Surfactant protein A and D polymorphisms and methylprednisolone pharmacogenetics in donor lungs

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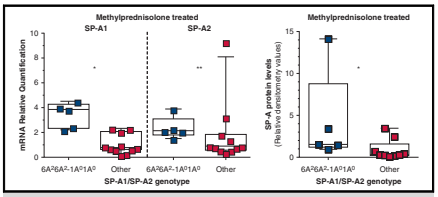
ABSTRACT

Objective: Surfactant proteins A and D are important molecules involved in lung allograft innate immunity. Genetic polymorphisms of surfactant proteins A and D are associated with various lung diseases. In this study, surfactant protein A and D expression responses were investigated during pharmacogenetics upon methylprednisolone treatment as observed during lung transplantation.

Methods: A human cell line (NCI-H441) and precision-cut lung slices from 16 human donors were incubated with methylprednisolone, and surfactant protein A1, surfactant protein A2, and surfactant protein D messenger RNA and surfactant protein A protein expression were assayed. Surfactant protein A1, A2, and D polymorphisms and surfactant protein A gene and protein expressions were determined.

Results: In NCI-H441 cells, methylprednisolone treatment at 10^{-5} M and 10^{-6} M reduced surfactant protein A1 and surfactant protein A2 messenger RNA and surfactant protein A protein expression ($P < .05$). A pharmacogenetic relationship was observed in human donor precision-cut lung slices between the surfactant protein A2 ($1A^x$) variants: Surfactant protein A1, A2, and D messenger RNA expression were greater for $1A^0$ versus $1A^1$ ($P < .05$); surfactant protein A1/surfactant protein A2 genotype $6A^26A^2/1A^01A^0$ (n = 5) showed greater surfactant protein A1, A2, and D messenger RNA expression and surfactant protein A protein expression compared with the other surfactant protein A1/surfactant protein A2 genotypes (n = 11) ($P < .05$).

Conclusions: The surfactant protein A genotype and methylprednisolone stimuli influence donor lung surfactant protein A and D expression. Lungs carrying the surfactant protein A2 variant $1A^0$ have a greater expression of surfactant protein A when treated with methylprednisolone. Surfactant protein A polymorphisms could be used to personalize immunosuppressive regimens. (J Thorac Cardiovasc Surg 2019;157:2109-17)



Methylprednisolone influences SP-A mRNA and protein expression according to SP-A genotypes.

Central Message

Our study, although acquired from a small cohort of human donor lungs, for the first time showed that the SP-A genotype and methylprednisolone stimuli influence donor lung SP-A and SP-D expression.

Perspective

This study, as the first reference for future pharmacogenetic studies in lung transplantation, may provide the basis for novel immunosuppressive regimens tailored according to the genetic background of the donor lung. Further studies are required on donor lungs for the purposes of identifying different genotypes to predict the response of these genotypes to methylprednisolone treatment.

See Commentaries on pages 2118 and 2119.

To date, lung transplantation is a good option to counter end-stage lung disease. Long-term outcome is still challenged by multiple dangerous events (ie, infections, rejection, and gastroesophageal reflux micro-aspiration) that contribute to a suboptimal survival.[1-3] Pulmonary surfactant provides the lung with one of its innate defense mechanisms consisting of a mixture of phospholipids and surfactant protein (SP)-A, SP-D, SP-B, and SP-C.[4-6] SP-A and SP-D (collectins serving as opsonins) have also been shown to orchestrate lung immunity by regulating cytokine production in macrophages and neutrophils while also providing direct or indirect modulation of lymphocyte

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Abbreviations and Acronyms
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
mRNA = messenger RNA
PCLS = precision-cut lung slices
SP = surfactant protein

proliferation.[7,8] Determining the regulation of these proteins in lung transplantation is important when selecting the optimal treatment strategy.

Human SP-A and SP-D genes reside in the same chromosome (10q22.2-23.1). SP-A is encoded by 2 genes, SP-A1 and SP-A2, with several polymorphisms within the coding region.[9,10] Three coding single nucleotide polymorphisms (SNPs) of the SP-D gene have been described for amino acid residue 11 (Met^{11}Thr), 160 (Ala^{160}Thr), and 270 (Ser^{270}Thr), the latter being infrequent.[11,12] Gene polymorphisms of the SP-A1, SP-A2, and SP-D proteins may be responsible for quantitative or qualitative differences that may include reduced levels of protein production and variations in protein functionality.[9,10] SP-A and SP-D gene variants are associated with respiratory distress syndrome, idiopathic pulmonary fibrosis, emphysema, and other lung diseases.[11-19]

Expression of SP-A and SP-D is regulated by cAMP, thyroid transcription factor-1, and glucocorticoids.[20-22] SP-A genes and variants have been suggested to display a variety of responses to glucocorticoids.[20-22] This is of interest in lung transplantation given that donor lungs are treated with elevated doses of methylprednisolone before organ retrieval and that the recipient standard immunosuppressive regimen also includes relatively high doses of prednisone.[23] Lung allografts with low levels of SP-A messenger RNA (mRNA) expression just before implantation are associated with reduced survival.[24] Furthermore, donor lung SP-A2 polymorphisms are associated with greater incidence of pneumonia and reduced survival.[25] In contrast, SP-D variants were associated with development of chronic lung allograft dysfunction.[26]

Given these observations, we hypothesized a pharmacogenetic relationship of SP-A and SP-D variants and methylprednisolone. This would be relevant in the context of lung transplantation because lung allografts are exposed to high levels of steroids both before procurement and during the first post-transplant period. By using donor precision-cut lung slices (PCLS) and cell cultures in vitro, we studied the effect of methylprednisolone on SP-A and SP-D expression according to the different genotypes as determined by the SP-A1 and SP-A2 haplotypes and the 2 most frequent SP-D variants, residue 11 (Met^{11}Thr) and residue 160 (Ala^{160}Thr).

