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Clinical Study

Association of a FGFR-4 Gene Polymorphism with Bronchopulmonary Dysplasia and Neonatal Respiratory Distress

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Background. Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease of premature birth, characterized by impaired alveolar development and inflammation. Pathomechanisms contributing to BPD are poorly understood. However, it is assumed that genetic factors predispose to BPD and other pulmonary diseases of preterm neonates, such as neonatal respiratory distress syndrome (RDS). For association studies, genes upregulated during alveolarization are major candidates for genetic analysis, for example, *matrix metalloproteinases (MMPs)* and *fibroblast growth factors (FGFs)* and their receptors *(FGFR)*. *Objective*. Determining genetic risk variants in a Caucasian population of premature neonates with BPD and RDS. *Methods*. We genotyped 27 polymorphisms within 14 candidate genes via restriction fragment length polymorphism (RFLP): *MMP-1, -2, -9, and -12, -16, FGF receptors 2 and 4, FGF-2, -3, -4, -7, and -18, Signal-Regulatory Protein α (SIRPA)* and *Thyroid Transcription Factor-1 (TTF-1)*. *Results*. Five single nucleotide polymorphisms (SNPs) in *MMP-9, MMP-12, FGFR-4, FGF-3, and FGF-7* are associated ($P < 0.05$) with RDS, defined as surfactant application within the first 24 hours after birth. One of them, in *FGFR-4* (rs1966265), is associated with both RDS ($P = 0.003$) and BPD ($P = 0.023$). *Conclusion*. rs1966265 in *FGF receptor 4* is a possible genetic key variant in alveolar diseases of preterm newborns.

1. Introduction

Great progress has been made in neonatal care over the last few decades, reflected by improving survival rates and clinical outcomes of preterm infants. Despite these advances, 45 years after its first description [1], bronchopulmonary dysplasia (BPD) remains a major complication of premature birth, causing ongoing morbidity and mortality: it is the most common neonatal chronic lung disease, affecting around 25% to 35% of VLBW neonates (very low birth weight, <1500 g) [2], and is associated with increased risk for rehospitalization [3, 4], cognitive delay, and neurosensory deficits [5].

Initially described by Northway et al. in 1967, the "old BPD" mainly affected modestly premature newborns suffering from respiratory distress and therefore mechanically ventilated with high levels of supplemental oxygen [1]. With the introduction of surfactant treatment, prenatal maternal use of glucocorticoids, improved nutrition, and ventilator strategies the clinical course and pathology of BPD have changed considerably. Unlike the original description, today's "new BPD" is mainly regarded as a disruption of distal lung growth [6, 7]. The underlying etiology is multifactorial. Thus, influenced by both genetic susceptibility [8, 9] and environmental factors [7] on the immature lung, the pathophysiology is characterized by inflammation, abnormal microvascularization, and impaired alveolarization [10].

Alveolar formation of the primitive saccules is a complex process of epithelial morphogenesis, capillary growth, and coordinated extracellular matrix (ECM) remodelling. At this, fibroblast growth factor (FGF) signalling and matrix metalloproteinase (MMP) activity play eminent roles.

MMPs are zinc-dependent proteolytic enzymes degrading all forms of ECM-components [11]. Some MMPs are upregulated in inflammatory environment and yet are involved

in pulmonary host defense [11]. There is evidence for some MMP isoforms being important determinants for alveolarization, especially MMP-2 [12], -9 [13], and -16 [14]: *MMP-2* deficient mice showed fewer and larger alveoli with thinner interstitial tissue [12]. Hadchouel et al. demonstrated an increase of MMP-16 activity during the alveolar stage and moreover found two SNPs within the *MMP-16* gene being associated with lower tracheal MMP-2 and -16 activity and to protect from BPD [14]. Prospecting further potential biomarkers for BPD, also MMP-9 shows some promise; for example, Harijith et al. highlighted a MMP-9-dependent lung injury pathway in an IFNγ-mediated animal model of BPD. Mice with a partial *MMP-9* deficiency showed a reversal of IFNγ-induced lung injury during hyperoxia [13].

