ALLELE-SPECIFIC GLYCOSYLATION OF ILE131THR VARIANT RETARDS SURFACTANT PROTEIN B PROCESSING AND SECRETION: BASIS OF PREDISPOSITION TO RESPIRATORY DISTRESS SYNDROME

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Background: Surfactant protein B (SP-B) is essential for normal lung function. Differences in SP-B levels may be influenced by variation in the encoding gene (*SFTPB*). *SFTPB* SNP Ile131Thr affects proSP-B N-terminal glycosylation.

Aims: To investigate whether proSP-B glycosylation affects propeptide processing and secretion kinetics, and to determine whether differences in amniotic fluid SP-B levels associate with *SFTPB* polymorphisms.

Methods: CHO cell lines expressing either proSP-B/Ile or proSP-B/Thr were generated. The effect of glycosylation on proSP-B secretion kinetics was investigated by pulse-chase labeling. Sample pairs of amniotic fluid and umbilical cord blood were collected from term pregnancies. SP-B in amniotic fluid was quantitated by Western analysis and immunoblotting. Relative protein levels were assessed to detect correlations with four *SFTPB* polymorphisms. To study whether there is any preferential transcription between the allelic variants of Ile131Thr, cDNA from heterozygous lung tissue samples was used for allelic expression imbalance assay.

Results: Glycosylated proSP-B/Thr was secreted from CHO cells at a significantly slower rate than proSP-B/Ile ($p=0.0012$). SP-B levels in amniotic fluid differed significantly among the Ile131Thr genotype groups ($p=0.016$). This SNP, together with gestational age and respiratory distress, was a significant covariate for SP-B levels in a recessive inheritance model. Expression levels of Ile and Thr variant mRNAs were equal in the heterozygote tissue samples, indicating no allelic expression imbalance.

Conclusion: Our results suggest that Ile131Thr variation-dependent proSP-B glycosylation retards the secretion of SP-B *in vitro* and *in vivo* via a post-transcriptional mechanism. Ile131Thr polymorphism may therefore predispose to respiratory distress syndrome.