MATERIALS AND METHODS

Cell Cultures

NCI-H441 cells (American Type Culture Collection, Manassas, Va) were cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, Calif). Cells were grown in T-25 flasks at 37°C in 5% CO_2 humidified atmosphere. Cells during passages 10 to 15 were plated at approximately 80% confluence and cultured for 24 hours using incremental doses ($10^{-5, -6, -7, -8, -9}$ M) of methylprednisolone (Sigma, St Louis, Mo). Control cells received the vehicle 0.01% (v/v) dimethyl sulfoxide.

Precision-Cut Lung Slice Cultures

Human PCLS were obtained in accordance with Columbia University Medical Center research ethics review board. The donor's proxy and transplant recipients consented for the use of lungs for research in adherence to the principles set forth in the Helsinki Declaration. Donor clinical data were not available. Lung samples from 16 donors were infused with 3% low-melting agarose solution (Sigma Aldrich, Munich, Germany) and cooled on ice. PCLS were obtained 300 μm thick and 8 mm in diameter (Krumdieck tissue slicer, Alabama Research and Development, Munford, AL). PCLS were cultured in Dulbecco's Modified Eagle's medium/nutrient mixture F-12 Ham with L-glutamine and 15 mmol/L HEPES (Sigma Aldrich, Munich, Germany) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, Calif). PCLS were cultured for 48 hours at 37°C in a humidified atmosphere of 5% CO_2. After 24 hours of culture, PCLS were supplemented with methylprednisolone (0 and 10^{-5} M) for 24 hours. Experiments were performed in duplicate or triplicate pending on tissue availability. After stimulations, PCLS were snap-frozen in liquid nitrogen and stored at −80°C before analysis.

Reverse Transcription-Polymerase Chain Reaction

RNA was isolated with RNeasy kits (QIAGEN, Valencia, Calif). RNA was reverse transcribed to cDNA using the High-Capacity cDNA Kit (Applied Biosystems, Carlsbad, Calif). Quantitative polymerase chain reaction was performed using the TaqMan Gene Expression Assays in an ABI 7300H polymerase chain reaction system (Applied Biosystems). TaqMan primers for SP-A1 (HS01921510_S1), SP-A2 (HS00359837_M1), SP-D (HS01108490_M1), glyceraldehyde-3-phosphate dehydrogenase (GADPH) (HS999999905M1), and 18S rRNA (Hs99999901_s1) were used. GADPH and 18S rRNA served as endogenes for cells and PCLS, respectively. Relative quantification was calculated.

Protein Quantification

Cells were homogenized in RIPA lysis buffer (Thermo Fisher, Rockford, Ill). The homogenate was centrifuged for 10 minutes at 13,000g. Proteins were quantified using the BioRad assay (BioRad, Hercules, Calif). Immunoblots were undertaken with 10 μg of human SP-A antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) at 0.2 μg/mL. Blots were detected by anti-goat horseradish peroxidase–conjugated secondary antibody, followed by enhanced horseradish peroxidase chemiluminescence (Thermo Fisher) and autoradiography. Bands were semiquantified using Quantity One Image software (Kodak MI 4.0). Cell protein levels were normalized to GADPH or cyclophilin A (Abcam, Cambridge, Mass).

Surfactant Protein Genotype

Donor lung DNA was analyzed in a blinded fashion. SP-A1 ($6A^x6A^x$), SP-A2 ($1A^x1A^x$) variant and SP-D gene variants Met^{11}Thr, Ala^{160}Thr were assessed using a pyrosequencing protocol as previously described.[10,19,27] Pyrograms were scored by pattern-recognition software comparing the predicted single nucleotide polymorphism pattern (histogram) with the observed pattern (pyrogram) (Pyrosequencing AB, Uppsala, Sweden).

Statistical Analysis

RNA and protein expression are reported as median (25th-75th percentile range). Box plot graphs represent the median at the 25th-75th percentile range and bars at the 10th and 90th percentile. We used Kruskal–Wallis tests to compare continuous variables across categories. When the Kruskal–Wallis test was P less than .05, we used the Mann–Whitney test to compare continuous variables between categories to preserve an overall alpha of 0.05. Statistical analysis was performed using SAS 9.2 software (SAS Institute Inc, Cary, NC).

