

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Bochud P-Y, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* 2008;359:1766-77.

Table S1. Univariate Analysis of the Association of TLR Alleles in Recipients and Donors with Cumulative Incidence of Invasive Aspergillosis in The Discovery Study.

Gene	Gene Region	SNP	Amino acid change	rs#	MAF	HWE	LD (R ²)	Cumulative Incidence of IA (36 months, %)					
								Patient (N=316)			Donor (N=242)		
								Allele Absent	Allele Present	P (Log rank)	Allele Absent	Allele Present	P (Log rank)
TLR2 (4q32)	Intr. 1	-16934	T/A -	rs4696480*	0.50	0.12		5	11	0.10	3	10	0.09
	Intr. 1	-15607	A/G -	rs1898830*	0.35	0.78		13	7	0.10	7	9	0.58
	Ex. 3	597	T/C N199N	rs3804099*	0.45	0.49		4	12	0.05	7	9	0.63
	Ex. 3	1350	T/C S450S	rs3804100*	0.07	0.11		9	15	0.25	8	10	0.68
	Ex. 3	2258	G/A R753Q	rs5743708	0.03	1.00		10			8	18	0.11
TLR3 (4q35)	5'	-8921	A/T -	rs5743303*	0.19	0.32		10	9	0.85	10	4	0.14
	5'	-8441	T/A -	rs5743305*	0.36	0.93		9	10	0.72	6	9	0.38
	Intr. 3	+2602	G/C -	rs5743314*	0.22	0.90		9	10	0.70	8	9	0.66
	Exon 4	1234	C/T L412F	rs3775291*	0.29	0.61		13	6	0.02	10	7	0.53
TLR4	5'	-3612	A/G -	rs2770150*	0.27	0.91		10	9	0.65	11	6	0.18

(9q32-q33)	5'	-2604	A/G	-	rs10759931*	0.34	1.00	}0.96	9	10	0.74	4	13	0.01
	5'	-1607	T/C	-	rs10759932*	0.13	0.58		10	8	0.52	7	11	0.34
	Ex. 4	1063	A/G	D299G	rs4986790	0.06	0.28		10	9	0.84	7	22	0.01
	Ex. 4	1363	C/T	T399I	rs4986791	0.07	0.07		10	9	0.82	7	22	0.01
	3'	+11381	G/C	-	rs11536889*	0.15	0.20		9	11	0.63	10	4	0.14
	3'	+12186	G/C	-	rs7873784*	0.16	0.44		9	10	0.72	10	5	0.19
TLR9	5'	-1486	T/C	-	rs187084*	0.41	0.38		10	9	0.92	11	7	0.28
(3p21.3)	5'	-1237	T/C	-	rs5743836	0.16	0.76		11	7	0.27	7	11	0.22
	Intr. 1	+1174	G/A	-	rs352139*	0.44	0.49	}0.96	8	11	0.49	6	9	0.44
	Ex. 2	1635	A/G	P545P	rs352140	0.45	0.49		8	11	0.57	7	9	0.48

Numbers indicate cumulative incidence of invasive aspergillosis (IA). SNP stands for single nucleotide polymorphism, MAF for minor allele frequency, HWE for Hardy-Weinberg equilibrium test, LD for linkage disequilibrium. Minor allele frequencies, Hardy-Weinberg equilibrium tests and linkage disequilibrium are calculated together for both Caucasian patients and donors. * indicate haplotype tagging SNPs. Since 20 out of 336 patients and 94 out of 336 donors were not Caucasian or had missing ethnicity, the denominators in the cohort study were 316 patients and 242 donors. SNPs located outside the coding region (introns, 5' or 3' boundaries) were numbered relative to their position in the gDNA sequence upstream ("-") or downstream ("+") the translational start site ("ATG", bp=1). SNPs located in the coding region were numbered relative to the translational start site on the mRNA sequence.

Table S2. Univariate Analysis of the Association of TLR Haplotypes in Recipients and Donors with Cumulative Incidence of Invasive aspergillosis in The Discovery Study.

		Cumulative Incidence of IA (36 months, %)						
		Patient (N=316)			Donor (N=242)			
				P			P	
				(Log			(Log	
		Haplotype	Haplotype	rank	Haplotype	Haplotype	rank)	
Gene		Absent	Present)	Absent	Present		
<i>TLR2</i>	1	TGTTG	12	8	0.19	9	8	0.82
	2	AACTG	8	12	0.18	7	10	0.44
	3	AATTG	10	10	0.84	7	10	0.46
	4	TACTG	10	8	0.62	10	2	0.09
	5	AACCG	9	15	0.22	8	10	0.68
	6	TATTG	10	7	0.66	9	5	0.58
<i>TLR3</i>	1	ATGC	8	12	0.16	8	9	0.64
	2	AACC	9	11	0.57	8	9	0.64
	3	ATGT	11	6	0.16	9	8	0.86
	4	TTGC	10	9	0.79	10	4	0.18
	5	AAGT	11	4	0.09	8	10	0.67
	6	AAGC	9	17	0.15	8	7	0.82
<i>TLR4</i>	H1	GATACGG	10	10	0.91	11	6	0.19
	H2	AATACGG	10	9	0.82	9	8	0.81
	H3	AATACCG	9	11	0.66	10	4	0.15
	H4	AGTACGC	10	11	0.65	9	5	0.35
	H5	AGCACGG	10	8	0.61	7	17	0.04

