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Pharmacogenetics of sunitinib in metastatic renal cell carcinoma

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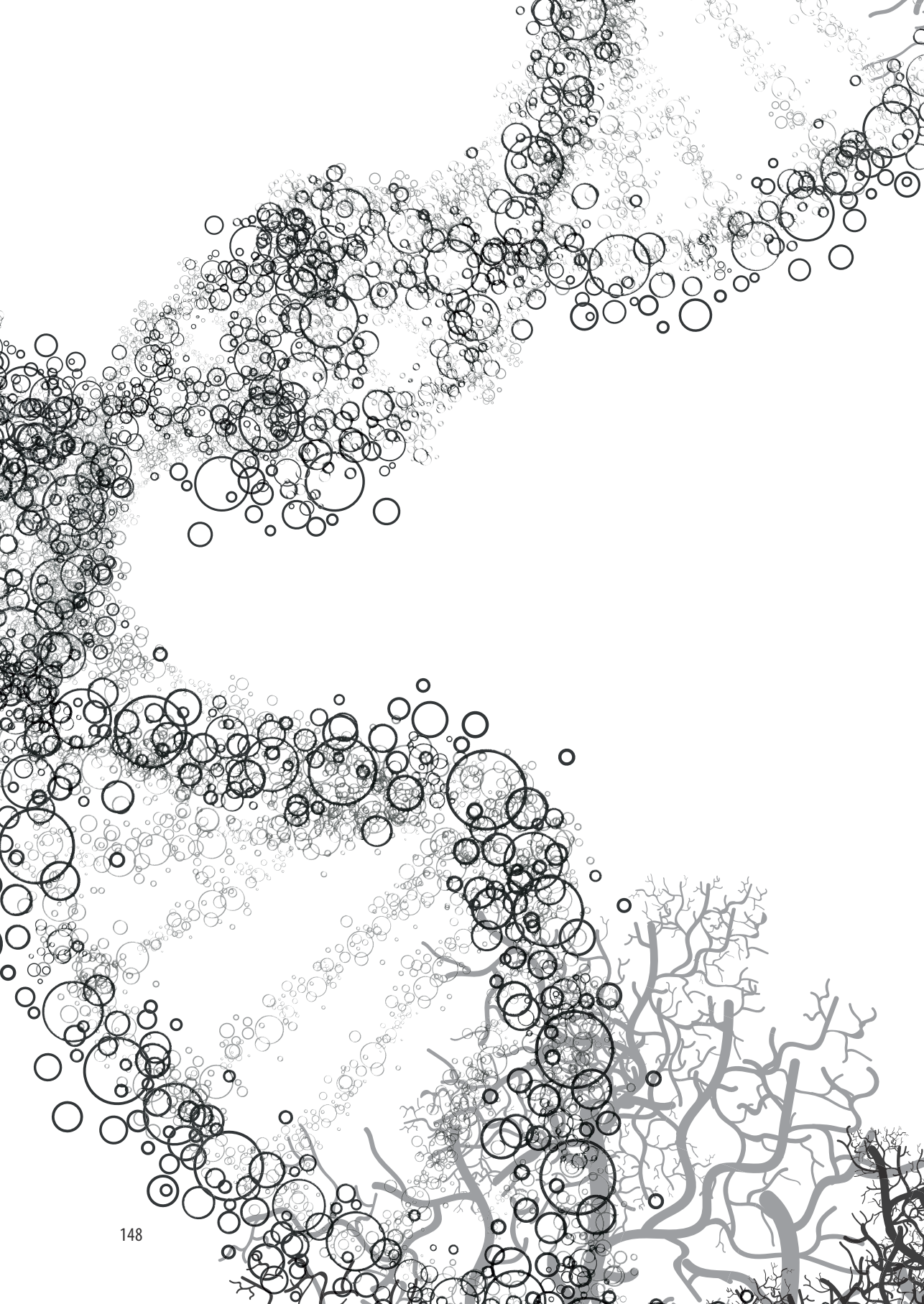


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CHAPTER 7

Evaluation of *KDR* rs34231037 as predictor of sunitinib efficacy in patients with metastatic renal cell carcinoma.