MMPs, particularly MMP-2 and -9, activate fibroblast growth factors (FGFs) by cleavage in the ECM, especially during angiogenesis [15]. In turn activated FGFs upregulate *MMP* expression [15]. FGFs are secreted glycoproteins involved in interactions between epithelium and mesenchyme regulating cell migration and proliferation in embryonic development, especially in fetal pulmogenesis [16, 17]. Their signalling depends on membrane-located receptors (FGFRs) with a tyrosine kinase domain, encoded by four different genes (*FGFR 1–4*) [18–20]. They are all translated in developing lungs and are suggested to play major roles in modifying distal lung patterns during alveolarization [21]; for example, *FGFR-3-FGFR-4* double-knockout mice show no alveolarization [22].

It has been assumed that heritable determinants contribute significantly to both BPD [8, 23] and RDS [24]. On this account, we were interested in identifying genetic risk factors in a Caucasian population of premature newborn with BPD and RDS. We genotyped 27 polymorphisms within fourteen candidate genes for BPD: *MMP-1, -2, -9, -12,* and *-16, FGF receptors 2 and 4, FGF-2, -3, -4, -7,* and *-18, signal-regulatory protein α (SIRPA), and thyroid transcription factor-1 (TTF-1)*. We also included *SIRPA* because of the known effect on surfactant proteins [25] and inhibition of macrophages [26], as well as *TTF-1* due to its effect on lung differentiation [27].

2. Material and Methods

2.1. Subjects. We recruited preterm neonates (≤28 weeks of gestation) born between January 1996 and September 2010 at the Centre for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Germany. To provide an ethnically homogenous population, all infants were of Caucasian origin. Twins and siblings were excluded from the study as were children with chromosomal aberrations, congenital heart defects, or other major congenital malformations. DNA was collected by buccal swabs or by routine blood sampling, between 2 weeks up to 2 years of age.

Medical charts of all recruited infants were reviewed and clinical data were recorded. This included gestational week, number of days with supplemental oxygen, need of mechanical ventilation and positive airway pressure, and need of surfactant therapy. As described previously [28], the subdivision of our BPD study population was based on the analysis by Lavoie et al. about the heritability of BPD [9] according to the consensus defined by the National Institute of Health [6]: the BPD population included all infants with moderate and severe BPD, that is, supplemental oxygen for at least 28 days plus need of oxygen and/or positive pressure at 36 weeks of gestation, whereas the control population consisted of all preterm neonates with no or mild BPD.

Recruiting neonates for the RDS population was targeted on severe cases of respiratory distress by including only newborns depending on surfactant within the first 24 hours after birth (see Supplementary Material available online at http://dx.doi.org/10.1155/2013/932356). At our Neonatal Intensive Care Unit (NICU) the following approach has been applied regarding the treatment with surfactant: avoiding of intubation independent of the gestational week. Therefore, even very premature infants are only intubated if they show failure of ventilation and/or need of supplemental oxygen above 40%. Once they required intubation during the immediate postnatal period, they receive surfactant within 2 hours. This practice was consistent during the whole study period.

2.2. Genotyping. DNA was extracted by standard procedures as previously described [28]. Genotyping was carried out by RFLP following polymerase chain reaction amplification (PCR). Studied SNPs are summarized in Table 1. We included a minority of polymorphisms that already had been tested for other pathologies (rs1799750 in *MMP1* [29] and rs2276109 and rs652438 in *MMP12* [30]).

For PCR reactions, genomic DNA was initially denatured at 94°C for 5 minutes and underwent 35–40 cycles of denaturation (94°C for 30 seconds), annealing (1 minute, corresponding temperatures displayed in Table 2), extension reaction (72°C for 1 minute), and a final extension step at 72°C for 8 minutes. In Table 2 primers, annealing temperatures, and restriction enzymes (RE) are shown. Some primers contain intended single nucleotide mismatches (mutagenic primers) to create sites for restriction enzymes.

