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FUT 2 polymorphism and outcome in very-low-birth-weight infants

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BACKGROUND: To determine whether the secretor gene fucosyltransferase (FUT)2 polymorphism G428A is predictive for adverse outcomes in a large cohort of very-low-birth weight (VLBW) infants.

METHODS: We prospectively enrolled 2,406 VLBW infants from the population-based multicenter cohort of the German Neonatal network cohort (2009–2011). The secretor genotype (rs601338) was assessed from DNA samples extracted from buccal swabs. Primary study outcomes were clinical sepsis, blood-culture confirmed sepsis, intracerebral hemorrhage (ICH), necrotizing enterocolitis (NEC) or focal intestinal perforation requiring surgery, and death.

RESULTS: Based on the assumption of a recessive genetic model, AA individuals had a higher incidence of ICH (AA: 19.0% vs. GG/AG: 14.9%, $P = 0.04$) which was not significant in the additive genetic model (multivariable logistic regression analysis; allele carriers: 365 cases, 1,685 controls; OR: 1.2; 95% CI: 0.99–1.4; $P = 0.06$). Other outcomes were not influenced by FUT2 genotype in either genetic model.

CONCLUSION: This large-scale multicenter study did not confirm previously reported associations between FUT2 genotype and adverse outcomes in preterm infants.

The secretor status, i.e., the ability to secrete ABH histo-blood group antigens into body fluids is determined by the enzyme fucosyltransferase 2 which is encoded by the FUT2 gene. About 80% of the Caucasian populations are secretors (either homozygous SeSe or heterozygous Sese) while the remaining 20% carry the homozygous 428G→A nonsense mutation in the FUT2 gene and are nonsecretors (sese). Several *in vivo* studies have revealed the clinical significance of this polymorphism and the secretor status in terms of susceptibility to infection and association with immunologically mediated diseases (1–6). Thorven *et al.* (1) were able to demonstrate that secretor-negative individuals are resistant to Norovirus infections possibly due to binding of Norovirus to secreted ABH histo-blood group antigens as indispensable condition for infection. Another study noted that secretor individuals are overrepresented in a cohort of patients with viral infections of the respiratory tract (2). So far the only investigation involving preterm infants found a strong association between secretor status and severe outcome (i.e., death, necrotizing enterocolitis (NEC), Gram-negative sepsis) in a cohort of 410 premature infants (6).

The aim of this study was to determine possible associations between the FUT2 genotype and short-term as well as long-term outcomes in a large cohort of 2,406 preterm infants with a birth weight < 1,500 g (very-low-birth weight (VLBW)) from the German Neonatal Network.

RESULTS

Genotype Frequencies

In the German Neonatal Network cohort including infants born between 2009–2011, $n = 2,566$ infants were enrolled (64.8% of eligible infants; early death occurred in 21% of nonenrolled infants). As ethnic differences for FUT 2 genotype distribution have been previously noted, we decided to exclude infants with Asian ($n = 48$), African ($n = 75$), and unknown background ($n = 37$). The genotype frequencies in the remaining cohort of 2,406 infants were in the expected range for Caucasian populations (6–8) and appropriate to allele frequencies, as determined by Hardy-Weinberg equilibrium (FUT2 428 GG: $n = 744$, 30.9%; FUT2 428 GA: $n = 1,193$, 49.6%; FUT2 428 AA: $n = 469$, 19.5%). The power of this study to detect a difference in mortality rates between AA and AG/GG genotype groups was 99.9885% at the two-sided 5% test-level. For power calculations, we used the marginal genotype frequencies of this study, the mortality rates reported by Morrow *et al.* (6), i.e., 12/95 in the AA genotype group and 13/299 in the AG/GG genotype group, and the continuity-corrected χ^2 test. The power exceeds 99% even at the two-sided 0.0002 test-level.

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Clinical Characteristics

The clinical data of the whole cohort are described in Table 1. We noted differences for causes of preterm delivery stratified to FUT-2 G428A genotype. The incidence of preterm labour was higher in AA individuals compared to GG/AG (39.7 vs. 32.2 %; $P = 0.002$), while the incidence for amniotic infection was not different. By contrast, GG/AG individuals were more often delivered due to pre-eclampsia (8.1 vs. 3.9%; $P = 0.002$) and had a higher likelihood of Caucasian maternal background (88.7 vs. 81.4%; $P = 0.001$) as compared to AA individuals.

