

Improvement of graft prognosis in penetrating normal-risk keratoplasty by HLA class I and II matching

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CLINICAL STUDY

Abstract

Background Owing to contradictory results, HLA matching in penetrating keratoplasty still is equivocal. Different surgical techniques in multicentre studies, missing risk differentiation in high-risk situations, and faulty HLA typing can be identified as main reasons for these contradictory results. In this monocentre study, the value of HLA class I and II matching (A, B, DR loci) was examined in a homogeneous group of 418 normal-risk keratoplasty patients using serological typing techniques for HLA class I and immunogenetic typing techniques for class II.

Methods Penetrating normal-risk keratoplasty was performed in two groups of patients (group I with 0–2, group II with 3–6 mismatches in the A/B/DR loci). All surgery was carried out by three experienced surgeons according to a standardized scheme. Furthermore, postoperative therapy and controls were standardized. There were no statistically significant differences between the two study groups with regard to the number of ABO or H-Y compatibilities, patient age, patient gender, ratio of previous intraocular surgery, ratio of triple procedures, indication for surgery, follow-up period, donor age, donor gender, post-mortem time of the graft, and endothelial cell density of the graft at the end of organ culture. All HLA typing was performed in a quality-controlled laboratory, serologically for HLA class I (A and B loci) and immunogenetically for HLA class II (DR locus).

Results At 4 years postoperatively, the ratio of clear and rejection-free graft survival was 92% in group I and 66% in group II (Kaplan–Meier estimation, log rank test, $P=0.03$). Monovariate analysis in the Cox model gave no influence of solitary HLA class I or II

matching, but only an influence of combined HLA class I and II matching ($P=0.03$).

Conclusions In this monocentre study with proper typing techniques, the beneficial effect of HLA class I plus II matching on clear and rejection-free graft survival could be demonstrated in a homogeneous group of normal-risk keratoplasty patients.

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Keywords: graft survival; HLA matching; HLA typing; immune reaction; keratoplasty

Background

Almost one century after the first successful penetrating keratoplasty,¹ immune reactions are still a major problem in normal-risk as well as in high-risk situations.^{2–4} Owing to the immune privilege of the cornea and the anterior chamber,^{5–8} topical corticosteroid prophylaxis over some months postoperatively has been supposed to limit this complication sufficiently.^{9,10} In normal-risk keratoplasty about 18% and in high-risk situations up to 75% of the patients experience, however, immune reactions, often with subsequent graft failure.^{2–4} In order to improve graft prognosis, topical and/or systemic immunosuppressives may be administered in the long run, and/or HLA matched grafts may be used. In contrast to the first approach, HLA matching is not associated with side effects.

The beneficial effect of HLA class I and II matching in renal transplantation is unequivocal.¹¹ Numerous penetrating keratoplasty studies performed within the past three decades gave, however, contradictory results.^{12–25} These contradictory results may be

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explained by three main reasons: (1) In multicentre studies with up to 200 centres and up to 400 surgeons, different surgical experience and techniques may influence the outcome.²⁶ (2) Most studies were performed in high-risk patients with an elevated risk of immune reactions. Such patients may have additional risk factors such as surface disorders (eg in atopic keratoconjunctivitis or limbal stem cell deficiency) or glaucoma problems or they may experience viral or microbial recurrences.²⁷ HLA matching has no influence on these additional risk factors, which may, of course, lead to irreversible graft failure, for example, in severest lime burn, the risk of graft failure from limbal stem cell deficiency alone may be 100% without necessity of an additional immune reaction. (3) In some studies, HLA typing was performed in laboratories that were not quality controlled. In the CCTS, concordance between original and retyping was only 55%.^{28,29} Völker-Dieben *et al*²⁹ demonstrated that faulty HLA DR typing in only 5% abrogates the beneficial effect of HLA DR matching. HLA typing on an immunogenetic basis is the safest way to avoiding typing faults in HLA class II. This is especially true when blood obtained up to 72 h after the donor's death is analysed.^{11,30} In previous keratoplasty studies, however, HLA typing had been performed almost exclusively serologically.

In this study, faults committed in previous studies as mentioned above were avoided by a monocentre design with only three experienced cornea surgeons, by choosing normal-risk keratoplasty patients as participants, thus excluding nonimmunological reasons for graft failure, and by performing HLA typing in an optimally quality-controlled laboratory, serologically for class I and immunogenetically for class II.

Methods

Penetrating normal-risk keratoplasty was performed in a homogeneous monocentre group of 418 patients after approval by the local ethics committee and after obtaining written informed consent by every patient.