RESULTS

Methylprednisolone Treatment of Human Lung Cells

The *NCI-H441* cells were used because they express SP-A[28] and were found to have an SP-A1/SP A2 genotype $6A^4 6A^4/1A^5 1A^5$.[29] SP-A1 and SP-A2 m RNA expression in this cell line was assayed after treatment with methylprednisolone at incremental doses. The data were expressed as relative quantification. SP-A1 mRNA expression was as follows: for methylprednisolone concentration of 10^{-5} M, median 0.38 (25th-75th percentile range, 0.25-0.5); for 10^{-6} M, 0.416 (0.4-1.2); for 10^{-7} M, 1.3 (0.3-2.1); for 10^{-8} M, 1.2 (0.9-2.3); for 10^{-9} M, 1.2 (0.9-1.6) (Kruskal–Wallis $P = .0087$; Mann–Whitney test for 10^{-5} M vs 10^{-8} M $P = .0043$ and vs 10^{-9} M $P = .0043$) (Figure 1, *A*). SP-A2 mRNA expression was as follows: for methylprednisolone, concentration 10^{-5} M, median 0.6 (0.45-0.6); for 10^{-6} M, 0.84 (0.5-3.6); for 10^{-7} M, 1.68 (0.9-3.4); for 10^{-8} M, 1.26 (0.85-5); for 10^{-9} M, 1.2 (1.0-2.8) (Kruskal–Wallis $P = .0506$; Mann–Whitney test for 10^{-5} M vs 10^{-8} M $P = .0152$ and vs 10^{-9} M $P = .026$) (Figure 1, *A*). The SP-A protein expression was as follows: with methylprednisolone 10^{-5} M, median 0 (0-0.02); for 10^{-6} M, 0.02 (0-0.3); for 10^{-7} M, 0.3 (0.01-1.82); for 10^{-8} M, 0.52 (0.14-1.24); for 10^{-9} M, 0.48 (0.03-1.4) (Kruskal–Wallis $P = .0051$; Mann–Whitney test for 10^{-5} M vs 10^{-7} M $P = .026$, 10^{-8} M $P = .0022$, and 10^{-9} M $P = .002$) (Figure 1, *B*).

SP-A variants affect methylprednisolone responses in human PCLS. SP-A1 and the SP-A2 variant frequency (Table 1) and genotypes (Table 2) were examined for each of the 16 human donors. In 5 donor lungs, the SP-A1/SP-A2 genotype was $6A^2 6A^2/1A^0 1A^0$. The donor PCLS were grouped for comparisons according to the presence or absence of the 2 most frequent variants for SP-A1 $6A^2$ (n = 7) and $6A^3$ (n = 4), for SP-A2 variants $1A^0$ (n = 8) and $1A^1$ (n = 4) (lungs with SP-A1 genotype $6A^2$-$6A^3$ and SP-A2 genotype $1A^0$-$1A^1$ were excluded from the groups), and according to SP-A1/SP-A2 genotype, $6A^2 6A^2/1A^0 1A^0$ (n = 5) to be compared with the remaining 11 lungs with a heterogeneous variety of genotypes grouped together and identified as ''other.''

Methylprednisolone treatment of the PCLS with high (10^{-5} M) or low (10^{-9} M) doses showed no difference in the SP-A1 and SP-A2 mRNA expression in all groups (Table 3). Subsequent data comparisons between groups were performed irrespective of the methylprednisolone dose.

After methylprednisolone treatment, donor PCLS grouped for SP-A1 variants showed that SP-A1 mRNA expression was for variant $6A^2$ median 2.9 (2.4-3.7) and for $6A^3$ median 1 (0.3-3.9). SP-A2 mRNA expression was

