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Donor Surfactant Protein A2 Polymorphism and Lung Transplant Survival

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Running Head: Donor lung SP-A2 genotype & Lung Transplant Survival

Keywords: surfactant protein A, polymorphism, lung transplant, survival.

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ABSTRACT

Purpose: Gene polymorphisms of surfactant proteins, key players in lung innate immunity, have been associated with various lung diseases. The aim of this study was to investigate the potential association between variations within the SP-A gene of the donor lung allograft and recipient post-transplant outcome. **Methods:** Lung-Tx pts (n=192) were prospectively followed by PFTs, bronchoscopies with BAL and biopsies. Donor lungs were assayed for SP-A1 ($6A^n$) and SP-A2 ($1A^n$) gene polymorphism by using the pyrosequencing method. Unadjusted and adjusted stratified Cox survival models are reported. **Results:** SP-A1 and SP-A2 genotype frequency and lung transplant recipient and donor characteristics as well as the cause of death are noted. Recipients were grouped per donor SP-A2 variants. Individuals that received lungs from donors with the SP-A2 $1A^0$ (n=102) versus $1A^1$ variant (n=68) or SPA2 genotype $1A^01A^0$ (n=54) versus $1A^0A^1$ (n=38) had greater survival at one year (logrank $p<0.025$). No significant association was noted for SP-A1 variants. Stratified adjusted survival models for one year survival and diagnosis showed a reduced survival for $1A^1$ variant and the $1A^01A^1$ genotype. Furthermore, when survival was conditional on one year survival no significance was observed, indicating that the survival difference were due to the first year's outcome associated with the $1A^1$ variant. **Conclusion:** Donor lung SP-A gene polymorphisms are associated with post-transplant clinical outcome. Lungs from donors with the SP-A2 variant $1A^1$ had a reduced survival at one year. The observed donor genetic differences, via innate immunity relate to the post-transplant clinical outcome.

Key words: lung transplant, gene polymorphisms, surfactant protein A, lung allograft rejection, lung immunity.

INTRODUCTION

Lung transplantation is a widely accepted therapeutic option for end stage lung disease. Clinical outcome is yet challenged by primary graft failure responsible for the majority of the early mortality, and by chronic allograft dysfunction and chronic rejection accounting for more than 30% of deaths after the third postoperative year. Lung transplantation suffers recipient and graft survival, significantly lower than for liver, kidney, and heart transplantation. The lung's ongoing exposure to the environment is likely the determining factor, with a significant role attributed to the performance of its defense mechanisms.

Pulmonary surfactant and the surfactant related proteins are primary components of the organ's specific innate immunity. As such they serve as one of the first host defense mechanisms the lung can mount against the various insults. Surfactant phospholipids in addition to lowering the alveolar surface tension, also serve as part of the physical mucosal barrier [1, 2]. Similarly, surfactant associated proteins A, B, C, and D play different specific roles with respect to surface tension lowering function, phospholipid homeostasis, and innate and adaptive immunity [3-8]. The hydrophilic surfactant proteins (SP) SP-A and SP-D play essential roles in innate host defense, interacting between the innate and the adaptive immune systems [3-8].

SP-A biologic activity seems to be genetically determined and its polymorphisms have been associated with several lung disease: respiratory distress syndrome, idiopathic pulmonary fibrosis, emphysema [9, 10]. The two *sftpa1* and *sftpa2* genes encoding the proteins SP-A1 and SP-A2 respectively, have been identified with several polymorphisms within the coding regions, SP-A1 (6A, $6A^{2-20}$) and SP-A2 (1A, $1A^{0-13}$) [11, 12]. In particular, SP-A2 is the predominant and very polymorphic SP-A protein present in the adult human airways [13].

We showed a differential SP-A protein expression in the peri lung transplant phase according to the SP-A2 variants and further showed a significant pharmacogenetic relationship with of the SP-A2 methylprednisolone treatment [14, 15].

These observations allowed us to formulate the main hypothesis for this project that focuses on pulmonary surfactant protein A2 polymorphisms determining lung allograft survival.