Patient selection

Only patients undergoing first keratoplasty within an avascular host cornea were included. All grafts with a diameter of 7.7 mm were positioned centrally. Indications for surgery were keratoconus, Fuchs' endothelial dystrophy, bullous keratopathy, and nonherpetic scars. None of the patients had a history of severe surface disorders, glaucoma, or herpetic eye disease.

Since 1995, HLA typing has been performed in all normal-risk keratoplasty patients of the clinic. All patients were set on a waiting list for a maximum of 6

months. If a graft with 0–2 mismatches (group I) on the HLA A, B, and DR loci had not been found within this period, a graft with 3–6 mismatches was assigned (group II). Group I comprised 66, group II 352 patients: one patient received a graft with zero, 28 with one, 37 with two, 70 with three, 124 with four, 114 with five, and 44 with six mismatches. All relevant patient, donor, graft, and surgery data of both study groups are given in Tables 1a and 2a.

HLA typing and matching

All serologic HLA A, B, and all immunogenetic HLA DRB, DRQB typings of donors and recipients were performed in a single laboratory accredited by the American Society for Histocompatibility and Immunogenetics.³⁰ For HLA matching, only broad antigens (class I: A, B; class II: DR) were considered (Tables 2b and c).

Distribution of blood group mismatches (ABO)

ABO typing was performed serologically in the same optimally quality-controlled laboratory. In group I 40/66 patients and in group II 184/352 patients received ABO-matched grafts (donor A/recipient A or AB, donor B/recipient B or AB, donor AB/recipient AB, donor O/recipient A, B, AB, or O) (χ^2 test, $P=0.64$) (Table 2a).

Table 1 (a) Patient data of the study. (b) Donor and graft data of the study

	0–2 mismatches	3–6 mismatches	P
(a)			
Age (years) ^a	54.8 ± 21.8	59.3 ± 19.4	NS
Gender (f/m) ^b	41/25	183/169	NS
Previous i.o. surgery ^a	25.8%	29.3%	NS
Triple/pkp ^b	15.2%	20.2%	NS
Indication for surgery (kc/ Fed/other) ^b	23/21/22	102/126/124	NS
Follow-up (days) ^a	588 ± 419	623 ± 466	NS
(b)			
Age (years) ^a	62.3 ± 18.8	61.3 ± 17.0	NS
Gender (f/m) ^b	32/34	133/219	NS
Post-mortem time (h) ^a	9.5 ± 10.1	13.1 ± 14.9	NS
Storage time in organ culture (days) ^a	17.6 ± 4.0	16.4 ± 4.4	0.03
Ecd directly after organ culture (cells/mm ²) ^a	2324 ± 273	2318 ± 246	NS

^aANOVA. ^b χ^2 test. NS=not significant, kc=keratoconus, Fed=Fuchs endothelial dystrophy, other=bullous keratopathy or nonherpetic avascular scars, Ecd=endothelial cell density.

Table 2a HLA mismatches (A, B, DR locus; broad), ABO and minor histocompatibility antigen H-Y mismatches

	Group I 0–2		Group II 3–6		P
	mismatches		Mismatches		
Patients with HLA mismatches (n)	0 mm	1	3 mm	70	
	1 mm	28	4 mm	124	
	2 mm	37	5 mm	114	
Patients with ABO ^a mismatches (n)	Unknown	8	Unknown	54	
	0 mm	40	0 mm	184	
	1 mm	17	1 mm	105	NS
	2 mm	1	2 mm	9	
Patients with H-Y ^a mismatches (n)	0 mm	47	0 mm	244	
	1 mm	19	1 mm	108	NS

^a χ^2 test. mm=Mismatches, NS=not significant.

Table 2b Considered HLA alleles in the group with 0–2 mismatches

Recipients		Donors	
A1	B22	A1	B22
A2	B27	A2	B27
A3	B35	A3	B35
A9	B37	A9	B37
A10	B40	A10	B40
A11	B41	A11	B41
A19	B53	A19	B46
A28	DR1	A28	B47
B5	DR2	B5	B53
B7	DR3	B7	DR1
B8	DR4	B8	DR2
B12	DR5	B12	DR3
B13	DR6	B13	DR4
B14	DR7	B14	DR5
B16	DR8	B15	DR6
B15	DR9	B16	DR7
B17	DR10	B17	DR8
B18		B18	DR9
B21		B21	DR10

Distribution of minor histocompatibility H-Y mismatches

In group I 47/66 patients and in group II 244/352 patients received H-Y-matched grafts (donor male/recipient male, donor female/recipient male or female) (χ^2 test, $P = 0.4$) (Table 2a).