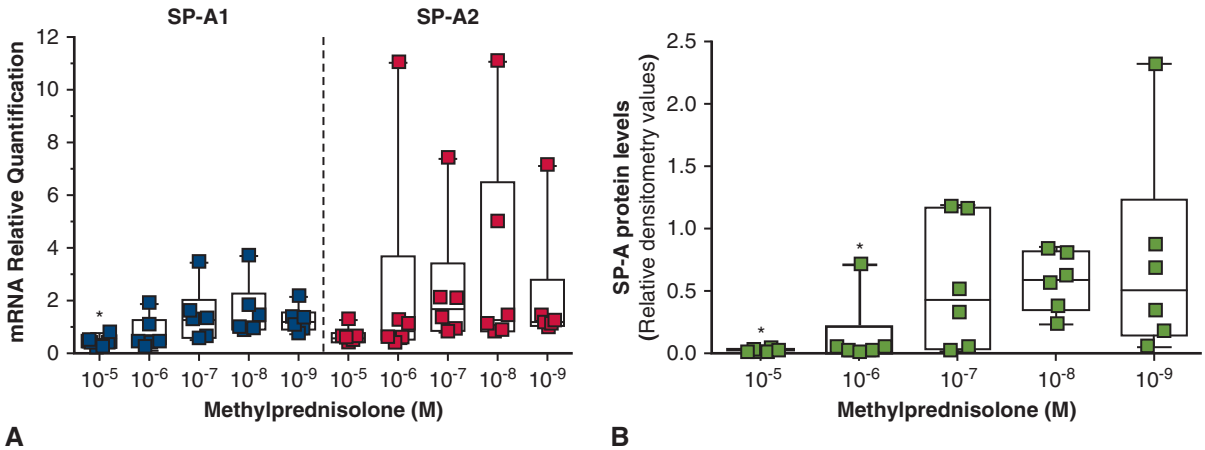

FIGURE 1. A, Relative quantification of SP-A1 and SP-A2 mRNA expression. For SP-A1, Kruskal–Wallis test showed $P < .009$; Mann–Whitney exact tests for 10^{-5} M versus 10^{-8} M showed *$P = .022$ and for 10^{-5} M versus 10^{-9} M $P = .043$. For SP-A2, Kruskal–Wallis test showed $P = .054$. The SP-A1 mRNA expression for cells without treatment (control) median was 0.75 (0.6-1.3); the SP-A2 for control median was 0.98 (0.8-1.2). B, Western blot analysis revealed that methylprednisolone at high concentrations (10^{-5} M to 10^{-6} M) had significantly decreased SP-A protein expression levels compared between groups. SP-A densitometry was normalized to GADPH and relative to control densitometry values were determined. Kruskal–Wallis test showed $P = .0051$. *Denotes $P = .0022$ for 10^{-5} M and $P = .0004$ for 10^{-6} M compared with vehicle treated cells by Mann–Whitney tests for protein. Cells without treatment (control) median: 0.9 (0.7-1.3). *SP*, Surfactant protein.

TABLE 1. Surfactant protein A1 ($6A^x$) and A2 ($1A^x$) allele frequency in human donor lungs

SP-A1 n (allele frequencies)		SP-A2 n (allele frequencies)	
$6A^2$	16 (50%)	$1A^0$	14 (44%)
$6A^3$	11 (34%)	$1A^1$	6 (20%)
6A	2 (6%)	1A	4 (12%)
$6A^4$	3 (9%)	$1A^2$	3 (9%)
		$1A^3$	2 (6%)
		$1A^5$	1 (3%)
		$1A^8$	1 (3%)
		1^{15}	1 (3%)

SP, Surfactant protein.

for $6A^2$ median 2 (0.5-5.4) and for $6A^3$ median 0.5 (0.07-2.2). No significant association was noted for the protein expression.

After methylprednisolone treatment, donor PCLS grouped according to SP-A2 variants showed (Figure 2, *A*) that SP-A1 mRNA expression was for group $1A^0$ median 2.33 (25th-75th percentile range 1.5-3.9) and for group $1A^1$ median 0.9 (0.5-1.6) ($P = .1$). The SP-A2 mRNA expression was for group $1A^0$ median 2.2 (1.8-3.6) and for group $1A^1$ median 0.74 (0.5-0.9) ($P = .017$). The SP-A protein expression was also lower in PCLS from SP-A2 variant $1A^1$ donors (median = 0.45, 25th-75th percentile range = 0.11-2.02) compared with $1A^0$ variant (median = 1.462, 25th-75th percentile range = 0.43-3.41), although this was not statistically significant (Mann–Whitney $P = .23$) (Figure 2, *B*). After treatment with methylprednisolone, donor PCLS grouped according to the SP-A1/SP-A2 genotype (Table 2) showed (Figure 3, *A*) that SP-A1 mRNA expression was as follows: for group $6A^26A^2/1A^01A^0$ median 3.9 (2.4-4.1) and for group "other" 0.8 (0.6-3.1.8) ($P = .004$). The SP-A2 mRNA expression was for $6A^26A^2/1A^01A^0$ median 2.1 (1.9-2.7) and for group "other" 0.9 (0.6-1.7) ($P = .047$). Similar to findings for SP-A2 variants, after methylprednisolone treatment, PCLS from donor lung with SP-A1/SP-A2 genotypes showed that SP-A protein expression was higher compared with the "other" group (Figure 3, *B*). In particular, SP-A protein expression was for genotype $6A^26A^2/1A^01A^0$ median 1.5 (1.2-8.7), and for the "other" genotypes was median 0.3 (0.2-1.5) (Mann–Whitney test $P = .042$).

Surfactant Protein D Variants and Methylprednisolone Responses in Human Precision-Cut Lung Slices

The polymorphisms for aa11 ($Met^{11}Thr$) and aa160 ($Ala^{160}Thr$) of the donor lungs are shown in Table 4. After methylprednisolone treatment, SP-D mRNA expression in the PCLS cultures showed no significant association with the aa11 and the aa160 variants (Figure 4). SP-D mRNA expression after treatment of PCLS with methylprednisolone was significantly associated with the SP-A polymorphic groups. PCLS were grouped according to the SP-A2 variants showing (Figure 4) SP-D mRNA expression for group $1A^0$ median 2.1 (0.8-6.2) and for group $1A^1$ median 0.7 (0.4-1.3) (Mann–Whitney test $P = .04$). No association was observed for the SP-A1 variant groups. SP-D mRNA expression in PCLS was grouped according to donor SP-A genotype (Figure 4): for genotype $6A^26A^2/1A^01A^0$ median 2.3 (2.1-7.9) and for the "other" median 0.6 (0.4-1.3) (Mann–Whitney test $P = .006$).