2.3. Sequencing. Accuracy of the RFLP was confirmed by sequencing via dideoxy chain termination method [31], respectively, three controls (homozygous wildtype, heterozygous, and homozygous mutation) for each polymorphism using the Big Dye Terminator cycle sequencing kit on an ABI 310 sequencer (Applied Biosystems). The RFLP analyses included these control references.

2.4. Statistical Analysis. Genotyping data of our case-control populations were analysed by using Armitage's trend test (ATT) for possible association with BPD and RDS as specified previously [28]. Moreover ATT was used to calculate Hardy Weinberg equilibrium (HWE) for each polymorphism.

2.5. Approval. The collection of blood/buccal swabs and the experimental procedures were approved by the Ethical Committee of the University of Freiburg. Parents were given written and verbal information about the study and a statement

Table 1: *Studied polymorphisms.* Genotyped polymorphisms, their corresponding reference SNP ID number, nucleotide substitution, and gene region. SNPs in exon regions are indicated as their amino acid substitution.

Gene	Polymorphism	Alleles	Gene region
MMP-1	rs1799750	G/GG	Promotor
	rs7125062	C/T	Intron
MMP-2	rs7201	A/C	3′UTR
	rs243865	C/T	Promotor
	rs17301608	C/T	Intron
MMP-9	rs20544	C/T	3′UTR
	rs3918242	C/T	Promoter
	rs17576	A/G	279 Gln/Arg
MMP-12	rs2276109	A/G	Promoter
	rs652438	A/G	357 Asn/Ser
MMP-16	rs2664352	C/T	Intron
FGFR-2	rs2981579	A/G	Intron
	rs1219648	A/G	Intron
FGFR-4	rs376618	A/G	136 Leu/Pro
	rs1966265	A/G	10 Ile/Val
FGF-2	rs11938826	C/G	Intron
FGF-3	rs11263591	G/C	Intron
	rs10796856	T/C	Intron
FGF-4	rs3740639	C/T	3′UTR
FGF-7	rs16962440	C/T	Intron
FGF-7, C15 or f33	rs4316697	G/A	Intron
	rs10519230	C/G	Intron
FGF-18	rs4559013	G/A	Intron
SIRPA	rs3828016	A/G	Intron
	rs4814734	A/T	Intron
TTF-1	rs11628131	A/C	Promoter
	rs999460	C/T	Downstream

of informed consent was signed by the parents of all enrolled children.

3. Results

The results of the 27 studied polymorphisms (Table 1) for association with bronchopulmonary dysplasia and neonatal respiratory distress are specified in Table 3 (BPD) and Table 4 (RDS).

3.1. Matrix Metalloproteinases. Among the 11 genotyped polymorphisms in different *MMP* genes (see Table 1) there was no BPD-associated polymorphism (Table 3) but two polymorphisms associated ($P < 0.05$) with RDS (rs20544 in *MMP-9*: $P = 0.033$; rs652438 in *MMP-12*: $P = 0.047$, see Table 4). Both SNPs show no significant deviation from Hardy-Weinberg equilibrium, neither in the control nor in the case population. Analysis of rs20544 (C/T) identifies the T allele as protective against respiratory distress. For the genotyping results of the amino acid substitution rs652438 (A/G, Asn357Ser) the complete absence of the G/G homozygous genotype in the respiratory distress case population must be taken in account. The other *MMP*-SNPs showed no association, inclusively rs2664352 in *MMP16*, that had been associated with protection from BPD [14].

3.2. Fibroblast Growth Factor Receptors. The *FGFR-4* SNP rs1966265, located in the exon region and causing an amino acid substitution of Isoleucine (Ile) for Valine (Val) is associated with both BPD ($P = 0.023$) and RDS ($P = 0.003$). Here the A/A genotype (Ile) could be identified as protective allele variant against our studied lung diseases. The association results from significant differences in allele frequencies: in both BPD and RDS analysis the G allele is more frequent in the disease populations (see Tables 3 and 4). The other SNPs in the *FGFR* genes showed no association with neither BPD nor respiratory distress.