Grafts

All grafts were preserved in organ culture according to the guidelines of the European Eye Bank Association.³¹ Preoperative evaluation of the graft endothelium was performed in hypotonic solution under the phase

Table 2c Considered HLA alleles in the group with 3–6 mismatches

Recipients		Donors	
A1	B22	A1	B27
A2	B35	A2	B35
A3	B37	A3	B37
A9	B40	A9	B40
A10	B41	A10	B41
A11	B42	A11	B46
A19	B47	A19	B53
A28	B53	A28	DR1
B5	B27	B5	DR2
B7	DR1	B7	DR3
B8	DR2	B8	DR4
B12	DR3	B12	DR5
B13	DR4	B13	DR6
B14	DR5	B14	DR7
B15	DR6	B15	DR8
B16	DR7	B16	DR9
B17	DR8	B17	DR10
B18	DR9	B18	
B21	DR10	B21	
B27		B22	

contrast microscope the day before penetrating keratoplasty.³² This examination was proven to deliver reproducible results.³³

Penetrating keratoplasty, postoperative treatment and controls

Surgery was performed by three experienced surgeons in retrobulbar anaesthesia according to a standardized scheme. Modified Franceschetti trephines with the diameters 7.5 mm (recipient) and 7.7 mm (donor) were used. Graft fixation was performed with a double running cross-stitch suture with Nylon 10.0.³⁴ If necessary, cataract surgery was carried out simultaneously (Table 1a). After surgery, gentamycin ointment was administered at least until the graft was covered with a complete epithelial layer. Then corticosteroid eye drops (prednisolone-21-acetate 1%) were given five times daily and tapered during the first five postoperative months. All patients received 1 mg/kg body weight/day of systemic fluocortolon tapered within 3 weeks postoperatively. Acetazolamide was administered in a daily dose of 500 mg for 5 days postoperatively. Controls of the graft at the slit lamp were scheduled 6 weeks, 4, 12, and 18 months postoperatively and thereafter annually.

Immune reactions

Endothelial immune reactions were diagnosed via endothelial precipitates and stromal oedema, and

stromal immune reactions via subepithelial infiltrates.³⁵ All patients received corticosteroid eye drops (prednisolone-21-acetate 1%) every hour until elimination of all precipitates. Furthermore, a subconjunctival injection with betamethasone-21-acetate was performed. Topical corticosteroids were tapered individually. In severe cases, systemic corticosteroids at a daily oral dose of 1 mg fluocortolone/kg body mass were administered additionally and tapered within 3 weeks.

Statistical analysis

All statistical evaluation was performed using SSPS Windows NT 4.0 (Microsoft Corp., Redmond, USA). Clear graft survival, ratio of grafts without immune reactions, and rejection-free graft survival were calculated according to Kaplan and Meier.³⁶ All Kaplan–Meier curves were compared via log rank test. Monovariate analysis was performed by entering patient, donor, graft, and surgery data into the Cox model.

Findings

At 4 years postoperatively, clear graft survival in group I was 100% and in group II 85% ($P = 0.065$), ratio of rejection-free grafts was 92% in group I and 72% in group II ($P = 0.048$) and ratio of clear and rejection-free graft survival was 92% in group I and 66% in group II ($P = 0.03$) (Figure 1a–c).

Reasons for graft failure in group II were irreversible immune reactions in eight patients and chronic endothelial cell loss in three patients. In group I, four immune reactions were recorded. All were reversible. In group II, 50 of 58 immune reactions were reversible (Table 3).

Monovariate analysis in the Cox model gave no influence of solitary HLA class I or II matching ($P = 0.31$ and 0.13 , respectively), but only an influence of combined HLA class I and II matching ($P = 0.03$) on clear and rejection-free graft survival (Table 4).

An influence of blood group or minor H-Y compatibility on clear and rejection-free graft survival could not be demonstrated (Table 4). The same is true for patient age, patient gender, preceding or simultaneous intraocular procedures, donor age, donor gender, post-mortem time of the graft, time of the graft in organ culture, or endothelial cell density at the end of the culture period (Tables 5a and b).