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ABSTRACT

Objective The identification of biomarkers able to predict clinical benefit from vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors is urgently needed. Recently, Maitland and colleagues described an association between *KDR*-rs34231037 and soluble VEGFR2 levels as well as pazopanib pharmacodynamics. We investigated in a well-characterized series of metastatic clear cell renal cell carcinoma patients whether rs34231037 could influence sunitinib response.

Methods Clinical data and DNA were available from an international series of 276 patients. *KDR*-rs34231037 association with sunitinib response, clinical benefit, and progression-free survival was analyzed using logistic and Cox regression analyses.

Results We found that G-allele carriers were over-represented among patients with clinical benefit during sunitinib treatment compared with those refractory to the treatment (odds ratio:3.78, 95%CI:1.02-14.06; $P=0.047$; multivariable analysis).

Conclusion In conclusion, rs34231037 variant carriers seemed to have better sunitinib response than wild-type patients. Moreover, the association with tumor size reduction suggests that this single nucleotide polymorphism might also identify patients with successful tumor downsizing under anti-VEGFR therapy.

INTRODUCTION

Renal cell carcinoma (RCC) accounts for 2-3% of all malignancies in adults. In most RCC, an aberrant activation of the vascular endothelial growth factor (VEGF) pathway is present because of inactivation of the Von Hippel–Lindau tumor-suppressor gene. This event leads to increased tumor vascularization that sustains tumor growth and metastasis. The development of drugs targeting VEGF or their receptors (VEGFR) has resulted in effective treatment options for patients with metastatic renal cell carcinoma (mRCC).¹ Sorafenib, sunitinib, pazopanib, axitinib, and cabozantinib are angiogenic inhibitors approved for the treatment of mRCC. These small-molecule tyrosine kinase inhibitors (TKIs) show high affinity for key proteins mediating tumor angiogenesis: VEGFR1, VEGFR2, VEGFR3, and platelet-derived growth factor receptor, among other targets. However, despite their effectiveness in RCC, a large interindividual variability has been observed in terms of drug response and toxicity.² Consequently, the identification of markers predictive of the response to these TKIs is urgently needed.

Antiangiogenic TKIs exert their effect on blood vessels rather than directly on tumor cells, suggesting that the patients' genomic profile could influence drug response. In fact, we and others have found single nucleotide polymorphisms (SNPs) associated with TKI therapy response.³⁻⁸ Recently, Maitland *et al.*⁹, through a Genome Wide Association Study, identified and validated an SNP in *KDR* (rs34231037; p.C482R) as the top hit associated with variations of soluble VEGFR2 (sVEGFR2) in blood. In addition, analysis of sVEGFR2 in RCC patients treated with pazopanib showed an association between the minor allele of this SNP and lower sVEGFR2 levels both at baseline and after 4 weeks of pazopanib treatment. sVEGFR2 is derived from an alternative splicing in the *KDR* gene.¹⁰ In patients in whom sVEGFR2 was measured at baseline and during TKI treatment, a greater decrease in sVEGFR2 has been associated with increased response to the treatment.^{11,12}

This suggests that rs34231037 might be predictive of anti-VEGFR2 drugs' outcome. However, to date, the association of rs34231037 with sunitinib response and progression-free survival (PFS) in patients remains unexplored. Here, we investigated the effect of *KDR*-rs34231037 on patient outcome in a large series of metastatic clear cell RCC patients treated with sunitinib.⁵

METHODS

Patients and sunitinib outcome

Patients' samples were collected by the Dutch SUTOX consortium, the Spanish Oncology Genitourinary Group (SOGUG), and the Taussig Cancer Institute of Cleveland Clinic

Foundation (CCF) as described previously.^{6,13-16} In brief, from the original series of 333 clear cell RCC patients previously described⁵, 42 samples were lost because of lack of DNA, 12 were eliminated because patients received previous anti-VEGF treatment, and three samples failed genotyping. Thus, 276 patients receiving sunitinib as first anti-VEGF therapy were analyzed for drug response and PFS. The study was approved by the corresponding ethical review boards.

Genotyping

Germline DNA was used to genotype rs34231037 (p.C482R). SUTOX and CCF samples were genotyped using a Taqman probe and SOGUG samples using the KASP genotyping chemistry (LGC, Teddington, UK). Genotype frequencies met Hardy–Weinberg equilibrium.