METHODS

The study was approved by the Institutional Review Board of the Columbia University Medical Center. Informed consent in adherence to the principles set forth in the Helsinki Declaration was obtained from each patient for the collection of blood samples from the donor of the lung allograft prior to implantation. We performed a retrospective analysis of prospectively banked samples. All samples were prospectively collected specifically to test the hypothesis of gene and

protein expression associations with post lung transplant outcomes. This study included 192 patients consecutively transplanted. Post lung transplantation patients underwent pulmonary function testing in the lung transplant clinic weekly for the first 3 months. Beyond 3 months, patients were seen monthly for the first year and on alternating months for the second year. Follow-up frequency was extended to every 3 months beyond the second year after surgery.

Recipient data were prospectively collected with regards to the development of CLAD (chronic lung allograft dysfunction) as determined by the permanent drop of the FEV1 by more than 20% from their post-transplant baseline and survival. CLAD development was monitored using routine pulmonary function tests. The interval of time from transplant to development of CLAD and survival after transplant was monitored.

Donor and recipient clinical information

Donor data were collected with regard to age, smoking history, gender, last pO2/FiO2 prior to procurement and duration of cold ischemic preservation.

All donors received 2g methylprednisolone. Recipient data were collected with regard to the development of primary graft dysfunction (PGD) in the first 3 post-transplant days; development of CLAD was determined by the permanent drop of the FEV1 by more than 20% from their post-transplant baseline and survival. The time from transplant to development of CLAD and death was monitored.

Biological samples from lung allograft

Blood samples from the donor of the lung allograft were collected at the time of lung procurement. Blood samples were stored at -80ºC for subsequent analysis. All samples were batched and assayed at once. Total DNA was extracted from blood with a DNA extraction kit (Qiagen, USA), according to the manufacturer's instructions. The quality of the DNA was assessed prior to assaying. Donor demographics and clinical data were collected by Organ Procurement Organization personnel and recorded in the donor medical record.

Lung allograft SP-A gene variants

The SP-A1 and SP-A2 gene polymorphisms were assessed in a blinded fashion for donor and recipient characteristics and for clinical outcomes. The SP-A1 and SP-A2 single nucleotide polymorphism (SNPs) assessment was done using a pyrosequencing protocol. The PCR–based RFLP genotype method for SP-A provided the basis for the pyrosequencing protocol, which is a primer–based DNA sequencing method. Pyrograms are scored by pattern–recognition software that compare the predicted SNP pattern (histogram) to the observed pattern (pyrogram) (Pyrosequencing AB, Uppsala, Sweden). Scoring of SP-A1 (6A, $6A^{2-20}$) and SP-A2 (1A, $1A^{0–13}$) gene variants was done as previously described [16, 17].

Statistical analysis

We analyzed categorical data using the Chi Square and Fisher's Exact tests. We constructed stratified Cox proportional hazards models to examine associations between genotype and time to events of interest (CLAD and survival) with strata for recipient disease and with adjustment for a priori purposefully selected recipient variables known to affect outcome after lung transplantation (i.e. precision variables). For time-to-death analyses, we censored follow-up time at the end of the study. For the CLAD analyses, we censored follow-up time at death and at end of study. Variants were assumed to have additive effects. There were no missing covariate data.

Differences were considered significant when the p value was less than 0.05. Continuous variables are expressed as medians and 25^{th} to 75^{th} percentile range. Statistical analysis was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Donor and Recipient information: Table 1 shows the donor lung SP-A1 and the SP-A2 variant frequencies seen in our cohort of lung transplant recipients. Table 1s shows the SPA1 and SPA2 genotype combinations. The overall characteristics of the 192 lung transplant recipients and of the donors are shown in Table 2.

The overall median follow-up time was 1290 days (823–1843). To date 60/192 patients have died, 4 within 30 days from the lung transplantation and 56 with a median survival of 719 days (274–1269). The 132 patients currently alive have a median follow-up of 1595 days (1091–2054). CLAD was diagnosed in 88 patients, 38 of whom have died with median survival of 902 days (575–1422) and 50 of whom are currently alive with a median follow-up of 1841 days (1450–2296). Patients alive and free of CLAD are 81 with a median follow-up of 1303 days (1006–1823). Table 2 shows the overall recipient and donor characteristics of the study cohort.