Discussion

Many studies were performed within the past three decades considering the effects of HLA matching in penetrating keratoplasty. They delivered contradictory

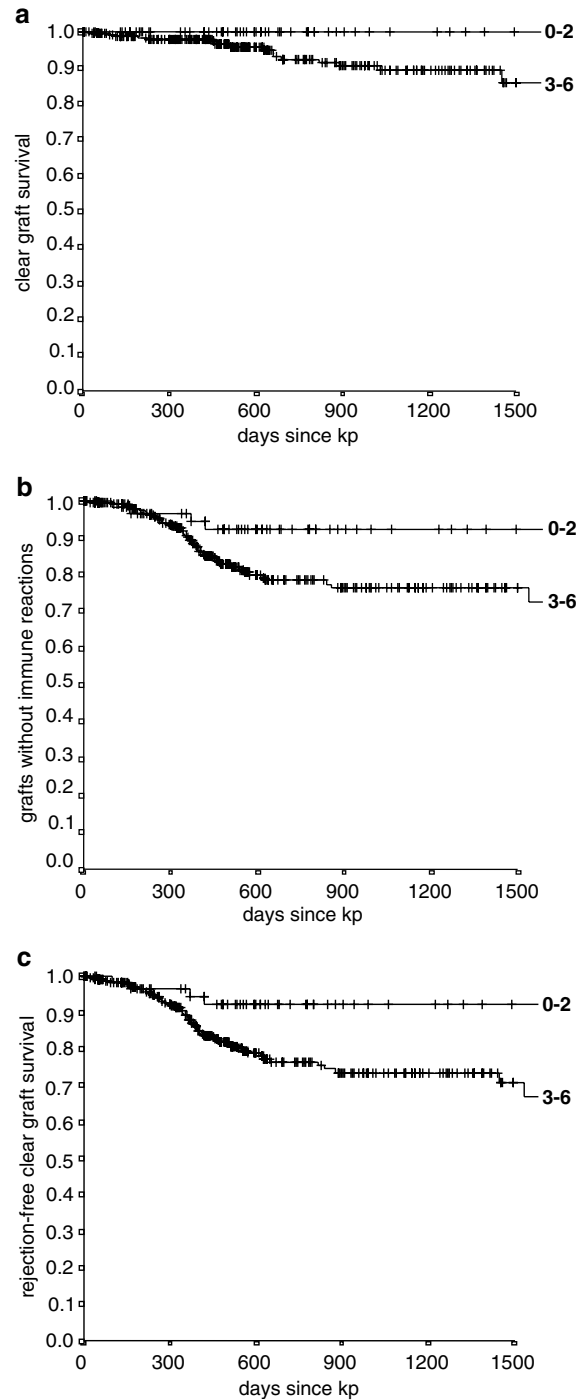


Figure 1 HLA matching in 418 normal-risk penetrating keratoplasty (kp) (a) clear graft survival, 0–2/3–6 mismatches, log rank test: $P = 0.065$; (b) grafts without immune reactions, 0–2/3–6 mismatches, log rank test: $P = 0.048$; (c) rejection-free clear graft survival, 0–2/3–6 mismatches, log rank test: $P = 0.03$.

results (Table 6a and b).^{12–25} In this study, the beneficial effect of HLA class I plus II matching on clear and rejection-free graft survival after penetrating normal-risk

Table 3 Immune reactions and reasons for graft failure in the two study groups

	0–2 <i>mismatches</i>	3–6 <i>mismatches</i>	P
All immune reactions ^a	4/66	58/352	0.016
Reversible immune reactions ^a	4/4	50/58	NS
Irreversible immune reactions ^a	0/4	8/58	NS
Chronic endothelial cell loss with subsequent irreversible graft failure ^a	0/66	3/352	NS

^aFisher test, NS=not significant.

Table 4 Influence of HLA matching, ABO- and minor H–Y compatibility on clear and rejection-free graft survival, mono-variate Cox model (discrete factors)

	P-value	Relative risk
HLA A/B/DR 0–2/3–6 mm	0.03	3.0
HLA A/B 0–2/3–4 mm	NS	1.3
HLA DR 0/1–2 mm	NS	2.2
ABO (blood group) 0/1–2 mm	NS	0.9
H–Y mm	NS	1.1

mm=mismatches, NS=not significant.

Table 5a Influence of patient factors on clear and rejection-free graft survival, monovariate Cox model (discrete factors)

	P-value	Relative risk
Age	NS	0.99
Gender	NS	1.4
Previous i.o. surgery	NS	1.6
Triple/pkp	NS	1.2

NS=not significant.