Statistical analysis

To investigate rs34231037 association with sunitinib response, patients were divided into groups according to their best response to this drug: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Logistic regression analysis was used to perform comparisons between CR/PR vs. SD/PD and between CR/PR vs. PD. In a subsequent analysis, we compared patients with clinical benefit defined as those with CR, PR, or SD longer than 1 year versus those who had PD or SD shorter than 1 year. Kaplan–Meier and Cox regression analyses were used to analyze PFS. In multivariate analyses, we adjusted for Heng prognostic group¹⁷, age, sex, and study center.

RESULTS

We analyzed a series of 276 clear cell RCC patients receiving sunitinib as first anti-VEGF therapy. The majority of patients were men (68%), White (96%), had nephrectomy (84%), had received no previous antitumor treatment (86%), and the median age at the start of sunitinib was 61 years. The median PFS during sunitinib treatment was 16.0 months (95% CI=13.3-18.8) and 9, 116, 99, and 35 patients showed a CR, PR, SD, and PD, respectively.

We analyzed the association of rs34231037 with sunitinib response using univariate and multivariable analyses. We found a trend toward a better response among G-allele carriers compared with wild-type patients (multivariable analysis, odds ratio (OR):2.58; 95% CI: 0.95–6.99; $P=0.062$ for CR, PR or SD vs. PD and OR: 3.17; 95% CI:0.62–16.18; $P=0.165$ for CR or PR vs. PD **Table 1**). Analyzing each subcohort of patients separately, we found the strongest association in the Spanish subcohort (**Figure 1** and **Supplementary**

Table 1, Supplemental digital content 1, <http://links.lww.com/FPC/B209>). When studying sunitinib clinical benefit, differences became statistically significant, with the G-allele carriers having better sunitinib outcome (OR:3.78; 95% CI: 1.02–14.06; $P=0.047$; **Figure 1** and **Table 1**).

In terms of PFS, time to progression of rs34231037 G-allele carriers was similar to that of wild-type individuals when considering the full series of patients (15.4 and 16.0 months, respectively; **Figure 1g** and **Table 1**). However, in the Spanish subcohort, rs34231037 G-allele carriers showed a longer PFS than wild-type patients (17.9 compared with 12.3 months; **Figure 1h**), although the difference was not statistically significant.

Table 1 Association of rs34231037 with response to sunitinib and progression-free survival

Clinical variables	Number of patients	Univariate		Multivariate ^a	
		OR/HR ^b (95% CI)	<i>P</i> value	OR/HR (95%CI)	<i>P</i> value
Response ^c					
CR, PR vs SD, PD	259	2.11 (0.81-5.46)	0.126	2.58 (0.95-6.99)	0.062
CR, PR vs PD	160	1.92 (0.41-8.92)	0.408	3.17 (0.62-16.18)	0.165
Clinical benefit ^c					
CR, PR, SD >1 years vs PD, SD <1 year	246	2.93 (0.83-10.30)	0.094	3.78 (1.02-14.06)	0.047
PFS	276	1.05 (0.61-1.82)	0.852	1.23 (0.7-2.15)	0.466

CR, complete response; HR, hazard ratio; OR, odds ratio; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; SOGUG, Spanish Oncology Genitourinary Group.

^aMultivariate analyses included as covariates Heng prognostic risk group, age, sex, and study center.

^bThe data correspond to the effect of the wild-type A-allele of rs34231037, where OR/HR >1.0 indicates an increased risk of poor response/shorter PFS.

^cLogistic regression comparing the groups of patients indicated.

Bold values indicate significant level $P<0.1$.