3.3. Fibroblast Growth Factors. Whereas no association could be detected between the eight *FGF*-SNPs and BPD, rs10796856 in *FGF-3* and rs4316697 in *FGF-7* showed associations with RDS. Correspondent *P* values are $P = 0.036$ (rs10796856) and $P = 0.044$ (rs4316697), and no deviations from Hardy-Weinberg equilibrium were detected (see Table 4).

3.4. SIRPA and TTF-1. The four SNPs in *SIRPA* and *TTF-1* showed no association with neither BPD nor RDS. Analysis of *TTF-1* rs999460 unfolds deviation from Hardy-Weinberg equilibrium in both case and one control populations in our Caucasian population (see Tables 3 and 4).

4. Discussion

Bronchopulmonary dysplasia and respiratory distress syndrome of preterm infants have complex pathogenic mechanisms. The aim of this study has been to identify genetic risk factors in an ethnically homogenous Caucasian population.

Genetic contribution to BPD is suggested on the basis of twin studies demonstrating that at least half of the susceptibility is hereditary [8, 9, 23]. Additionally, Lavoie et al. [9] could differentiate in their study that mild BPD (according to the National Institute of Child Health and Human Development consensus definition [6]) had been mainly attributable to shared environmental factors whereas moderate or severe BPD had been attributable to genetic influence. Following these findings, we defined our control population as neonates with no BPD or mild BPD, whereas our BPD population included neonates with moderate or severe BPD. Furthermore, we recruited only preterm neonates ≤28 weeks of gestational age for the BPD population to avoid false associations based on the fact that BPD hardly develops in newborn older than 30 weeks of gestational age.

In contrast to BPD, the results of twin studies on RDS susceptibility showed mostly contradictory results [24, 32–35]. A twin study by Levit et al. with 332 twin pairs of a heterogeneous population has been the first one to include and assess the influence of several independent covariates,

Table 2: PCR conditions: primers, annealing temperatures, number of cycles and restriction enzymes (RE) for RFLP analysis.