Grouping of lung transplant recipients; SP-A2 variant and genotype associations:

Lung transplant recipients were grouped according to the two most frequent donor SP-A1 ($6A^2$ and $6A^3$); and SP-A2 variants ($1A^o$ and $1A^1$). No association with clinical outcomes was noted for the SP-A1 variants. Figure 1 shows the non-adjusted survival curves for patient grouped according to the SP-A2 variants as well as the curves conditional to one-year survival. No association was noted for freedom from CLAD. The characteristics of the patient grouped according to the SP-A2 single nucleotide polymorphic variants for $1A^0$ and $1A^1$ shown in Table 3 include recipient age, gender, end stage pulmonary disease, type of transplant, cytomegalovirus (CMV) mismatch and gender mismatch. With regard to the distribution of the recipient diagnosis some differences were noted: in particular, for cystic fibrosis (CF) 24% for variant $1A^0$ versus 17% for variant $1A^1$, for COPD 25% for variant $1A^0$ versus 34% for variant $1A^1$ whereas for ILD and other were approximately half and half of each variant (Table 3).

Figure 2 shows the non-adjusted survival curves for patient grouped according to the SP-A2 genotypes $1A^0 1A^0$ and $1A^0 1A^1$ as well as the curves conditional to one-year survival. The characteristics of the patient grouped according to the SP-A2 genotypes for $1A^0 1A^0$ and $1A^0 1A^1$ shown in Table 4 include recipient age, gender, end stage pulmonary disease, type of transplant, cytomegalovirus (CMV) mismatch and gender mismatch. With regard of the distribution of the recipient diagnosis some differences were noted: for cystic fibrosis (CF) 28% for genotype $1A^0 1A^0$ versus 18% for variant $1A^0 1A^1$, although for COPD 24% for genotype $1A^0 1A^0$ versus 37% for $1A^0 1A^1$ and for ILD and other about half and a half with each genotype (Table 4).

One-year survival: Table 5 shows the one-year survival models stratified for recipient diagnosis, with adjustment for donor polymorphic variation in $1A^1$, recipient age, gender, procedure type, CMV status, and gender mismatch. The data showed a significant effect in recipients with regards to the presence of donor variant $1A^1$ ($p=0.02$), age ($p=0.04$) and bilateral lung Tx ($p=0.04$) on the one-year survival. Table 5 also shows the one-year survival models for the SP-A2 genotypes stratified for recipient diagnosis, with adjustment for donor polymorphic variation in $1A^1$, recipient age, gender, procedure type, CMV status and gender mismatch. The SP-A2 $1A^0 A^1$ genotype was associated with a significant overall reduction in survival ($p=0.02$) in the first year. Table 6 shows the cause of death within the first year, according to the donor $1A^1$ and $1A^0$ variants as well as according to the donor SP-A2 genotypes $1A^0 1A^0$ and $1A^0 1A^1$.

DISCUSSION

The role of the genetic background of the donor lung has not been sufficiently explored in relation to lung transplant recipient outcomes. This study reports novel findings with regards to the role played by SP-A gene polymorphisms in clinical outcomes post-lung transplantation. Donor lung SP-A polymorphic variants, may serve as predictors of post-transplant recipient survival. The SP-A1 and SP-A2 gene polymorphisms were investigated, uncovering a significant association between the donor lung SP-A2 variants and post lung transplant recipient survival.

We have previously reported that donor lung SP-D polymorphisms predict chronic lung allograft dysfunction, although no association to survival was reported for any of those variants [18]. Hence, the two hydrophilic surfactant proteins SP-D and SP-A may have a distinct impact on the overall innate and adaptive response to pathogens.

The human SP-A locus consists of two functional genes, *sftpa1* and *sftpa2* encoding SP-A1 and SP-A2, respectively. Each gene has been identified with several polymorphisms within the coding region, which may or may not be subject to amino acid substitutions, SP-A1 (6A, $6A^{2}$-^{20}), and SP-A2 (1A, $1A^{0}$-^{13}) [16, 12]. Associations of SP-A1 and SP-A2 variants have been shown for several pulmonary diseases [9, 10] and mutations in these genes are found in patients with interstitial lung disease and lung cancer. Despite such frequency, their pathologic mechanism is poorly understood [19]. Gene polymorphisms of SP-A1 and SP-A2 may be responsible for both quantitative and qualitative differences in

levels of protein synthesis, variations in protein functionality, or an altered ratio between the two proteins SP-A1 and SP-A2 [20-24]. Hence, within the context of organ specific innate immunity, a compromised surfactant proteomic composition may largely contribute to a deficiency in first-line response to various insults.