TABLE 2. Combinations of the observed polymorphic surfactant protein A1 ($6A^x6A^x$) and A2 ($1A^x1A^x$) genotypes in human donor lungs

	SP-A1 genotype							
SP-A2 genotype	**$6A6A^2$**	**$6A6A^4$**	**$6A^26A^2$**	**$6A^26A^3$**	**$6A^26A^4$**	**$6A^36A^3$**	**$6A^36A^4$**	**Total**
$1A1A^0$	1	0	0	0	0	0	0	1
$1A1A^1$	0	0	0	0	0	1	0	1
$1A1A^2$	0	1	0	0	0	0	0	1
$1A1A^3$	0	0	0	1	0	0	0	1
$1A^01A^0$	0	0	**5**	0	0	0	0	5
$1A^01A^1$	0	0	0	0	0	1	0	1
$1A^01A^2$	0	0	0	1	0	0	0	1
$1A^01A^5$	0	0	0	0	1	0	0	1
$1A^11A^1$	0	0	0	0	0	1	0	1
$1A^11A^2$	0	0	0	0	0	0	1	1
$1A^11A^8$	0	0	0	1	0	0	0	1
$1A^31A^{15}$	0	0	0	1	0	0	0	1
Total	1	1	5	4	1	3	1	16

SP, Surfactant protein.

TABLE 3. Surfactant protein A1 and A2 mRNA expression and methylprednisolone doses

Methylprednisolone	SP-A1 mRNA			SP-A2 mRNA		
	10^{-9} M	10^{-5} M	*P*	10^{-9} M	10^{-5} M	*P*
All lungs (n = 16)	0.97 (0.6-2.2)	1 (0.7-2.5)	.6	1.4 (0.8-2)	1.3 (0.7-2.9)	.7
SP-A1 variant groups						
$6A^2$ (n = 11)	2.5 (0.7-3.2)	2.3 (0.6-3.5)	.9	1.8 (0.3-2.8)	1.3 (0.5-4.6)	.9
$6A^3$ (n = 4)	2.5 (0.4-5)	1 (0.2-2.5)	.4	0.5 (0.2-1.6)	1 (0.4-2.2)	.6
SP-A2 variant groups						
$1A^0$ (n = 8)	1.7 (0.9-3.3)	2.2 (1-2.9)	.7	1.6 (1.4-2.9)	2.9 (1.1-4.6)	.4
$1A^1$ (n = 4)	0.7 (0.3-1.1)	1 (0.66-2.2)	.4	0.5 (0.3-1)	0.7 (0.5-1)	.6
Genotype groups						
$6A^26A^2$-$1A^01A^0$ (n = 5)	2.6 (2-5.9)	2.3 (1.9-3.8)	.7	1.6 (1.5-3.2)	2.6 (1.3-3.5)	.7
Other (n = 11)	0.8 (0.61)	0.9 (0.5-1.7)	.4	1.1 (0.6-1.9)	1.1 (0.6-1.9)	.9

mRNA, Messenger RNA; *SP*, surfactant protein.

DISCUSSION

This study is the first attempt to investigate the pharmacogenetic relationship between SP-A and SP-D polymorphisms and the impact of methylprednisolone on SP-A and SP-D expression in human lung parenchyma, particularly within lung transplantation. Our data further support the notion that SP-A polymorphisms provide regulation of the SP-A expression in the lung. We documented in a human pulmonary cell line, and as shown in the Graphical Abstract, in human donor PCLS the relationship between SP-A gene polymorphisms and the influence of methylprednisolone on SP-A expression. The findings revealed that the SP-A expression after treatment with methylprednisolone differed according to the SP-A2 polymorphism. Human lungs with SP-A2 variant $1A^0$ (Figures 2 and 3) had a significantly greater SP-A expression when treated with methylprednisolone. We also observed that the SP-A2 pharmacogenetics also associated with SP-D expression. This pilot study stimulates further research investigating immunosuppressive regimens also tailored according to the genetic background of the donor lung.