Primary Outcomes Investigated in a Recessive Genetic Model

Based on the assumption of a recessive genetic model (GG/AG vs. AA), we found no differences according to genotype for clinical sepsis, blood-culture confirmed sepsis, necrotizing enterocolitis or focal intestinal perforation requiring surgery, and death (Table 2). In line with this, the pathogenic spectrum of blood-culture proven sepsis episodes was not different between GG/AG and AA individuals (Table 3). AA individuals were observed to have a nonsignificant association with early-onset sepsis (GG/AG: 1.2%, AA: 2.4%, $P = 0.06$, Fisher's exact test). Interestingly, AA individuals with preterm premature rupture of membranes (PPROM) carried a higher risk to early-onset sepsis as compared to GG/AG individuals with PPROM (5.5 vs. 1.8%, $P = 0.036$). We also analyzed the subgroup of VLBW infants < 32 wk ($n = 2,076$) to exclude over-representation of small-for gestational age infants and found no significant risk for early-onset sepsis in that subgroup (GG/AG: 1.4%, AA: 2.3%; $P = 0.08$, Fisher's exact test). In the recessive genetic model, AA individuals were noted to have a higher incidence of intracerebral hemorrhage (ICH; whole cohort: GG/AG: 14.9 %, AA: 19.0%, $P = 0.04$, Fisher's exact test; VLBW infants < 32 wk gestational age: GG/AG: 16.8, AA: 21.6%, $P = 0.03$, Fisher's exact test).

Multivariable Logistic Regression Analysis (Additive Genetic Model)

Alternatively, we assumed an additive genetic model to test the effect of FUT2 adjusted for well-known clinical risk factors (gestational age per week, birth weight per 100 g steps, gender, multiple birth, inborn delivery, exposure to antenatal steroids) for the outcomes of interest in a multivariable logistic regression analysis. In the cohort of VLBW infants < 32 wk the FUT2 genotype was noted to have a nonsignificant effect on the development of ICH (365 cases, 1,685 controls; OR: 1.2; 95% CI: 0.99–1.4; $P = 0.06$). Likewise, other outcomes including clinical and blood-culture proven sepsis, NEC/focal intestinal perforation requiring surgery and death were not influenced by FUT2 genotype (Table 4). In our cohort, gestational age proved to be the most important predictor for outcome.

Table 1. Clinical characteristics of very-low-birth weight cohort stratified to genotype

Clinical characteristics	All	GG	AG	AA	*P* value GG/AG vs. AA
Number of infants	2,406	744	1,193	469	
Gestational age (weeks), mean (SD)	28.8 (2.8)	28.7 (2.8)	28.8 (2.7)	28.7 (2.8)	
Birth weight (g), mean (SD)	1,067 (306)	1,058 (316)	1,073 (304)	1,076 (299)	0.72
Male gender (%)	50.0	47.7	51.8	49.0	0.64
Multiple birth (%)	32.5	31.2	32.1	35.4	0.13
Mode of delivery (%)					
Vaginal	9.5	9.2	8.6	12.3	0.07
Caesarean section (C/S)	81.2	81.6	82.0	78.6	
Emergency C/S	9.3	9.2	9.4	9.1	
Cause of preterm birth (%)[a]					
PPROM	28.0	25.0	31.0	28.0	0.77
Preterm labor	**33.7**	**32.1**	**32.3**	**39.7**	**0.002**
Amniotic infection	21.4	20.8	21.5	22.0	0.7
Pre-eclampsia	**7.3**	**8.3**	**8.0**	**3.9**	**0.002**
HELLP syndrome	**8.6**	**9.5**	**9.1**	**5.8**	**0.02**
Pathological CTG	19.8	18.8	20.5	19.7	0.91
IUGR/path. Doppler	23.4	23.1	24.6	21.0	0.16
Placental abruption	7.1	6.7	7.6	6.5	0.55
Antenatal antibiotics (%)	48.2	46.3	49.3	48.1	0.99
Antenatal steroids (%)	**89.6**	**91.6**	**89.8**	**85.9**	**0.004**
Maternal ethnicity (%)					
Caucasian	88.8	89.9	87.9	81.4	**0.001**
Turkey/Middle East	11.2	10.1	12.1	18.6	

[a]The cause of preterm birth was set by the attending obstetrician, the documentation of multiple causes was possible; bold text indicates significant associations.
C/S, Caesarean section; CTG, cardiotocography; HELLP syndrome (complication of pregnancy including *h*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelets); IUGR, intrauterine growth restriction.