Recent animal studies have shown that not only SP-A1 and SP-A2 variants distinctively affect the alveolar macrophage miRNome [25], but most relevantly they also differentially affect lung function and survival after infection [26, 27]. It is therefore conceivable that SP-A gene variants may contribute to the complex etiologic pathogenesis of lung allograft dysfunction. This retrospective study, although with a potential bias from uneven distribution of patient characteristic that was taken in account in the adjusted statistical analysis, documents this association for the first time showing a significantly greater risk of death within the first-year post lung transplant for recipients of donor lungs with SP-A2 variant $1A^1$ and genotype $1A^0 1A^1$ compared to others (see Figure 1 and Table 5). This appears to be due to a greater incidence of death for infection (see Table 6). Interestingly, Dominic and colleagues [28] demonstrated that homozygosity of the SP-A2 variant, $1A^1$, was associated with an increased risk of meningococcal disease, suggesting a recessive effect of this variant. The carriage of another SP-A2 variant, $1A^5$, was significantly associated with a reduced risk of infection, suggesting a dominant effect of this variant. Variants $1A^1$ and $1A^5$ are identical at the codons that encode amino acids 9 and 140, but they differ at amino acids 91 and 223 [28]. The change in amino acid 223 between these two variants is significant. Variants $1A^5$ and $1A^0$ that were associated with reduced infection risk

($1A^5$) [28] and better survival ($1A^0$) in the present study and in a recent mouse study [27] have the same amino acid, namely a glutamine. This charged amino acid may contribute to a better outcome.

Low levels of SP-A mRNA measured in the lung allograft just prior to implantation appeared to be a negative predictor with respect to post-transplant survival [14]. An association between donor lung SP-A mRNA expression levels and SP-A2 genotype has been previously observed [14]. In particular, the SP-A2 genotype $1A^0A^0$ had greater levels of SP-A mRNA expression in the allograft prior to implantation [14]. Lung donors are treated with very high levels of steroids prior to organ procurement. We recently reported a significant pharmacogenetic relationship between SP-A2 variants and methylprednisolone. Precision cut lung slices from organs with SP-A2 variant $1A^0$ and genotype $1A^01A^0$ showed a significantly greater protein expression when treated with methylprednisolone [15]. These observations are consistent with the present results where recipients from donors with $1A^0$ variant and $1A^01A^0$ genotype exhibited greater survival, indicating that the total SP-A levels determined by donor lung genotype may play a role in post-transplant recipient survival. Interestingly, the survival advantage identified in this study was observed within the first-year during which recipient's immunosuppressive regimes include the greatest levels of steroids.

Lung as with intestine compared to other solid organs suffer the disadvantage of a continuous exposure to the environment, thus they rely on a more active organ specific innate immunity for the first line protection from the ongoing external pathogens [29-32]. Interactions between innate and adaptive immune responses

in these organs in the setting of transplantation are likely a major contributor to the increased graft dysfunction that is seen. It is conceivable that the capability of the lung allograft to withstand the various transplant related insults is driven by its genetic background, and especially that of donor innate immunity. The innate immune molecules, SP-A1 and SP-A2, are subject to differential and complex regulation as shown in vitro and fetal lung explants [33-36].

In conclusion, donor lung SP-A2 gene polymorphisms are associated with post-transplant recipient survival. Further studies are needed to explore the different roles of SP-D and SP-A polymorphisms within the innate and adaptive immune response post-lung transplantation. This study was not designed for a detailed assessment of confounding factors or interaction effects, nevertheless the interesting findings reported significantly add weight to further hypothesis generation regarding the potential impact of genetic polymorphisms for key proteins in the donor lung influencing post lung transplant outcome(s). In fact our findings suggest a constitutive role of the donor innate immunity towards lung transplant recipient outcome.