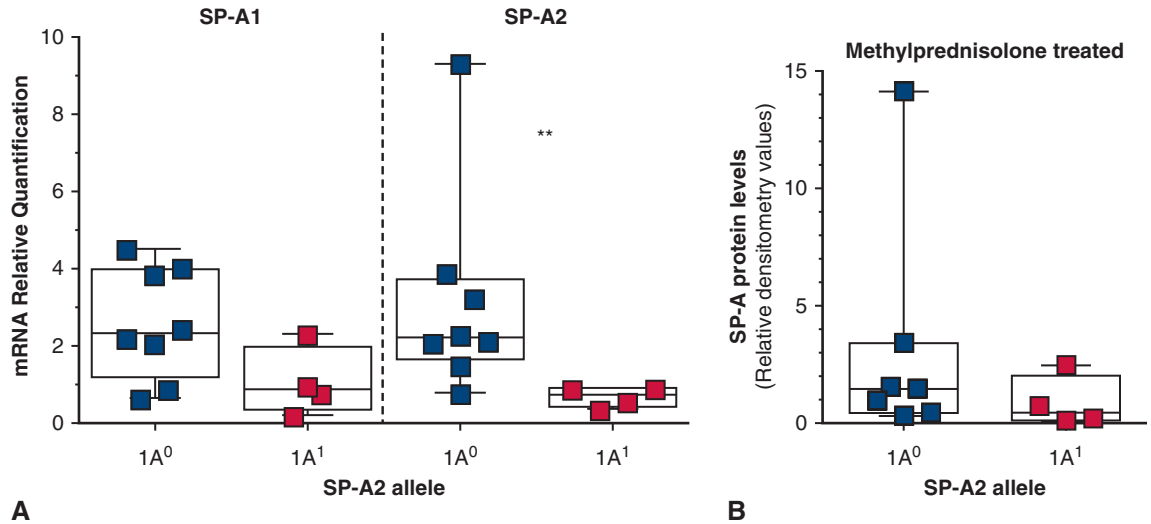

FIGURE 2. A, Quantitative reverse transcription-polymerase chain reaction assessment of the mRNA expression for SP-A1 and SP-A2 after incubation for 24 hours of PCLS with methylprednisolone. Relative quantification was expressed according to the presence or absence of the 2 most frequent SP-A2 variants: group $1A^0$ and group $1A^1$ (donor lung with genotype 1A01A1 was excluded from the groups). Mann–Whitney exact test showed for $^{**}P = .02$. In PCLS without treatment (control) the SP-A1 mRNA expression was for group $1A^0$ median was 0.78 (0.6-1.7) and for group $1A^1$ median was 1.18 (0.3-1.5). The SP-A2 expression in control PCLS was for group $1A^0$ median was 0.45 (0.4-2.3) and for group $1A^1$ median was 1.05 (0.2-1.8). B, Western blot analysis revealed that SP-A protein levels are reduced in PCLS with a $1A^1$ SP-A2 variant treated with methylprednisolone (10^{-5} M and 10^{-9}M) compared with $1A^0$ SP-A2 variant treated with methylprednisolone (10^{-5} M and 10^{-9} M). SP-A densitometry was normalized to cyclophilin A, relative densitometry values were determined, and representative blots are shown. Mann–Whitney test was used to determine significant changes for gene expression and for protein changes, with * denoting a *P* value = .23. The SP-A protein levels in PCLS without treatment (control) was for group $1A^0$ median 0.68 (0.2-2.0) and for group $1A^1$ median 0.96 (0.05-2). *SP*, Surfactant protein.

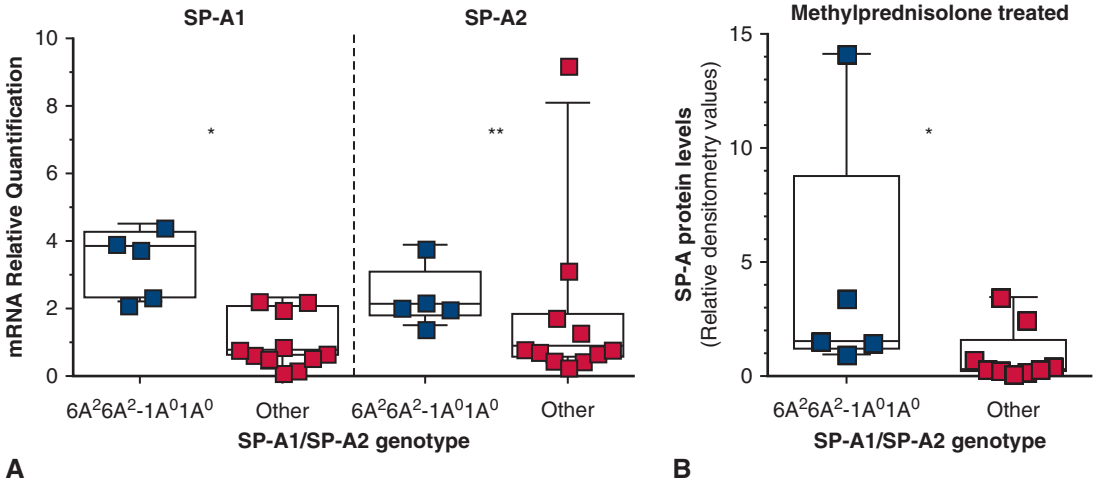

FIGURE 3. A, Quantitative reverse transcription-polymerase chain reaction assessment of the mRNA expression for SP-A1 and SP-A2 after incubation for 24 hours of PCLS with methylprednisolone. Relative quantification was expressed according to the genotype $6A^2 6A^2/1A^0 1A^0$ and the remaining 11 grouped as "other." Mann–Whitney exact test showed for * $P = .002$ and for ** $P = .0002$. In PCLS without treatment (control), the SP-A1 mRNA expression for group $6A^2 6A^2/1A^0 1A^0$ was median 0.86 (0.4-1.7) and in "other" median was 0.94 (0.4-1.8); the control SP-A2 mRNA for group $6A^2 6A^2/1A^0 1A^0$ median was 0.52 (0.4-1.9) and in "other" median was 0.64 (0.3-1.6). B, Western blot analysis revealed that SP-A protein levels are greater in PCLS with $6A^2 6A^2/1A^0 1A^0$ genotype treated with methylprednisolone compared with the remaining 11 grouped as "other" treated with methylprednisolone. SP-A densitometry was normalized to cyclophilin A, relative densitometry values were determined, and representative blots are shown. The Mann–Whitney test was used to determine significant changes, with * denoting a $P = .042$. The SP-A protein levels in control PCLS for group $6A^2 6A^2/1A^0 1A^0$ was median 0.37 (0.29-2.0) and for the "other" group control median 0.5 (0.18-1.2). *SP*, Surfactant protein.