Gene	Polymorphism	Forward-primer	Reverse-primer	PCR	RE
MMP-1	rs7125062	5′-ATC TCT GTC GGC AAA TTC GT-3′	5′-AGC AAC AAG AAG GAG CTG GA-3′	56.5°C, 35 cycl.	*BseG1*
MMP-2	rs7201	5′-TGC CCA AGA ATA GAT GCT GA-3′	5′-CTG TGA GCC ACA GAA GGT TG-3′	59.1°C, 35 cycl.	*MboI*
	rs243865	5′-CTG ACC CCC AGT CCT ATC TG-3′	5′-GAG CTG AGA CCT GAA GAG CCA AA-3′	59.1°C, 40 cycl.	*BstXI*
	rs17301608	5′-GCA GTG TAG ACA CTA GAG GAA GGA T-3′	5′-GTC ACC AAG GCC TGT ACC AC-3′	59.1°C, 40 cycl.	*DpnII*
MMP-9	rs20544	5′-AGT GCC CTG AGG ACT AGA G-3′	5′-CAT ACA TGC ATA CAT ACG TGC ATA C-3′	54.2°C, 40 cycl.	*SacI*
	rs3918242	5′-GCC TGG CAC ATA GTA GGC CC-3′	5′-CTT CCT AGC CAG CCG GCA TC-3′	67°C, 40 cycl.	*XceI*
	rs17576	5′-AAT TCA CCC TCC CGC ACT CT-3′	5′-GTT TTG GGG GCC AAT ACA TGA-3′	56.5°C, 35 cycl.	*SmaI*
MMP-16	rs2664352	5′-GGT AGG CCT GCT TTG GTT CT-3′	5′-GCC TTC CAA ACT CCA CTC ACA T-3′	56.5°C, 35 cycl.	*NlaIII*
FGFR-2	rs2981579	5′-CCA CTC AAG TCC CTG CTT GT-3′	5′-GTG GGG ACT GAT CAG AGG AA-3′	56.5°C, 40 cycl.	*AccI*
	rs1219648	5′-GCA CGC CTA TTT TAC TTG ACA CGC-3′	5′-CAT TGT GGT GAT CCT TCA CG-3′	59.1°C, 40 cycl.	*HhaI*
FGFR-4	rs376618	5′-GGG GGT AAC TGT GCC TAT TCTA-3′	5′-TAG CAG GGA GTG AAG GGA TG-3′	56.5°C, 40 cycl.	*XbaI*
	rs1966265	5′-CAA AGG TGC ACG TGT AGC AG-3′	5′-TCC CAC CTC AGA AGC CAT AC-3′	56.5°C, 40 cycl.	*BamHI*
FGF-2	rs11938826	5′-GAT GAT TTC TCA GGG CCA AA-3′	5′-GCT TCA CAT GCC TAT TTG CTT-3′	59.1°C, 40 cycl.	*AluI*
FGF-3	rs11263591	5′-GGT GTG TGG GCT GAA GAG AT-3′	5′-CAC CCT GAC ACT CCT GGT CT-3′	61.8°C, 40 cycl.	*TaqI*
	rs10796856	5′-CTT CCC CTC CTA GGC TTC AG-3′	5′-GAA TGC CAG GTT AGC AAA GG-3′	59.1°C, 40 cycl.	*HhaI*
FGF-4	rs3740639	5′-AAA AAC ACA CCC GCA GAA CT-3′	5′-TGA AGG TCA CCC ACT TCC TC-3′	59.1°C, 40 cycl.	*HinfI*
FGF-7	rs16962440	5′-TGT CGA ACA CAG TGG TAC CTG-3′	5′-GCA AAA GGA AGA TGA GAA AAG AT-3′	56.5°C, 40 cycl.	*EcoRV*
FGF-7, C15 or f33	rs4316697	5′-TGG CTC TTT CCA GCA ATT AGA-3′	5′-CCC TTT CTC TCC CAG GTT CT-3′	59.1°C, 40 cycl.	*TaiI*
	rs10519230	5′-AAT GTG ACC GCA TAT TGG TG-3′	5′-CAT TTT GGG AAC TGG GGT AA-3′	56.5°C, 35 cycl.	*TaqI*
FGF-18	rs4559013	5′-GAG GCA CAG AAT GAG GAA GG-3′	5′-TAC CTG GGC TCT CAA CAT CC-3′	56.5°C, 35 cycl.	*Alw26I*
SIRPA	rs3828016	5′-TCA AAC CCA GGC TTT CTG AC-3′	5′-AAC CAA GGC TGG CAC ATA AG-3′	63°C, 40 cycl.	*NlaIII*
	rs4814734	5′-CTG TGT ACA TCC TCT TAA ATG GAC-3′	5′-TGC TGT GTG CCA GAG TAT GC-3′	59.1°C, 40 cycl.	*HinfI*
TTF-1	rs11628131	5′-TGC AGC CTC ATT CAG CTT TA-3′	5′-GGT TGG AGA GGA GGA AAG GA-3′	56.5°C, 40 cycl.	*TaqI*
	rs999460	5′-ACC TGG GCG AGA AGT GAG TA-3′	5′-AGA GAG GGG AGA AGG AAT GG-3′	56.5°C, 40 cycl.	*SspI*

revealing that 50% of the variance to RDS susceptibility is hereditary [24].

Given these lines of evidence for genetic contribution, we have chosen the candidate-gene approach for our association study based on the hypothesis that genes fundamental in lung organogenesis and alveolar remodelling, that is, *MMP and FGF*, determine susceptibility to BPD and RDS.

