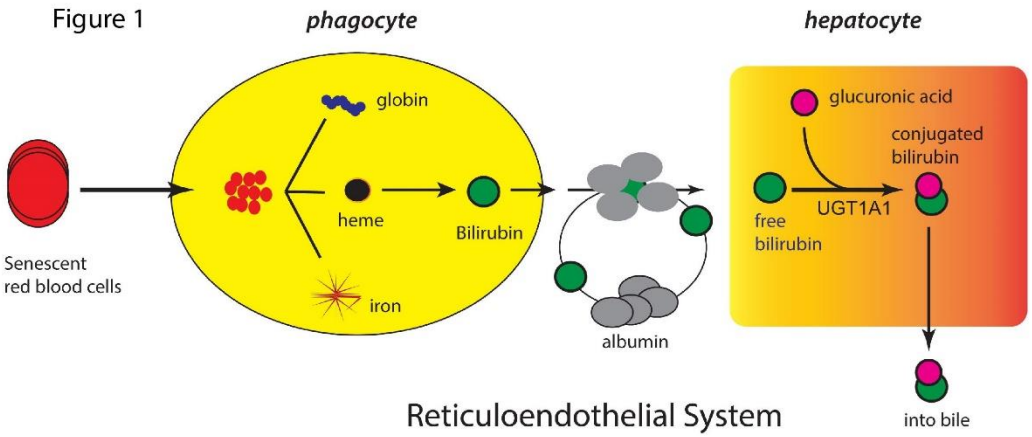

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Figure 2

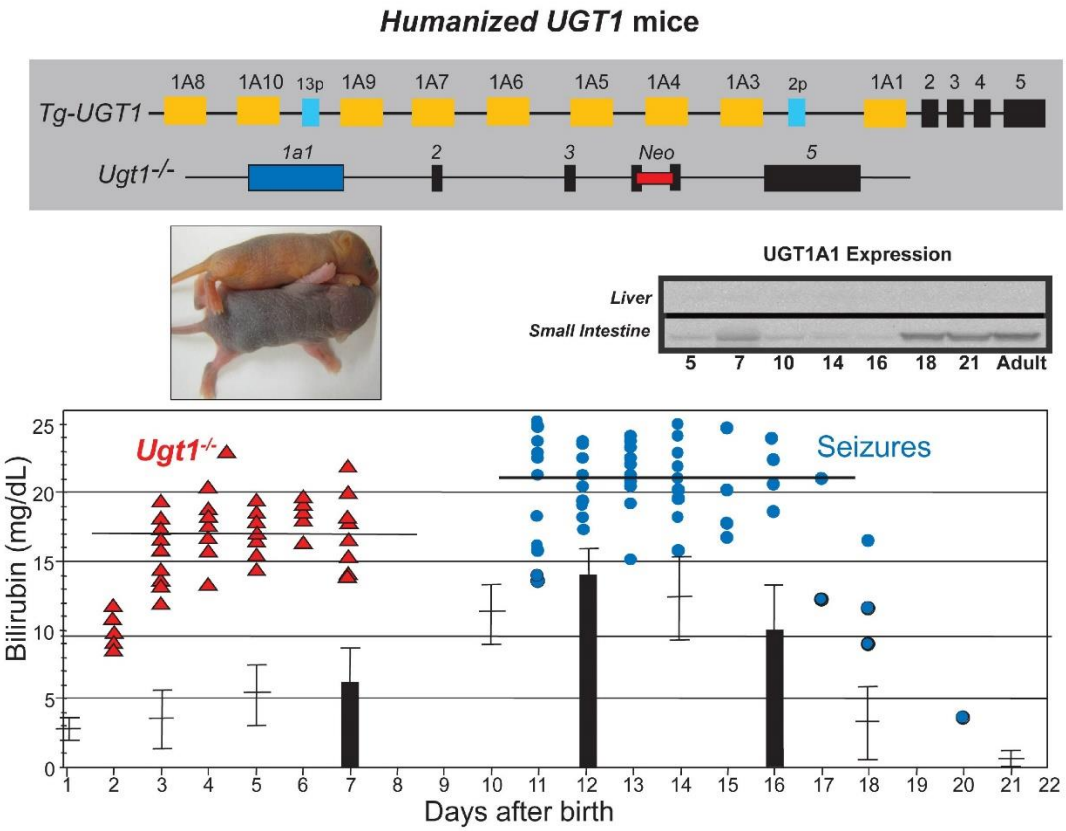

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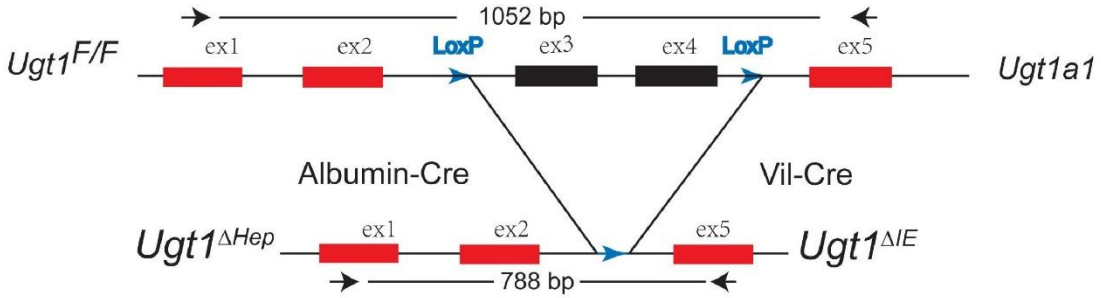