We initially tested human lung adenocarcinoma NCI-H441 cells. These cells were chosen because they are the only cells that express SP-A in a consistent manner.[28-31] Of note, the NCI-H441 human cell line expresses an uncommon SP-A1/SP-A2 genotype ($6A^4 6A^4/1A^5 1A^5$).[32] NCI-H441 cells were treated with methylprednisolone at a 10-fold incremental dose from 10^{-9} M to 10^{-5} M. Methylprednisolone at high concentrations significantly reduced the mRNA and protein expression of SP-A compared with lower doses. These observations confirm what was previously reported using dexamethasone in the same cell line.[28,29] Methylprednisolone and dexamethasone, although both are glucocorticoids, have different clinical applications and dexamethasone rarely is used in clinical lung transplantation because it is associated with worse clinical outcomes. A similar investigation was then conducted in a human donor PCLS culture model. This is a more difficult model to implement, particularly from a logistic standpoint given that human donor lungs available for research are a scarce resource, although it is the most relevant biological model from which meaningful clinical conclusions can be drawn. The SP-A1 and SP-A2 variant frequencies seen in our donor lung study population (Table 1) reflect that of the larger patient populations described in the literature.[10,13,27] Within the cohort of 16 donor lungs tested, 5 lung grafts (Table 1) had the SP-A1/SP-A2 genotype $6A^2 6A^2/1A^0 1A^0$, which is the most common genotype identified in the human population.[10,13,27]

TABLE 4. Observed frequency for surfactant protein D variants residue 11 ($Met^{11}Thr$) and residue 160 ($Ala^{160}Thr$) in human donor lungs

	SP-D variant			
	$Ala^{160}Ala$	$Ala^{160}Thr$	$Thr^{160}Thr$	Total
$Met^{11}Met$	3	1	2	6
$Met^{11}Thr$	6	3	0	9
$Thr^{11}Thr$	1	0	0	1
Total	10	4	2	16

Values are the number of patients with the specified SP-D variant. *SP*, Surfactant protein.

When investigating PCLS in human donors and the pharmacogenetic relationship between methylprednisolone treatment and the SP-A1 and SP-A2 most frequent variants, our results indicated that the SP-A1 variants $6A^2$ and $6A^3$ had no influence on the response of the SP-A1 and SP-A2 mRNA expression. In contrast, PCLS obtained from lungs grouped according to the presence of SP-A2 alleles $1A^0$ or $1A^1$ showed a different response: SP-A2 variant $1A^0$ had a greater mRNA and protein expression after methylprednisolone treatment and seems to be stimulated by methylprednisolone treatment, although variant $1A^1$ seems to be inhibited by the steroid (Figure 2, *A*). Donor lungs with the SP-A1/SP-A2 genotype $6A^2 6A^2/1A^0 1A^0$ compared with the other donor lungs that were identified by the various less frequent genotypes

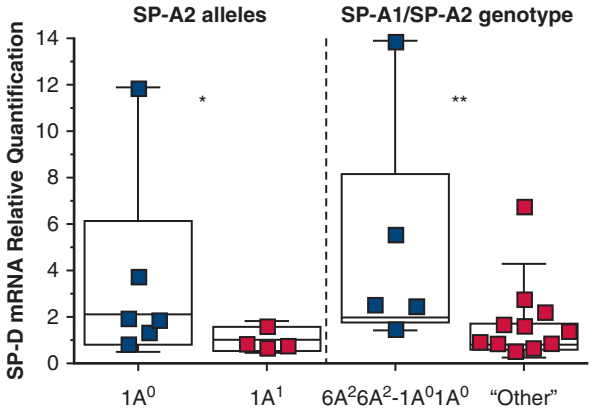

FIGURE 4. SP-A2 variants and genotypes influence SP-D expression after methylprednisolone treatment. Quantitative polymerase chain reaction was performed for SP-D mRNA expression after incubation for 24 hours of the PCLS with methylprednisolone. Relative quantification of the SP-D mRNA is expressed as fold differences of mRNA expression relative to the untreated control samples in PCLS grouped according to SP-A2 variants $1A^0$ and $1A^1$ and according to the SP-A1/SP-A2 genotype $6A^26A^2/1A^01A^0$, and the remaining 11 grouped as "other." Only methylprednisolone-treated analyses are shown (10^{-5} M and 10^{-9} M combined data). Mann–Whitney test $*P = .04$, $**P = .006$. The SP-D mRNA expression in PCLS without treatment (control) was for group $1A^0$ median 0.6 (0.3-1.9) and for group $1A^1$ median 1 (0.6-1.3); for group $6A^26A^2/1A^01A^0$ median 1.1 (0.3-1.56); and for the "other" group median 1 (0.6-1.2). *SP*, Surfactant protein.