Table 5b Influence of donor factors on clear and rejection-free graft survival, monovariate Cox model (discrete factors)

	P-value	Relative risk
Age	NS	0.99
Gender	NS	1.5
Post-mortem time	NS	1.0
Storage time in organ culture	NS	0.96
Ecd directly after organ culture	NS	1.0

Ecd=endothelial cell density, NS=not significant.

keratoplasty could be demonstrated. Main difference between the two study groups was the number of HLA mismatches. All other factors possibly influencing study outcome were comparable. The study is unique because of five reasons:

1. In a monocentre design, only three experienced surgeons performed all transplantations according to a standardized scheme. Furthermore, all postoperative therapy and all postoperative examinations were performed in a standardized manner.

2. Only patients undergoing normal-risk keratoplasty were considered for the study in order to minimize the influence of nonimmunological risk factors. Another possibility would have been to study high-risk patients with an elevated risk for immune reactions as the only risk factor. Such patients are, however, much rarer than normal-risk patients.

3. Various patient, donor, and graft parameters may influence clear and rejection-free graft survival. In this study, patient age, patient gender, ratio of previous intraocular surgery, ratio of triple procedures, indication for surgery, and follow-up did not show statistically significant differences between the two study groups. The same is true for donor age, donor gender, post-mortem time, and endothelial cell density of the graft at the end of standardized organ culture. Only storage time in organ culture was statistically significantly longer in group I. If this had an influence on the study results, one might expect worse results in the group with the longer storage period.^{37,38} The contrary, however, was observed.

4. In the past, the influence of blood group compatibility on graft prognosis was discussed controversially.²² It may be important, therefore, that the number of patients with blood group compatibilities was comparable in both study groups. An influence of minor histocompatibility mismatches on graft prognosis was found by Streilein³⁹ in the mouse penetrating keratoplasty model. The only minor histocompatibility antigen investigated in this study was H–Y. In the two study groups, the number of patients with minor histocompatibility antigen H–Y mismatches was similar.

5. All HLA typing was performed in only one quality-controlled laboratory, serologically for class I and immunogenetically for class II. In the past, serological typing of class I was shown to deliver reliable results.³⁰ This is not the case for HLA class II, even if material from heart-beating donors is analysed.^{28,29} In penetrating keratoplasty, however, blood from cornea donors is often obtained up to 72 h after death. In this study, therefore, the DR locus was determined exclusively by immunogenetic means.³⁰

The expression of class I and II antigens in the cornea is still controversially discussed. Streilein⁵ identified reduced expression of MHC antigens as one reason for the immune privilege of corneal grafts. Newsome *et al*⁴⁰ demonstrated the presence of class I antigens on epithelial, stromal, and endothelial corneal cells. In

Table 6 (a) Previous studies investigating the influence of HLA class I or II matching on graft prognosis after penetrating keratoplasty

Trial	Gibbs et al (1974)	Vannus (1975)	Stark et al (1978)	Ehlers et al (1979)	Foullks et al (1983)	Santfilipo et al (1986)	Baggesen et al (1991)
Mono/multicentre	Mono I A	Mono I A/B	Mono I A/B	Mono I A/B	Mono I A/B	Mono I A/B	Mono II DR
HLA I/II loci	Serological	Serological	Serological	Serological	Serological	Serological	Immunogenetic
Methods of typing	-	+	+	+	+	+	-
Antibody-screening/crossmatching	155	46	103	222	46	97	51
Patients (n)							
Risk profile							
Hrk	95	+	103	106	46	97	51
Nrk	60	+	-	106	-	-	-
Follow-up (m)	24 ^a	Not reported	14	Not reported	18 ^a	72 ^a	20 ^a
Effect	Beneficial, P < 0.05	No statistical evaluation	No statistical evaluation	Beneficial, P < 0.005	Beneficial, P = 0.001	Beneficial, P < 0.01	Beneficial, P = 0.003

^aSurvival calculated according to Kaplan and Meier.³⁶
Nrk=normal-risk keratoplasty, Hrk=high-risk keratoplasty, m=months.

contrast, class II antigens are regularly expressed only on epithelial Langerhans cells.⁴¹ After incubation with gamma-interferon, however, class II antigens are expressed on all corneal cells including the endothelium.^{42,43} CD4 T cells are the main source of gamma-interferon.⁴⁴ In the rat penetrating keratoplasty model, CD4 T cells were found even in syngeneic grafts.⁴⁵ Possibly, an elevation of gamma-interferon levels in the graft may already be caused by a surgical trauma such as penetrating (normal-risk) keratoplasty.