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TABLES

Table 1. SP-A1 and SP-A2 variant and genotype frequency

SP-A1				**SP-A2**			
variant frequency		**genotype frequency**		**variant frequency**		**genotype frequency**	
$6A^2$	53% (199)	**$6A6A^2$**	5% (10)	**$1A^0$**	**52% (195)**	**$1A^01A$**	9% (18)
$6A^3$	34% (126)	**$6A6A^4$**	0.5% (1)	**$1A^1$**	**19% (73)**	**$1A^01A^0$**	**28% (54)**
6A	6% (23)	**$6A^26A^2$**	28% (53)	1A	10% (38)	**$1A^01A^1$**	**20% (38)**
$6A^4$	7% (28)	**$6A^26A^3$**	34.5% (66)	$1A^2$	9% (33)	**$1A^01A^2$**	6% (12)
		$6A^26A^4$	9% (17)	$1A^3$	2% (9)	**$1A^01A^3$**	1.5% (3)
		$6A^36A^3$	11% (21)	$1A^5$	6% (23)	**$1A^01A^5$**	7.5% (14)
		$6A^36A^4$	4% (8)	$1A^8$	1% (3)	**$1A^11A^5$**	3% (6)
		6A 6A	0.5% (1)	$1A^9$	0.5% (1)	**$1A^11A^8$**	0.5% (1)
		$6A6A^3$	5% (10)	$1A^{10}$	0.5% (1)	**1A1A**	0.5% (1)
		$6A^46A^4$	**0.5% (1)**			**$1A1A^1$**	5% (9)
		Blank	**2% (4)**			**$1A1A^2$**	2% (4)
						$1A^01A^9$	0.5% (1)
						$1A1A^3$	1.5% (3)
						$1A^11A^1$	2.5% (5)
						$1A^11A^2$	5% (10)
						$1A1A^8$	1% (2)
						$1A^21A^2$	1.5% (3)
						$1A^21A^5$	0.5% (1)
						$1A^31A^5$	1% (2)
						$1A^31A^{10}$	0.5% (1)
						Blank	2% (4)
		Total pts	100% (192)			**Total pts**	100% (192)

Numbers in brackets (n) represent the total number of such variant or genotype in the studied patient population. Blank indicate lung in which the genotype could not be determined.

Table 2. Lung transplant recipient and donor characteristics.

Recipients **192**		**Lung donor**	
Males	90 (47%)	Males	99 (52%)
Age	57 (43-62)	Age	35 (23-47)
Bilateral	141 (73%)	Last pO2	457 (402-506)
Disease		Smoking	63 (33%)
COPD	56 (29%)		
ILD	76 (40%)		
CF	37 (19%)		
PPH	6 (3%)		
Bronchiectasis	6 (3%)		
Sarcoidosis	7 (4%)		
Scleroderma	4 (2%)		
CMV mismatch	52 (27%)		
PGD-3 at 72 h	16 (8%)		

Table 3. Donor and recipient characteristics according to the donor SP-A2 variants.

Recipients	**$1A^0$**	**$1A^1$**
n.170	102 (60%)	68 (40%)
Age	56 (43-61)	57 (42-63)
Female	57 (56%)	36 (53%)
Bilateral Tx	78 (76%)	51 (75 %)
CMV Mism.	26 (25%)	21 (31%)
Gender Mism.	36 (35%)	22 (32%)
Disease		
CF	24 (24%)	12 (17%)
COPD	26 (25%)	23 (34%)
ILD	39 (38%)	25 (37%)
Other	13 (13%)	8 (12%)
Donor		
Age	36 (24-47)	40 (22-51)
Smoking	28%	34%
Last pO2	468 (415-504)	425 (393-492)

Table 4. Donor and recipient characteristics according to the donor SP-A2 genotypes.

Recipients	**$1A^0 1A^0$**	**$1A^0 1A^1$**
n.92	54 (59%)	38 (41%)
Age	54 (41-61)	55 (42-62)
Female	30 (56%)	20 (53%)
Bilateral Tx	37 (69%)	30 (79%)
CMV Mism.	13 (24%)	13 (34%)
Gender Mism.	23 (43%)	10 (27%)
Disease		
CF	15 (28%)	7 (18%)
COPD	13 (24%)	14 (37%)
ILD	19 (35%)	13 (34%)
Other	7 (13%)	4 (11%)
Donor		
Age	37 (21-47)	41 (30-55)
Smoking	33%	43%
Last pO2	470 (416-510)	463 (399-507)

Table 5. Adjusted Survival Models for one-year survival stratified per recipient diagnosis.