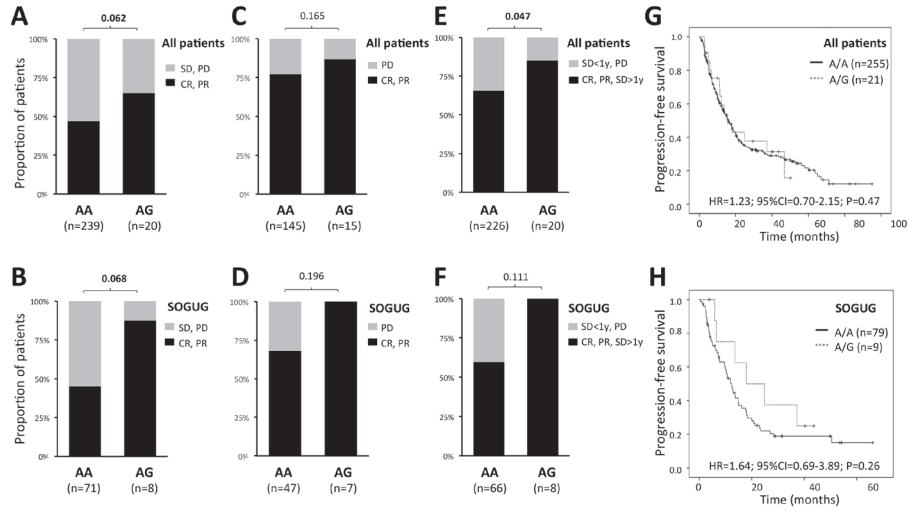


Figure 1 Effect of *KDR* rs34231037 on sunitinib response and PFS in metastatic ccRCC patients. Sunitinib response (responder defined as those with CR or PR) in the full series of patients (a) and in SOGUG series (b). Sunitinib response (responders defined as those with CR, PR, or SD longer than 1 year) in the full series of patients (c) and in SOGUG series (d). Sunitinib clinical benefit in the full series of patients (e) and in SOGUG series (f). PFS in the full series of patients (g) and in SOGUG series (h) according to the rs34231037 genotype. The P values correspond to multivariate analyses. ccRCC, clear cell renal cell carcinoma; CI, confidence interval; CR, complete response; HR, hazard ratio; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; SOGUG, Spanish Oncology Genitourinary Group; Time, time from the start of sunitinib treatment until PD (months).

DISCUSSION

Recently, Maitland *et al.*⁹ identified rs34231037 as the most relevant SNP associated with sVEGFR2 baseline levels as well as with the extent of decline in sVEGFR2 upon pazopanib treatment. Our results suggest that rs34231037 variant carriers might have a better response to sunitinib treatment (**Figure 1**).

The relatively low MAF of rs34231037 in the White population results in a relatively small number of patients carrying the variant allele (21 out of 276) and decreased the statistical power of the analysis. Thus, the weak association found in this study indicates that *KDR* rs34231037 does not have a strong effect, although variant carriers seem to respond better to sunitinib. The association with tumor size reduction suggests that

this SNP might also be relevant to identify patients in whom tumor downsizing with TKI therapy before surgery would be successful.

We observed variability in the results among the series. In the SOGUG group, all patients received sunitinib as first line, whereas 20 and 22% of SUTOX and CCF patients received previous antitumor treatments. There were also differences in prognosis, sunitinib response, and PFS among the series. 11, 23, and 36% of SOGUG, SUTOX, and CCF patients had a poor prognosis; 19, 14, and 6% of patients had PD as the best response; and the median PFS was 13, 15, and 35 months in these series, respectively. This suggests that differences in the basal characteristics of the patients could be relevant for the effect of the SNP.

The sVEGFR2 soluble protein has been shown to be a physiologic inhibitor of developmental and reparative lymphangiogenesis in mice.¹⁰ As carriers of the *KDR* rs34231037 G-allele have lower sVEGFR2 levels⁹, the improved sensitivity to anti-VEGFR drugs might be explained by an intrinsic increased tumor lymphangiogenesis; however, this remains to be investigated.¹⁰ How rs34231037 leads to decreased expression of sVEGFR2 is unknown. As sVEGFR2 derives from an alternative splicing of the *KDR* gene, one possibility would be that rs34231037, located in the immunoglobulin-like domain V of the VEGFR2, might be altering the splicing site signals in exon 11. Alternatively, it could lead to decreased stability of the mRNA or protein product. In addition, rs34231037 has been described as a risk factor for infantile hemangioma and when present in full-length VEGFR2 protein, it has been shown to alter the angiogenesis, decreasing the formation of complexes with β 1 integrin and decreased VEGFR1 expression.¹⁸

In *VEGFR2* immunoglobulin-like domain V, there is one high-frequency missense polymorphism (rs1870377, p.Q472H) for which we described a trend toward shorter PFS and OS and an increased risk of hypertension upon sunitinib treatment in mRCC^{5,6}, and others have described higher levels of VEGF in sera.¹⁹ In addition, in the immunoglobulin-like domain V of *VEGFR3*, a common missense variant (rs307826; p.T494A) has been associated with sunitinib response and PFS in RCC patients^{6,7} and with altered VEGFR3 protein expression in tumors.²⁰ These results, together with the fact that the extracellular domain V of VEGFRs is essential for dimer formation and activation²¹, suggest that variants altering this domain may influence the effect of anti-VEGFR TKIs.