	H6	AGTGTGG	10	10	0.92	7	22	0.01
	H7	AGCACGC	10	11	0.79	9		
	H8	AGTACGG	10	13	0.67	8	22	0.11
	S1	-A--C-G	6	10	0.40	13	8	0.35
	S2	-G--C-C	10	11	0.73	10	4	0.18
	S3	-G--C-G	10	9	0.73	6	17	0.01
	S4	-G--T-G	10	9	0.84	7	22	0.01
<i>TLR9</i>	1	TTGA	9	10	0.68	6	9	0.42
	2	CTAG	10	10	0.91	10	7	0.28
	3	TCAG	11	7	0.37	7	12	0.19

Numbers indicate cumulative incidence of invasive aspergillosis (IA). Abs. stands for haplotype absent, Pres. for haplotype present. Since 20 out of 336 patients and 94 out of 336 donors were not Caucasian or had missing ethnicity, the denominators in the cohort study were 316 patients and 242 donors.

Table S3. Multivariate Analysis of the Association of Donor's *TLR4* Polymorphisms with the Risk of Invasive Aspergillosis in Both Studies

SNPs (amino-acid change)	Discovery Study			HR (95%CI)	P	Validation Study			OR (95%CI)	P
	IA N=20	No IA N=222	All N=242			IA N=103	No IA N=263	All N=367		
-3612 A/G (-)	35	51	49	0.53 (0.21-1.34)	0.18	46	52	50	0.64 (0.37-1.10)	0.11
-2604 A/G (-)	80	50	52	4.19 (1.39-12.64)	0.01	61	50	53	1.87 (1.10-3.20)	0.02
-1607 T/C (-)	33	23	24	1.70 (0.64-4.53)	0.29	24	24	24	1.01 (0.55-1.86)	0.97
1363 C/T (T399I)	25	8	10	7.97 (2.51-25.32)	<0.001§	16	9	11	2.12 (1.00-4.50)	0.05
+11381 G/C (-)	15	31	30	0.39 (0.11-1.35)	0.14	18	27	25	0.61 (0.32-1.14)	0.12
+12186 G/C (-)	15	29	28	0.43 (0.12-1.46)	0.17	40	30	33	1.83 (1.06-3.15)	0.03
Haplotypes										
H1 GATACGG	35	51	49	Ref.		48	53	51	Ref.	
H2 AATACGG	40	43	43	1.38 (0.47-4.10)	0.56	50	45	46	1.51 (0.88-2.58)	0.13
H3 AATACCG	15	31	29	0.99 (0.29-3.36)	0.99	18	26	24	0.90 (0.43-1.87)	0.77
H4 AGTACGC	15	24	24	0.93 (0.23-3.74)	0.92	28	21	23	2.03 (1.05-3.93)	0.04

H5	AGCACGG	35	16	17	2.49 (0.98-6.32)	0.05	11	15	14	0.76 (0.32-1.83)	0.55
H6	AGTGTGG*	25	8	10	7.00 (1.89-25.94)	0.004§	16	8	10	2.91 (1.26-6.72)	0.01
H7	AGCACGC		6	6			12	10	10	1.68 (0.65-4.29)	0.28
H8	AGTACGG	10	3	4	5.44 (0.86-34.37)	0.07	2	4	4	0.63 (0.12-3.37)	0.59
S1	-A--C-G	80	88	87	Ref.		90	88	89	Ref.	
S2	-G--C-C†	15	29	28	0.65 (0.19-2.23)	0.49	40	29	32	1.74 (1.04-2.93)	0.04
S3	-G--C-G	45	20	22	2.20 (1.14-4.25)	0.02	13	19	17	0.64 (0.30-1.34)	0.23
S4	-G--T-G††	25	8	10	6.16 (1.97-19.26)	0.002§	16	9	11	2.49 (1.15-5.41)	0.02

Numbers indicate the percentage of minor allele or haplotype carriers. Hazard Ratios and Odd Ratios are indicated for the presence versus the absence of the minor allele or haplotype. The effect of recipient TLR2 and TLR3 SNPs were lost when these SNPs were entered in multivariate models with TLR4 donor polymorphism.

Results in the discovery study (Cox regression model) were adjusted for CMV positive serostatus in donor and/or recipient and acute GVHD (as a time-dependant covariate). Results for genotypes in this table slightly differ from table 3 because only one SNP was assessed in each model in the present table, while several SNPs were assessed in the model of Table 3.

Results in the validation study (conditional logistic regression) were adjusted for underlying disease groups, CMV positive serostatus in donor and/or recipient, antifungal prophylaxis (itraconazole or voriconazole) and donor/recipient sex. The model was also adjusted for acute and chronic GVHD, as well as CMV disease, occurring during the time from transplantation to invasive aspergillosis in cases, and the corresponding time in matched controls.

Simplified *TLR4* haplotypes were indicated by the letter S (3 loci haplotypes). See figure 1A for genetic organization of *TLR4*.

† S2 contained individuals carrying the minor alleles of SNPs +12186 G/C

†† S4 contained individuals carrying the minor alleles of SNPs 1063 A/G and 1363 C/T (both in strong LD, $R^2=0.96$)

§ The significance level after adjusting for multiple testing using the Bonferroni method in the discovery study is 0.00277 for SNPs (considering 18 independent tests) and 0.0125 for haplotypes (considering 4 independent tests)