Patient grouped according to SP-A2 variant $1A^0$ and $1A^1$				
		HR	**95% CI**	**p- value**
DEATH	**$1A^1$**	2.9	1.2- 6.9	**0.02**
	Recipient Age	1	1.0 – 1.1	**0.04**
	Female	0.7	0.27 – 1.8	0.4
	Bilateral Lung-Tx	5.4	1 – 27.5	**0.04**
	CMV Mismatch	0.8	0.3 – 2.2	0.7
	Gender Mismatch	1.1	0.4 – 2.9	0.8
Patient grouped according to SP-A2 genotype $1A^0 1A^0$ and $1A^0 1A^1$				
DEATH	**$1A^0$-$1A^1$**	11.3	1.6- 80.5	**0.02**
	Recipient Age	1.1	1.0 – 1.2	**0.1**
	Female	1.1	0.3 – 4.5	0.9
	Bilateral Lung-Tx	2.9	0.5 – 15.8	**0.2**
	CMV Mismatch	0.4	0.07 – 2.5	0.4
	Gender Mismatch	1.1	0.2 – 6.2	0.9

HZ: Hazard ratio; CI: Confidence Interval

Table 6. Cause of death within first year.

	$1A^1$ (n=12)	$1A^0$ (n=7)
Pneumonia	**8**	4
MOF	2	1
CLAD	1	1
PE	1	1
	$1A^0$-$1A^1$ (n=7)	$1A^0 1A^0$ (n=1)
Pneumonia	**5**	0
MOF	2	0
CLAD	0	0
PE	0	1

MOF = Multi Organ Failure; CLAD = Chronic Lung Allograft Dysfunction; PE = Pulmonary embolism

LEGENDS

Fig. 1 Non-adjusted lung transplant patient actual survival curves of recipients grouped according to the lung allograft donor SP-A2 polymorphic variants $1A^0$ and $1A^1$. A significant greater survival during the first year post lung transplantation was noted for recipients of donor lungs with SP-A2 variant $1A^1$. Mantel-Cox Logrank test showed p=0.015 for the overall survival analysis, although no significant difference for survival in patients that were alive at one year after lung transplantation.

Fig. 2 Non-adjusted lung transplant patient actual survival curves for recipients grouped according to the lung allograft donor SP-A2 polymorphic genotypes $1A^01A^0$ and $1A^01A^1$. A significant greater survival during the first year post lung transplantation was noted for recipients of donor lungs with SP-A2 genotype $1A^01A^0$. The Mantel-Cox Logrank test showed (p=0.016) for the overall survival, although no significant difference in patients that were alive at one year after lung transplantation.