Known genetic risk factors for RDS are mostly allelic polymorphisms of the genes encoding surfactant proteins SP-A1, SP-A2, and SP-B [36]. Anyhow, other determinants than

Table 3: *Results for BPD.* Genotype distribution as numbers of individuals for each genotype. *P* values are given in accordance with Hardy-Weinberg equilibrium (HWE) and for association with BPD by Armitage's trend test.

Gene	Polymorphism	Genotypes BPD	HWE BPD	Genotypes controls	HWE controls	*P* values for association
MMP-1	rs1799750	16, 25, 14	0,590	34, 44, 39	0.009	0.532
	rs7125062	41, 15, 3	0,364	74, 43, 11	0.241	0.126
MMP-2	rs7201	21, 28, 9	1,000	43, 64, 21	0.860	0.747
	rs243865	35, 18, 6	0,170	80, 35, 12	0.013	0.675
	rs17301608	27, 25, 11	0,280	51, 63, 21	0.857	0.769
MMP-9	rs20544	17, 19, 17	0,053	27, 60, 37	0.856	0.508
	rs3918242	35, 12, 4	0,077	76, 36, 4	1.000	0.894
	rs17576	24, 18, 12	0,042	57, 46, 22	0.032	0.639
MMP-12	rs2276109	44, 13, 2	0,328	86, 38, 2	0.522	0.574
	rs652438	52, 8, 0	1,000	10, 19, 1	0.587	0.615
MMP-16	rs2664352	12, 31, 19	1,000	40, 57, 35	0.120	0.185
FGFR-2	rs2981579	21, 32, 10	0,799	51, 58, 25	0.281	0.858
	rs1219648	19, 33, 12	0,804	52, 61, 20	0.854	0.215
FGFR-4	rs376618	35, 23, 5	0,744	84, 40, 10	0.132	0.432
	rs1966265	47, 15, 1	0,473	79, 45, 9	1.000	0.023
FGF-2	rs11938826	48, 14, 1	1,000	96, 38, 1	0.305	0.557
FGF-3	rs11263591	25, 22, 15	0,037	45, 61, 29	0.382	0.710
	rs10796856	17, 26, 22	0,135	37, 59, 40	0.125	0.632
FGF-4	rs3740639	33, 23, 7	0,365	74, 46, 15	0.086	0.815
FGF-7	rs16962440	17, 35, 12	0,614	42, 70, 25	0.730	0.656
FGF-7, C15 or f33	rs4316697	28, 30, 4	0,377	65, 55, 15	0.550	0.868
FGF-7, C15 or f33	rs10519230	37, 23, 3	1,000	73, 50, 11	0.517	0.421
FGF-18	rs4559013	22, 23, 17	0,045	45, 62, 22	1.000	0.387
SIRPA	rs3828016	20, 31, 12	1,000	48, 59, 27	0.288	0.788
	rs4814734	16, 37, 10	0,206	46, 62, 25	0.598	0.553
TTF-1	rs11628131	20, 27, 16	0,311	54, 50, 29	0.012	0.287
	rs999460	34, 19, 11	0,019	60, 51, 24	0.040	0.414

components of the surfactant system might also affect the liability to RDS. Genes encoding for growth factors or enzymes that account for alveolarization through proper secondary septation and extracellular remodeling might affect the gas-exchange and therefore aggravate respiratory distress at birth.

Supposed genetic risk factors for BPD are mostly genes encoding components of innate immunity and antigen-presentation, cytokines, antioxidant defences, and angiogenic growth factors such as: mannose-binding lectin (MBL2) [37], tumor necrosis factor-alpha (TNF-α) [28, 38], human leucocyte antigen (HLA)-A, -B, and -C alleles [39], glutathione-S-transferase-P1 [40], and vascular endothelial growth factor (VEGF) [28]. Some years ago, two *MMP-16* gene polymorphisms were demonstrated to protect from BPD and moreover to be associated with lower tracheal MMP-2 and -16 levels [14].