Table 2. Outcomes of very-low-birth weight cohorts stratified to FUT 2 genotype

Outcome measures	All	GG	AG	AA	*P* value GG/AG vs. AA
Number of infants	2,406	744	1,193	469	
Clinical sepsis (%)	33.0	34.3	32.2	33.2	0.93
B/C proven sepsis	12.0	12.1	12.0	12.0	0.97
Early-onset sepsis (%)	**1.5**	**0.7**	**1.6**	**2.4**	0.08[a]
Late-onset sepsis (%)	11.3	11.8	11.2	10.6	0.63
Pneumonia (%)					0.12
Early onset	0.7	0.4	0.6	1.4	
Late onset	3.1	2.3	3.3	3.7	
Surgery for NEC/focal intestinal perforation (%)	4.5	4.2	4.6	4.4	0.93
Higher stage NEC (%)	4.9	4.3	5.4	4.4	0.61
ICH (%)	**15.7**	**13.9**	**15.6**	**19.0**	**0.04[a]**
Grade 1	6.0	5.4	6.1	6.5	
Grade 2	4.0	3.5	3.5	5.9	
Grade 3	1.9	1.6	2.1	2.0	
Grade 4	3.8	3.2	3.9	4.6	
Periventricular leukomalacia (%)	3.0	2.3	3.0	4.0	0.16
Surgery for VP-shunt (%)	1.7	0.8	2.2	2.0	0.65
Bronchopulmonary dysplasia (%)	14.6	13.9	15.5	13.3	0.4
Pneumothorax (%)	4.9	5.3	4.7	4.8	0.93
Death (%)	3.6	3.9	3.3	3.9	0.69
Nonsurvivors with sepsis (%)	7.4	7.9	6.4	9.3	0.56

The χ^2 test was used for statistical comparison, if not otherwise indicated ([a]Fisher's exact test). Bold text indicates significant associations.
B/C, blood culture.

We also performed a multivariable regression analysis in ethnically different subgroups (Caucasian, Turkey/Middle East). The FUT2 genotype had no effect on outcomes in the Caucasian population. In the much smaller subgroup of infants from Turkey/Middle East, A allele carriers were noted to have a potentially decreased risk of death during the primary stay in hospital (eight cases, 232 controls; OR: 0.21; 95% CI: 0.05–0.8, $P = 0.026$, data not shown).

Treatment Strategies

With regard to antibiotic treatment, infants with AA genotype were more often treated with cefotaxime (39.9 vs. 32.6%; $P = 0.003$) and tobramycin (16.1 vs. 11.8%, $P = 0.01$) as compared to GG/AG individuals, but no differences were observed for total use of antibiotics and administration of third line antibiotics such as carbapenems. In line with that, the FUT2 genotype did not influence the need for vasoactive drugs, diuretics, analgetics, inhalative drugs, surfactant, dexamethasone, insuline, or blood products. FUT2 genotype also had no impact on the need for mechanical ventilation, duration oxygen supplement, time until full enteral feeds (150 ml/kg), and duration of primary stay in hospital (data not shown).

Table 3. Pathogenic spectrum in blood-culture proven sepsis episodes

Pathogen in blood culture (number of episodes, (%))	GG or GA $n = 1,655$	AA $n = 386$	*P*
CoNS	126 (7.7)	32 (8.3)	0.5
Group B streptococci	6 (0.4)	1 (0.3)	0.8
Staphylococcus aureus	42 (2.5)	6 (1.6)	0.3
Enterococcus species	19 (1.1)	4 (1)	0.9
Other streptococci	1(0.1)	1 (0.3)	0.3
Pneumococcus	0	0	—
Escherichia coli	15 (0.9)	3 (0.8)	0.8
Klebsiella species	23 (1.4)	3 (0.8)	0.3
Enterobacter cloacae	10 (0.6)	4 (1)	0.4
Serratia	1 (0.1)	0	0.6
Proteus mirabilis	0	0	—
Pseudomonas aeruginosa	3 (0.2)	1 (0.3)	0.8
Listeria monocytogenes	1 (0.1)	0	0.2
Candida	11 (0.7)	2 (0.5)	0.8
ESBL *E. coli*	2 (0.1)	1 (0.3)	0.5
Others	31 (1.9)	13 (3.4)	0.07

The *P* values were derived from Fisher's Exact test.