(Table 2) consistently showed a significantly greater SP-A1 and SP-A2 mRNA (Figure 3, *A*) and SP-A protein expression after methylprednisolone treatment (Figure 3, *B*). Moreover, SP-D expression was also subject to the SP-A pharmacogenetic interaction with methylprednisolone (Figure 4). In contrast, no pharmacogenetic relationship was noted with the 2 most frequent SP-D single nucleotide polymorphisms (aa11 and aa160) that were investigated. It is not understood how the pharmacogenetic relationship between methylprednisolone and SP-A2 variants is associated with SP-A 1 and SP-D expression. SP-A2 was recently shown to regulate microRNA, which we speculate could in turn regulate the SP-A and SP-D expression.[33]

The results derived from the human donor PCLS model were different from the findings using the human lung cancer cell model with an SP-A1/SP-A2 genotype $6A^46A^4/1A^51A^5$. The cell line showed a significant reduction of SP-A expression when methylprednisolone was used at high doses (Figure 1), but this was not observed at low doses. The human cancer cells we have used in this study express a rare SP-A genotype. Although the results are from different models (immortalized adenocarcinoma cells and PCLS cultures), it is possible that some of the less frequent or rare genotypes encountered in the human population may eventually show a similar response as observed in NCI-H441 cells.

Glucocorticoids have been shown to have a biphasic regulation of the SP-A expression either stimulating or inhibiting accumulation in cultured human fetal lungs depending on the dose and time of exposure.[30,31] Moreover, there appears to be a differential regulation of the SP-A1 and SP-A2 genes.[24,25,31,32] Glucocorticoid treatment in vivo using a preterm sheep model triggered SP-A expression.[34] The concentrations of methylprednisolone used in our experiments mimic those reported in the literature investigating in vitro the hormonal regulation of SP-A expression.[28-32,34] How they relate to the doses used in the clinical transplant setting is difficult to extrapolate. It is not possible to exactly compare the doses used regularly in clinical treatment administered intravenously according to patient weight with the concentration of the drug administered in the culture media in our cell and PCLS cultures, and not knowing the weight of the cells and tissues.

Our results in the human PCLS model showed no influence of the methylprednisolone dose but did show a definite diverse pattern of SP-A expression according to the SP-A polymorphic variants. Our results suggest that lung allografts according to their SP-A genotypes may be able to provide potentially greater or less active surfactant innate immune system after lung transplantation, given the same regimen of steroid treatment. In particular, lungs with SP-A genotype $6A^26A^2/1A^01A^0$ showed a significantly greater SP-A expression after methylprednisolone treatment (Figure 3, *A* and *B*).

The information generated from our study is of interest given that the lung-specific innate immunity provided by surfactant and its proteins plays an important role in the first line of defense from the various noxious events encountered in lung transplantation.[1-3] SP-A was reduced in a rodent model of lung transplant ischemia–reperfusion injury, and endobronchial treatment with SP-A–enriched surfactant improved lung function.[35] Moreover, in humanized transgenic mice in which each was carrying a different SP-A variant, differences in lung function were observed after infection.[36] We reported that the donor lung allograft SP-A and SP-D polymorphism influence the post-transplant outcomes.[24-26] In particular, SP-A2 variant $1A^0$ has a protective effect toward survival. In humanized transgenic mice, those carrying SP-A2 variant 1A0 had greater survival after Klebsiella pneumonia infection.[37] Lung transplant recipients of allografts with SP-A2 genotype $1A^0$-$1A^0$ had significantly greater SP-A mRNA expression before implantation, which resulted in improved clinical outcomes.[24,25] Of note, donors before organ procurement are treated with 1 to 2 g of intravenous methylprednisolone every 12 hours, and lung transplant recipients are treated with 0.5 mg/kg of methylprednisolone early post-transplant, which is slowly tapered over the first year, thereafter reaching a

maintenance dose of 5 to 10 mg twice per day. Reduced levels of SP-A and SP-D proteins in the bronchoalveolar lavage collected at surveillance bronchoscopies in lung transplant recipients are associated with the development of lung allograft dysfunction.[38]

CONCLUSIONS

Our findings, although acquired from a small cohort of human donor lungs, show for the first time and serve as proof-of-concept that there is a significant pharmacogenetic relationship between the SP-A polymorphic genotype and the methylprednisolone treatment on SP-A expression. Of note, these findings are donor lung related and not recipient driven. Further studies are required on donor lungs for the purpose of identifying different genotypes that are associated with different patterns of expression from that observed in the current cohort of human donor lungs or the human cancer cell line genotype as a response of these genotypes to methylprednisolone treatment. These findings will need to be confirmed in vivo. The findings from this and future pharmacogenetic studies in lung transplantation may stimulate further research investigating immunosuppressive regimens tailored according to the genetic background of the donor lung. These putative future regimens could then be implemented in the routine management of lung transplant recipients.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: lung transplant, methylprednisolone polymorphism, pharmacogenetics, surfactant protein A and D