Matrix metalloproteinases are a family of zinc-dependent endopeptidases [11], and they degrade extracellular components and play a crucial role in lung development, especially during alveolarization. Particularly MMP-2 and -9 (so-called gelatinases A and B) seem to be relevant in extracellular remodeling and even pulmonary host defense. They degrade type IV collagen, fibronectin, elastin, and denatured collagen (gelatin). *MMP-2* deficient mice show an abnormal saccular development with larger and simplified alveoles [12]. In line with this finding, newborns developing BPD showed low MMP-2 tracheal levels at birth [41, 42]. Recently, MMP-9 could be identified as a pathogenic key mediator in a murine model of BPD [13]. On the other hand, increased tracheal levels of MMP-9 early after birth have been associated with resolving RDS, suggesting that increase in MMP-9-activity is a physiologic repair response [43]. Dik et al. demonstrated that increased MMP-9 activity in neonatal lungs early after birth correlated with resolving respiratory distress syndrome, demonstrating a likely role of MMP-9 in pulmonary host defense [43].

In our study we identified an SNP (rs20544) in the *MMP-9* gene to be associated ($P = 0.033$) with RDS, but not BPD. Respiratory distress syndrome has been defined as need of surfactant (see Supplementary Material). On one hand, ethnically homogenous populations like our Caucasian population are favourable to detect possible pathogenetic determinants, but one must bear in mind that the size of our RDS population is limited and the total numbers of neonates studied for each polymorphism vary slightly according to the recruiting time point. Furthermore, association studies

Table 4: *Results for RDS.* Genotype distribution as numbers of individuals for each genotype. *P* values are given for Hardy-Weinberg equilibrium (HWE) and for association with RDS.

Gene	Polymorphism	Genotypes RDS	HWE RDS	Genotypes controls	HWE controls	*P* value for association
MMP-1	rs1799750	25, 44, 20	1,000	24, 29, 33	0.004	0.163
	rs7125062	51, 36, 10	0.003	66, 21, 9	0.330	0.076
MMP-2	rs7201	30, 49, 16	0.678	34, 43, 16	0.067	0.648
	rs243865	58, 26, 11	0.012	58, 27, 7	0.139	0.540
	rs17301608	42, 47, 18	0,422	37, 43, 14	0.827	0.838
MMP-9	rs20544	29, 34, 24	0,828	14, 44, 30	0.052	0.033
	rs3918242	55, 23, 6	0,178	57, 25, 2	1.000	0.421
	rs17576	38, 30, 21	0,005	45, 34, 13	0.153	0.164
MMP-12	rs2276109	67, 27, 2	1,000	64, 23, 1	1.000	0.595
	rs652438	89, 10, 0	1,000	73, 17, 1	1.000	0.047
MMP-16	rs2664352	26, 46, 27	0,546	26, 43, 27	0.312	0.998
FGFR-2	rs2981579	33, 40, 20	0,840	40, 52, 15	0.289	0.351
	rs1219648	42, 49, 16	0,837	30, 47, 16	0.833	0.347
FGFR-4	rs376618	71, 27, 9	0,018	51, 36, 6	1.000	0.286
	rs1966265	76, 28, 2	1,000	50, 35, 8	0.605	0.003
FGF-2	rs11938826	80, 26, 1	0,690	67, 26, 1	0.684	0.586
FGF-3	rs11263591	31, 48, 25	0,438	40, 37, 19	0.085	0.127
	rs10796856	33, 50, 26	0,442	21, 38, 36	0.092	0.036
FGF-4	rs3740639	57, 41, 7	1,000	50, 30, 16	0.009	0.209
FGF-7	rs16962440	30, 57, 21	0,566	30, 49, 17	0.836	0.588
FGF-7 C15 or f33	rs4316697	56, 43, 7	1,000	38, 44, 12	1.000	0.044
FGF-7, C15 or f33	rs10519230	61, 41, 5	0,790	51, 33, 9	0.301	0.418
FGF-18	rs4559013	32, 45, 26	0,236	35, 43, 13	1.000	0.079
SIRPA	rs3828016	34, 53, 21	1,000	37, 38, 19	0.134	0.487
	rs4814734	31, 58, 18	0,334	32, 43, 17	0.674	0.671
TTF-1	rs11628131	36, 47, 23	0,326	41, 30, 22	0.002	0.453
	rs999460	56, 37, 16	0,027	38, 36, 19	0.082	0.121

on RDS are prone to confounding factors. Other pulmonary conditions such as a transient tachypnea provoked by wet lung syndrome or pulmonary infection might mimic respiratory distress syndrome caused by surfactant deficiency and thereby hamper the results of our study.