Infection-Related Morbidities in the First Year of Life

As part of our follow-up, we sent questionnaires to parents of German Neonatal Network infants and compared subjectively reported infection-related morbidities in the first year of life with the data of a representative cross-sectional German health survey (KIGGS) (9). 1,411/2,406 questionnaires were answered for infection-related items. We found no genotype-related differences for incidence of common cold, herpes-virus infection, bronchitis, croup, gastroenteritis/diarrhea, urinary tract infection, purulent conjunctivitis, and oral/diaper thrush. Parents noted that AA individuals were more often diagnosed with throat infection/tonsillitis (GG: 0.8%, $n = 3$, AG: 1.1%, $n = 6$; AA: 4.5%, $n = 9$; GG/AG vs. AA; $P < 0.001$; fisher´s exact test).

DISCUSSION

In a large well-characterized cohort of VLBW infants, we investigated the influence of the FUT2 428 A/G polymorphism on outcome measures. In contrast to previously published data (6), the FUT 2 genotype was not predictive for infections and other adverse outcomes.

The G428A polymorphism in the FUT2 gene is, in the Caucasian population, determining the so called secretor status. The FUT2 gene encodes for a α1,2-fucosyltransferase which is responsible for expressing ABH blood group antigens on mucosal surfaces and secretion of these antigens in body fluids. The polymorphism confers a nonsense mutation which results in a stop codon and subsequently leads to a nonfunctioning protein. The distribution of the polymorphism in our cohort is in concordance with existing literature (7,8). Mothers of 6.2% of enrolled infants had Asian, African, or unknown ethnicity and the infants were excluded from analysis. In other populations, different

Table 4. FUT 2 effect on neonatal outcomes adjusted for well-known clinical risk factors (multivariable regression analysis; very-low-birth weight infants < 32 wk with Caucasian background)

Outcome/ risk factors	ICH	Clinical sepsis	Proven sepsis	NEC surgery	Focal intestinal perforation surgery	death
Number cases/ controls	317/1,459	631/1,147	224/1,534	58/1,711	34/1,734	70/1,711
FUT2 genotype	1.2 (0.99–1.4); $P = 0.05$	1.0 (0.8–1.1); $P = 0.6$	1.0 (0.8–1.2); $P = 0.3$	1.2 (0.9–1.7); $P = 0.4$	0.8 (0.5–1.3); $P = 0.4$	1.1 (0.8–1.6); $P = 0.5$
Female gender	**0.7 (0.5–0.9); $P = 0.008$**	**0.8 (0.6–0.96); $P = 0.02$**	1.0 (0.7–1.3); $P = 0.9$	0.8 (0.5–1.4); $P = 0.5$	0.7 (0.3–1.4); $P = 0.3$	0.7 (0.4–1.2); $P = 0.2$
Multiple birth	1.2 (0.9–1.6); $P = 0.2$	0.9 (0.7–1.1); $P = 0.2$	1.0 (0.7–1.3); $P = 0.8$	1.2 (0.7–2.2); $P = 0.5$	1.1 (0.5–2.3); $P = 0.9$	1.2 (0.7–2.1); $P = 0.5$
Inborn	0.6 (0.3–1.2); $P = 0.2$	1.0 (0.6–1.9); $P = 0.9$	1.0 (0.5–2.5); $P = 0.8$	1.2 (0.3–5.4); $P = 0.8$	1.1 (0.1–8.8); $P = 0.9$	1.0 (0.2–4.6); $P = 0.9$
Gestational age per week	**0.7 (0.6–0.7); $P < 0.001$**	**0.84 (0.8–0.9); $P < 0.001$**	**0.8 (0.7–0.9); $P < 0.001$**	**0.8 (0.7–0.9); $P = 0.03$**	**0.7 (0.5–0.9); $P = 0.007$**	0.9 (0.7–1.0); $P = 0.1$
Birth weight per 100 g	1.07 (0.99–1.1); $P = 0.05$	**0.9 (0.8–0.9); $P < 0.001$**	0.9 (0.9–1.0); $P = 0.2$	0.9 (0.8–1.0); $P = 0.2$	0.9 (0.7–1.1); $P = 0.3$	0.7 (0.6–0.8); $P < 0.001$
Antenatal steroids	**0.5 (0.4–0.8); $P = 0.004$**	**0.6 (0.4–0.9); $P = 0.005$**	**0.6 (0.4–0.96); $P = 0.03$**	0.5 (0.3–1.2); $P = 0.1$	1.1 (0.3–3.9); $P = 0.9$	1.1 (0.4–2.1); $P = 0.5$