Figure 3



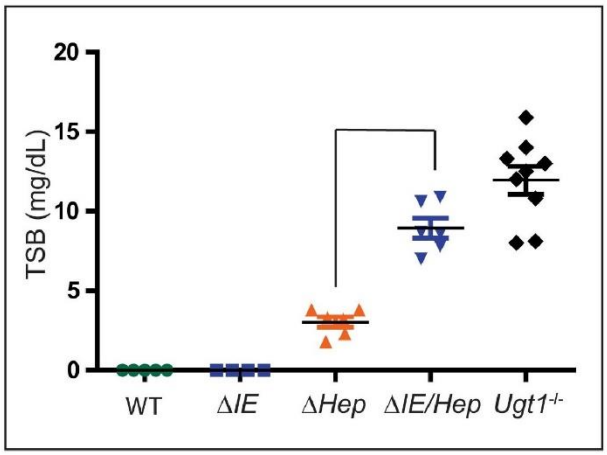


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Figure 4

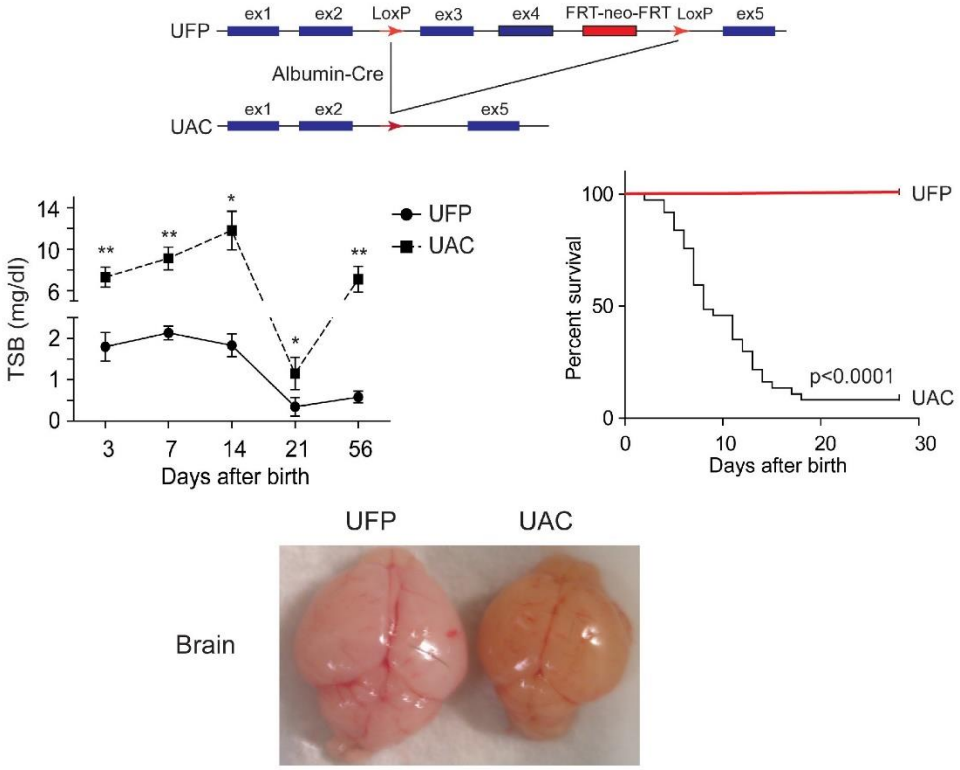

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Figure 5