The limitations of our study include that patients were treated with sunitinib, but in the study by Maitland *et al.*⁹, patients were treated with pazopanib. Although both sunitinib and pazopanib inhibit VEGFRs, the binding affinity is not identical.²² Thus, we can only speculate that rs34231037 would exert a similar effect on pazopanib-treated and sunitinib-treated patients. In addition, although the effect of rs34231037 on sVEGFR2 was large (explaining 23% of the variance⁹), the effect of the SNP on tumor response to TKI seems smaller. Because of the lack of serum samples in this study, measurement of sVEGFR2 levels during sunitinib treatment was not possible.

In conclusion, in this study, in a large and well-characterized series of sunitinib-treated patients, we report a potential moderate effect for *KDR*-rs34231037 on sunitinib response. Although Maitland and colleagues proposed that rs34231037 influenced pazopanib treatment outcome, this remains to be determined. Large series of patients treated with different anti-VEGFR TKIs and with sVEGFR2 pharmacodynamics and tumor response data are needed to validate these results and establish the clinical relevance of rs34231037 as a marker for antiangiogenic response.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 Results of rs34231037 for response to sunitinib and PFS in the different study centers.

Clinical variable	Series of patients	Number of patients	Univariate		Multivariate ^a	
			OR/HR ^b (95%CI)	P value	OR/HR (95%CI)	P value
Response CR, PR vs SD, PD	All series	259	2.11 (0.81-5.46)	0.126	2.58 (0.95-6.99)	0.062
	SUTOX	116	2.09 (0.53-8.26)	0.291	2.50 (0.61-10.29)	0.204
	SOGUG	79	8.53 (1.00-73.01)	0.050	7.74 (0.86-69.56)	0.068
	CCF	64	0.26 (0.02-3.10)	0.286	0.33 (0.03-4.25)	0.398
Response CR, PR vs PD	All series	160	1.92 (0.41-8.92)	0.408	3.17 (0.62-16.18)	0.165
	SUTOX	61	0.88(0.15-5.03)	0.881	1.28 (0.19-8.50)	0.799
	SOGUG	54	1.22 (1.05-1.41)	0.171 ^f	1.22 (1.05-1.41)	0.196 ^g
	CCF	45	NC ^e	1.000	NC ^e	1.000
Clinical benefit^d CR, PR, SD>1y vs PD, SD<1y	All series	246	2.93 (0.83-10.30)	0.094	3.78 (1.02-14.06)	0.047
	SUTOX	110	2.11 (0.42-10.69)	0.367	2.51 (0.47-13.50)	0.282
	SOGUG	74	1.20 (1.06-1.36)	0.044^f	NC ^e	0.111 ^g
	CCF	62	0.57 (0.05-6.70)	0.652	0.83 (0.04-17.88)	0.908
PFS	All series	276	1.05 (0.61-1.82)	0.852	1.23 (0.7-2.150)	0.466
	SUTOX	124	1.01 (0.44-2.32)	0.988	0.89 (0.38-2.08)	0.803
	SOGUG	88	1.59 (0.68-3.70)	0.281	1.64 (0.69-3.89)	0.261
	CCF	64	0.57 (0.14-2.42)	0.45	0.61 (0.14-2.65)	0.512

^a Multivariate analyses included as covariates Heng prognostic risk group, age, gender and study center.

^b The data corresponds to the effect of the wild type A-allele of rs34231037 where OR/HR>1.0 indicates increased risk of poor response/ shorter PFS.

^d Logistic regression comparing patients with CR, PR or SD >1 year versus patients with SD <1 year/ PD upon sunitinib treatment.

^e The associated risk could not be calculated (NC, not calculated).

^f Since all variant carriers belonged to one group, regression analysis results were not conclusive and Fisher exact test was used.

^g Since all variant carriers belonged to one group Mantel-Haenszel test was used.