The evolution in keratoplasty has been such that the early attempts to exploit the theoretical advantages of HLA matching had unavoidably too often been technically weak so that the probability of negative or questionable results was high. On the other hand, the introduction of more and more efficient systemic immunodrugs has brought about such a quick and important breakthrough in the prognosis especially of high-risk keratoplasties that the current 'neglect' of the HLA system is understandable from this experience.

We have shown that even patients with a normal-risk keratoplasty benefit from a good HLA A/B plus HLA DR match. As others^{29,46} have recently published that HLA matching is beneficial also in high-risk and mixed-risk groups, these unisono reports mean that HLA matching must now be regarded as basically beneficial for all keratoplasty patients.

It does not follow that HLA matching must actually be required for every patient. For the time being, the practical consequences will anyway be limited because the logistics for a widespread application of matching principles in keratoplasty do simply not yet exist in most parts of the world. It must be discussed, however, whether it will be worthwhile to establish such logistics. We vigorously favour such a project.

For normal-risk patients, we would expect that their transplants' lifespan, which currently on an average is not longer than about 10–20 years, will be considerably expanded. This is extremely important for all patients under the age of about 60 years. For high-risk patients, efficient HLA matching will help to bring about conditions in which tolerance of the graft by the host is established more easily and more efficiently and longer lasting than with systemic immunomodulative drugs alone. The application of the latter is often limited by severe side effects, or their effect may be insufficient when given as the only measure.

Thus, all keratoplasty patients should benefit to various degrees from HLA matching, and it is our hope that this paper together with those of others^{29,46} will lead to a re-evaluation and reappraisal of HLA matching in keratoplasty.

Table 6 (b) Previous studies investigating the influence of HLA class I and II matching on graft prognosis after penetrating keratoplasty

<i>Trial</i>	<i>Hoffmann and Pahlitzsch</i>	<i>Völker-Dieben et al (1989)</i>	<i>Boisjoly et al (1990)</i>	<i>Beekhuis et al (1991)</i>	<i>CCTS (1992)</i>	<i>Hoffmann et al (1994)</i>	<i>Vail et al (1997)</i>	<i>Völker-Dieben et al (2000)</i>	<i>Present study</i>
Mono/multicentre	Mono	Mono	Mono	Mono	Multi	Mono	Multi	Mono	Mono
HLA loci	I B II DR	I AB II DR	I AB II DR	I AB II DR	I AB II DR	I AB II DR	I AB II DR	I AB II DR	I AB II DR
Methods of typing	Not reported	Serological	Serological	Not reported	Serological	Serological	Not reported	Serological	I Serological II PCR
Antibody-screening/ crossmatching	–	–	+	–	+	–	–	+	–
Class I+II typed patients (n)	137	257	350	33	419	248	602	558	418
<i>Risk profile</i>									
Hrk	54	+	+	+	+	83	+	+	–
Nrk	83	+	+	–	–	165	+	+	418
Follow-up (m)	Not reported	36 ^a	36 ^a	36 ^a	36 ^a	18	12	58	60 ^a
<i>Effect</i>									
Class I	Beneficial, <i>P</i> <0.05	No statistical evaluation	<i>Nrk</i> beneficial, <i>P</i> =0.004; <i>Hrk</i> beneficial, <i>P</i> =0.0009	No statistical evaluation	Not beneficial, <i>P</i> =0.35	<i>Nrk, B locus.</i> beneficial, <i>P</i> <0.01; <i>Hrk, B</i> locus, <i>P</i> <0.01	Beneficial	<i>Nrk</i> beneficial, <i>P</i> =0.05; <i>Hrk</i> beneficial, <i>P</i> =0.0001	Combined beneficial effect of class I+II matching, <i>P</i> =0.03
Class II	Beneficial, <i>P</i> <0.05	No statistical evaluation	Not beneficial, <i>P</i> =0.32	No statistical evaluation	Not beneficial, <i>P</i> =0.95	<i>Nrk</i> beneficial, <i>P</i> <0.05; <i>Hrk</i> beneficial, <i>P</i> <0.05	Negative effect <i>P</i> <0.05	<i>Nrk</i> not beneficial, <i>P</i> =0.14; <i>Hrk</i> beneficial, <i>P</i> =0.02	Combined beneficial effect of class I+II matching, <i>P</i> =0.03

^aSurvival calculated according to Kaplan and Meier. PCR=polymerase chain reaction.³⁶

Nrk=normal-risk keratoplasty, Hrk=high-risk keratoplasty, m=months

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