Fig.1

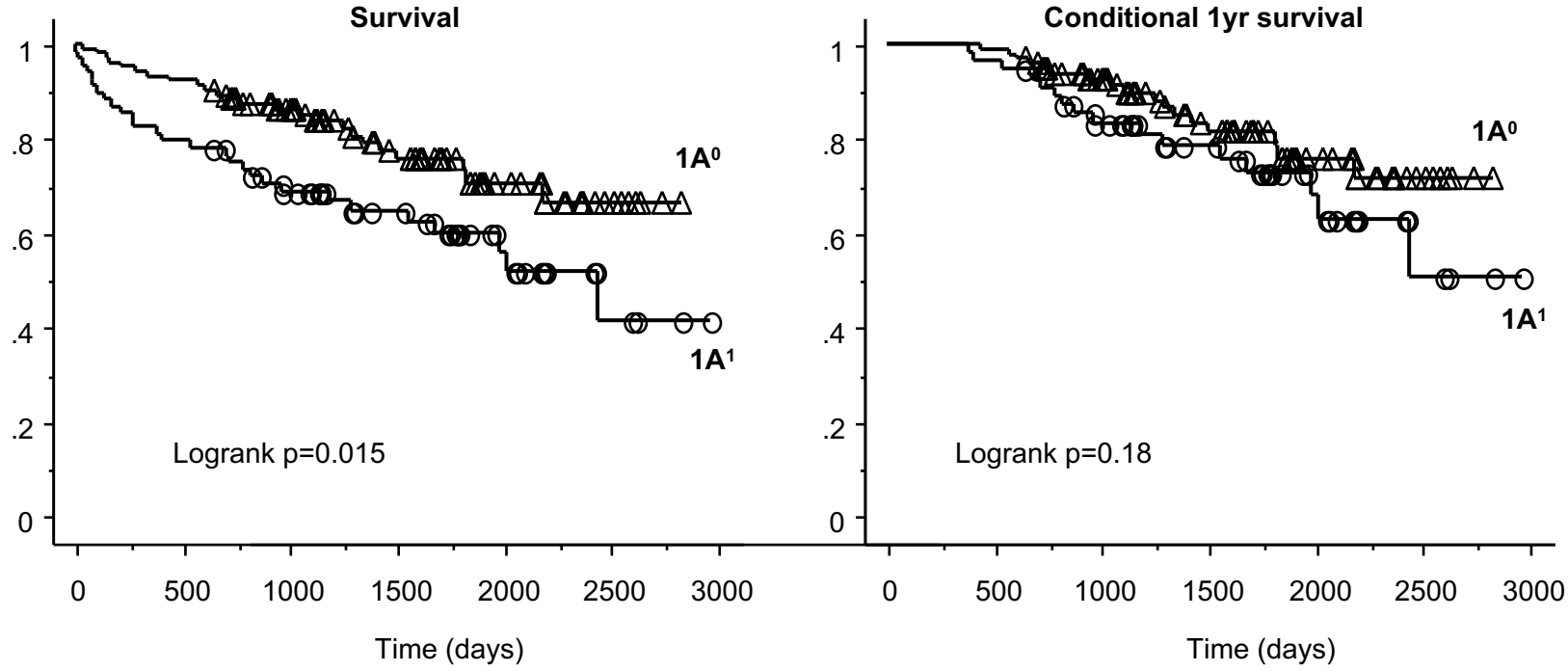
Survival
1
.8
.6
.4
.2
0
1A⁰
1A¹
Logrank p=0.015
0
500
1000
1500
2000
2500
3000
Time (days)
Conditional 1yr survival
1
.8
.6
.4
.2
0
1A⁰
1A¹
Logrank p=0.18
0
500
1000
1500
2000
2500
3000
Time (days)