Effect sizes are given as odds ratios (95% confidence interval) for each outcome; bold text indicates significant associations.
ICH, intracerebral hemorrhage; NEC, necrotizing enterocolitis.

polymorphisms (e.g., C571T) prevail within the FUT2 gene and determine secretor status.

Many data were published in recent years focusing on the secretor status and its association with mainly infectious and even other inflammatory diseases. Morrow *et al.* (6) were first to publish data on secretor status in VLBW infants and demonstrated an association between secretor genotype and death, Gram-negative sepsis, and NEC. This study included 410 preterm infants < 32 gestational age. Given that death occurred in 13% of AA individuals ($n = 12$ out of 95) and 4% in GG/AG individuals ($n = 13$ out of 299) in the Morrow study (6), our study had a statistical power of > 99% to detect the estimated difference (469 AA and 1,937 GG/AG individuals). In our study, none of the primary outcome measures proved to be associated with FUT2 genotype. In the first set of tests (GG/AG vs. AA), we assumed a recessive genetic model where two copies of the A allele are required to have an impact on the outcome of interest. We observed a tendency toward higher rates of early onset sepsis in AA individuals which was in part attributable to a combined effect of FUT 2 and PPROM. This observation underlines that future studies are needed to address potential interactions between maternal and infant genotypes related to adverse pregnancy outcomes, particularly preterm delivery, and PPROM. AA individuals were also noted to have a higher incidence of ICH. In a second set of tests, we assumed an additive genetic model and performed regression analysis in the subgroup of infants < 32 wk of gestation in order to avoid over-representation of small-for gestational age infants and to improve comparability to Morrow's data. We found no specific genetic effect of FUT2 on outcomes of interest apart from a protective value of the A allele against death in the subgroup of infants with Turkish/Middle Eastern background. This subgroup, however, is very small and our data do not warrant a robust conclusion. In comparison with our setting, the study by Morrow *et al.* (6) was a single-center trial supported by functional data on the secretor status. Notably, secretor genotype and phenotype determined by salivary H-antigen production are not always concordant. Low salivary H antigen production was observed among many heterozygous (GA) and some homozygous secretor (GG) individuals. Strengths of our present study are that it includes a large cohort of infants from multiple centers and includes well-defined in-hospital and follow-up clinical data obtained prospectively in a systematic manner. The major weakness is failure to measure the functional expression of the FUT2 gene (H-antigen). There are differences in population genetics, culture, and environment that clearly can account for the differences observed between this multicenter study and the previous study (6). We finally conclude that FUT2 genotype is not a good independent predictor of adverse infectious and inflammatory outcomes complicating prematurity.

With regard to infection-related morbidities in the first year of life, parents (1,411/2,406 questionnaires) noted that infants with AA genotype were more often diagnosed with throat infection/tonsillitis. This observation is interesting, in particular with respect to previous studies on the link of FUT2 and respiratory illness (2,4,5), but this hypothesis needs to be evaluated in a prospective trial with larger cohorts.

Future studies regarding the secretor status and potential link with disease in preterm infants need to involve microbiota investigations, as previous reports demonstrated the link of mucosa-associated microbiota with FUT2 genotype (10,11). It would also be interesting to study the interaction of fucosyltransferase activity with different aspects of the immune system in preterm infants, e.g., the regulation of assembly of the pre-B cell receptor and intracellular signalling for B-cell maturation (12).