In our study, we included *MMP-16* polymorphisms that had been associated with BPD in a French population (rs2664352) [14]. In our population rs2664352 did not show any association.

The *FGF-3* polymorphism rs10796856 showed association with RDS ($P = 0.036$). Up to now, the role of FGF3 has been mainly studied in cancer diseases, that is, lung cancer [44], but its exact role in pulmogenesis remains elusive. FGF-3 is encoded by the *fgf3/int2* gene. There is evidence for *FGF-3* upregulation to be associated with alveolar type 2 cell hyperplasia [45] and downregulation to be associated with an excessive recruitment of free alveolar macrophages [45] which might lead to symptoms of respiratory distress. Furthermore it has been shown that FGF-3 stimulates the secretion of MMP-2 and -9 propeptides in vitro [46].

FGFR-4 polymorphism rs1966265 showed association with both respiratory distress ($P = 0.003$) and bronchopulmonary dysplasia ($P = 0.023$). The A/A genotype (encoding for Isoleucin instead of Valine) has been protective in our association study. The exact-test showed no deviation from Hardy Weinberg equilibrium for this SNP in both case and control populations, suggesting that the association does not result from population admixture or genotyping errors.

FGFR-1 to *FGFR-4* are expressed in the lung and FGFR-3 and -4 signalling, in particular, appears to be fundamental in alveolar formation. Weinstein et al. demonstrated that mice deficient in both *FGFR-3* and *-4* show a completely blocked alveolarization and fail to show any formation of secondary septae, whereas solely *FGFR-4(−/−)* animals exhibit no significant abnormalities, revealing a cooperative effect of FGFR-3 and -4 in lung development [22]. Hyperoxia-exposed (FiO2 0.85) mice show a BPD-like lung pattern of enlarged airspaces and furthermore a reduced expression of *FGFR-3* and *-4,* suggesting a pathogenic role in arrested lung development [47]. Srisuma et al. [48] replicated these results in *FGFR-3* and *-4* deficient mice and demonstrated in addition that FGFR-3/-4 signaling contributes to excessive elastin production and its alveolar accumulation, which is another typical feature of BPD. But these abnormalities have not been due to fibroblast defects but due to increased expression of paracrine factors of alveolar type 2 cell (AT2) [48].

If a reduction in *FGFR-3* and *-4* expression affects distal lung development, a functionally significant polymorphism within the correspondent gene possibly alters the susceptibility to alveolar disease such as BPD and RDS. Powell et al. showed that there is a peak of *FGFR-4* expression at the day of birth, when respiratory distress syndrome occurs [21]. This supports the conclusion that defective FGFR-4 signalling possibly results in neonatal lung diseases.

Our associations do not justify general interpretation. False-positive results can only be excluded by replications in other study populations.

In conclusion, we describe five SNPs in *MMP-9, MMP-12, FGFR-4, FGF-3,* and *FGF-7* that are associated ($P < 0.05$) in our Caucasian population with respiratory distress syndrome of the newborn, defined as surfactant application within the first 24 hours after birth. Among these polymorphisms one polymorphism in *FGFR-4* (rs1966265) is additionally associated with bronchopulmonary dysplasia, demonstrating its possible role in the pathogenesis of newborn lung diseases on grounds of pulmonal immaturity.

Consent

The experiments with genetic material used from humans for this paper were undertaken with the understanding and written consent of each subject. The study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

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