Fig.2

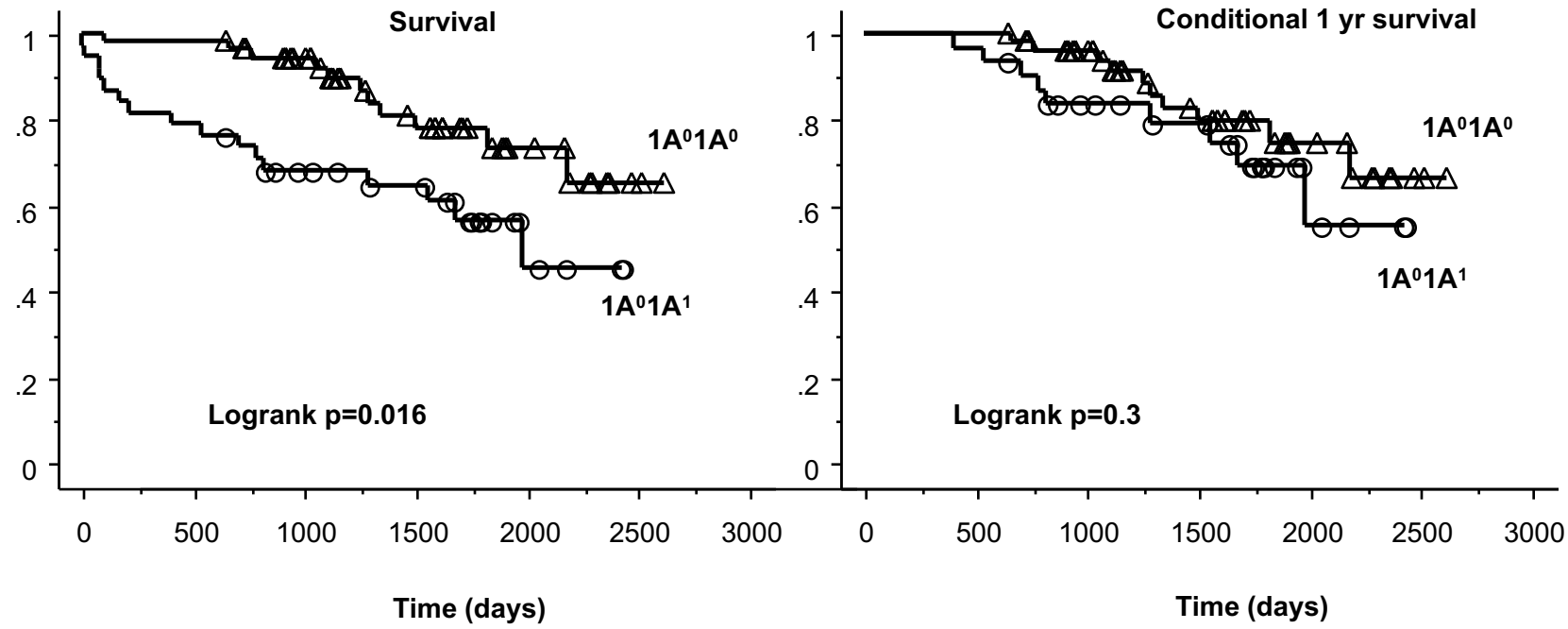
Survival
1
.8
.6
.4
.2
0
1A⁰1A⁰
1A⁰1A¹
Logrank p=0.016
0
500
1000
1500
2000
2500
3000
Time (days)
Conditional 1 yr survival
1
.8
.6
.4
.2
0
1A⁰1A⁰
1A⁰1A¹
Logrank p=0.3
0
500
1000
1500
2000
2500
3000
Time (days)

Table 1s. SP-A1 and SP-A2 genotype combinations

	6A6A	6A6A^2	6A6A^3	6A6A^4	6A^{2}6A^2	6A^{2}6A^3	6A^{2}6A^4	6A^{3}6A^3	6A^{3}6A^4	6A^{4}6A^4	Blank	**Total pts**
1A1A	1	0	0	0	0	0	0	0	0	0	0	1
1A1A^0	0	9	3	1	0	4	1	0	0	0	1	18
1A1A^1	0	1	5	0	0	1	0	2	0	0	0	9
1A1A^2	0	0	2	0	0	0	0	1	0	0	0	4
1A1A^3	0	0	0	0	0	2	0	0	0	0	1	3
1A1A^8	0	0	0	0	0	1	0	1	0	0	0	2
1A^{0}1A^0	0	0	0	0	47	5	0	1	0	0	1	**54**
1A^{0}1A^1	0	0	0	0	3	31	0	4	0	0	0	**38**
1A^{0}1A^2	0	0	0	0	0	12	0	0	0	0	0	12
1A^{0}1A^3	0	0	0	0	2	1	0	0	0	0	0	3
1A^{0}1A^5	0	0	0	0	0	0	13	0	1	0	0	14
1A^{0}1A^8	0	0	0	0	0	1	0	0	0	0	0	1
1A^{0}1A^9	0	0	0	0	1	0	0	0	0	0	0	1
1A^{1}1A^1	0	0	0	0	0	1	0	3	0	0	1	5
1A^{1}1A^2	0	0	0	0	0	2	2	4	2	0	0	10
1A^{1}1A^5	0	0	0	0	0	1	0	2	3	0	0	6
1A^{2}1A^2	0	0	0	0	0	0	0	2	1	0	0	3
1A^{2}1A^5	0	0	0	0	0	0	0	0	0	1	0	1
1A^{3}1A^5	0	0	0	0	0	0	1	0	1	0	0	2
1A^{3}1A^{10}	0	0	0	0	0	1	0	0	0	0	0	1
Blank	0	0	0	0	0	3	0	1	0	0	0	4
Total pts	1	10	10	1	**53**	**66**	17	21	8	1	4	192

Blank indicate lungs in which the genotype could not be determined.