In conclusion, we performed a large-scale study which did not confirm previous data on FUT2 genotype and infectious morbidity in preterm infants. It is important to acknowledge

known clinical risk factors for infection in this vulnerable cohort of infants, i.e., gestational age and birth weight (13), in particular for future genome-wide association studies (14).

METHODS

Genetic Association Study

We prospectively studied the influence of the FUT-2 G428A polymorphism on several outcomes in 2,406 VLBW infants enrolled in a multicenter trial involving 47 neonatal intensive care units in Germany from March 2009 until December 2011 (German Neonatal Network). The inclusion criteria were as follows: birth weight < 1,500 g and gestational age ≤36 + 6 wk, exclusion criteria: lethal malformations, e.g., trisomy 13 and trisomy 18). After written informed consent was given by the parents, a DNA-sample of the infant was obtained by buccal swab and/or cord tissue and transferred to the study center (University of Lübeck). Antenatal and postnatal treatment and outcome data were recorded by according data sheets at the participating centers. After discharge, data sheets were sent to the study centre. A physician trained in neonatology evaluated the data quality by annual on site monitoring of completed data sets every 6 mo.

DNA was extracted using a commercial DNA purification kit (Quiagen, Hilden, Germany). The DNA was washed twice and eluted. Secretor genotype was determined at a single null mutation FUT2 G428A, Trp149 ->STOP (rs601338) by the TaqMan 5′ nuclease assay (Applied Biosystems, Foster City, CA) and the 7900HT Fast Real-Time PCR System.

Definition of Outcome Measures

Clinical sepsis was defined as sepsis with at least two clinical signs (15); i.e., temperature > 38 °C or < 36.5 °C, tachycardia > 200/min, new onset or increased frequency of bradycardias or apneas, hyperglycemia > 140 mg/dl, base excess < −10 mval/l, changed skin color, increased oxygen requirements) as well as antimicrobial therapy for at least 5 d, no proof of causative agent in blood culture, no obvious site of infection, and one laboratory sign (C-reactive protein > 2 mg/dl, immature/neutrophil ratio > 0.2, white blood cell count < 5/nl, platelet count < 100/nl).

Blood culture confirmed sepsis was defined by at least two clinical signs and one laboratory sign as mentioned above plus confirmation of causative agent in blood or cerebrospinal fluid (15).

All-cause mortality was defined as death occurring after admission to Neonatal Intensive Care Unit before discharge home. Bronchopulmonary dysplasia was diagnosed when needing oxygen or assisted ventilation evaluated at 36 wk of postmenstrual age. ICH grades I–IV were diagnosed according to the ultrasound criteria of Papile (16). Cystic periventricular leukomalacia was defined as periventricular lesions. Clinical higher stage NEC was definied as NEC with Bell stage II/III. A further outcome parameter was surgery required for NEC or focal intestinal perforation (17). Higher stage retinopathy of prematurity was noted when treatment was required (18).

Statistical Analysis

Data analysis was performed using the SPSS 20.0 data analysis package (IBM, Munich, Germany). Hypotheses were evaluated with χ^2 test, Fisher's exact test and Mann–Whitney *U*-test. In the first set of tests (GG/AG vs. AA), we assumed a recessive genetic model where two copies of the A allele are required to have an impact on the outcome of interest. A P value < 0.05 was considered as statistically significant for single tests.

In the second set of tests, we assumed an additive genetic model which was investigated in a multivariable logistic regression analysis. In order to allow for adjustment of more than one clinically important covariate, we included gestational age per week, birth weight per 100g steps, gender, multiple birth, inborn delivery, and exposure to antenatal steroids in order to describe the overall impact of the FUT2 genetic variant on the risk of the outcomes studied. Effect sizes were given as odds ratios and 95% confidence interval. The regression analysis was performed for VLBW infants < 32 wk with full datasets. In addition, we performed regression analyses for subgroups of VLBW infants based on maternal ethnicity.

Ethics

The study parts were approved by the local committee on research in human subjects of the University of Lübeck and the local ethical committees at the other study centers.

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