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Original Research

Measured and genetically predicted plasma YKL-40 levels and melanoma mortality

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Abstract ***Purpose:*** High plasma levels of YKL-40 might be associated with mortality in patients with melanoma, and it is unknown if YKL-40 is causally related to mortality.
Experimental design: We studied two cohorts: 2618 patients with melanoma from hospital clinics and 1413 general population patients with melanoma, totalling 4031 patients followed up for mortality end-points for up to 20 years. All were genotyped for *CHI3L1* rs4950928, highly predictive of lifelong plasma YKL-40, and plasma YKL-40 levels were measured in 2165 patients. We tested the hypotheses that measured and genetically predicted high plasma YKL-40 are associated with increased mortality in patients with melanoma.

Abbreviations: AJCC, American Joint Committee on Cancer; SNP, single-nucleotide polymorphism; WHO, World Health Organisation; ICD-7 and ICD-10, International Classification of Diseases edition seven and ten; ELISA, sandwich-type enzyme-linked immunoabsorbent assay; CI, confidence interval; IL-2, interleukin-2; IFN-α, interferon-alpha; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study.

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Results: For the hospital melanoma cohort, age- and sex-adjusted hazard ratios for death in individuals with measured plasma YKL-40 in the 96–100th percentile versus 1–95th percentile and per 10-percentile increase were 1.52 (95% confidence interval, 1.07–2.16) and 1.07 (1.02–1.11), respectively, most pronounced for patients with localised melanomas. Each C-allele of the *CHI3L1* rs4950928 genotype was associated with plasma YKL-40 level increases of 32% in the hospital melanoma cohort ($p = 6 \times 10^{-48}$) and 43% in the general population melanoma cohort ($p = 7 \times 10^{-13}$). Multifactorially adjusted ratios for these increases in the combined cohorts were 1.04 (1.00–1.09) observationally for measured plasma YKL-40 and 0.98 (0.86–1.12) for the genetically predicted plasma YKL-40.

Conclusion: Measured, but not genetically predicted, increasing plasma YKL-40 was associated with increased mortality in patients with melanoma. Plasma YKL-40 is a marker but less likely to be a cause of increased mortality in patients with melanoma.

1. Introduction

The incidence of melanoma is increasing worldwide and is the main cause of skin cancer–associated deaths in industrialised countries [1]. Despite heightened awareness, and improved therapeutic options, the number of deaths due to melanoma remains high [2,3].

Patients with localised melanoma comprise more than 90% of melanoma cases [4–6] and have a 5-year survival rate of more than 96% for the thinnest lesions (defined as the American Joint Committee on Cancer [AJCC] stage I) [4,5,7]. However, up to 20% of the patients with the thickest tumours (up to 1.0 mm Breslow thickness) die from the disease within 10 years [7]. Moreover, as stage I patients comprise the majority, a high number of deaths occur among patients with thin lesions [7,8].

At present, the clinical problem is that we cannot reliably predict which patients with low-stage melanoma progress and die of the disease.

High plasma levels of YKL-40 are associated with poor survival in patients with localised or metastatic melanoma in some [9–11], but not in other [12–16] studies, totalling 2270 patients. Thus, it is currently not established if high levels of YKL-40 in the circulation are associated with mortality in patients with melanoma.

Plasma YKL-40 levels are inheritable, and rs4950928, a single-nucleotide polymorphism (SNP) in the proximal promoter of the *CHI3L1*, coding for YKL-40, explains up to 14% of the variation in plasma YKL-40 levels in the Danish general population [17]. Using this genotype, it is possible, in principle, to study the isolated effect of lifelong high levels of plasma YKL-40 and to rebut or confirm the hypothesis that high plasma YKL-40 is a cause of adverse prognosis. This is possible because genetic variance is generally not associated with any of the established prognostic factors, which therefore are less likely to confound analyses, and because reverse causation, meaning that melanoma changes a person's genes, is not possible [18].

The purpose of this study was to test the hypotheses that measured and genetically predicted high plasma levels of YKL-40 are associated with increased risk of mortality in patients with melanoma. To assess this, we performed cohort studies of 2618 patients with melanoma from hospital clinics and 1413 individuals diagnosed with melanoma from general population cohorts.

2. Material and methods

2.1. Individuals

2.1.1. Hospital melanoma cohort

The hospital melanoma cohort was enrolled prospectively from February 1997 to December 2014 at four hospital departments in Denmark: Department of Oncology at Odense University Hospital, plastic surgery departments at Aarhus University Hospital, Herlev and Gentofte Hospital and Rigshospitalet, Copenhagen. We also included individuals diagnosed with cutaneous melanoma from two independent prospective studies of the general population: the 1991–1994 examination of the Copenhagen City Heart Study and the Copenhagen General Population Study recruiting individuals from 2003 to 2013 [19,20] (Supplementary file).

2.2. Verification of diagnoses and vital status

2.2.1. Hospital melanoma cohort

The nationwide Danish Melanoma Database [21,22] provided detailed information on tumour characteristics, stage of disease at the time of diagnosis, treatment and information on disease relapse. Melanoma diagnoses were defined according to the World Health Organisation (WHO) International Classification of Diseases edition ten (ICD-10). Detailed histopathological information necessary to classify patients according

to relevant AJCC stage was mainly provided by the Danish Melanoma Database. However, for the few individuals for whom the Danish Melanoma Database lacked information on the histopathological diagnosis and the histopathological grading, it was obtained from the national Danish Pathology Register [23]. Vital status was obtained from the national Danish Civil Registration System.

2.2.2. General population melanoma cohort

Patients diagnosed with melanoma were found in the national Danish Cancer Registry, and the diagnoses were defined according to WHO ICD-7 and ICD-10. Vital status was obtained from the national Danish Civil Registration System.

2.3. Ethics

The study was conducted according to the Declaration of Helsinki. All participants gave written informed consent, and local Danish ethical committees approved the studies (KA-02152, KF-100.2039/91 and KF-01-144/01).

2.4. Plasma YKL-40 levels

Plasma levels of YKL-40 were determined in duplicate by a commercial two-site, sandwich-type enzyme-linked immunoabsorbent assay (ELISA) (Quidel Corporation, San Diego, CA, USA) [20]. The recovery of the ELISA was 102%, and the detection limit was 10 μg/L. The intra-assay coefficients of variations were 5% at levels of 40 μg/L and 4% at 104 μg/L and 155 μg/L. The inter-assay coefficient of variation was <6% [20].

2.5. Single-nucleotide polymorphism genotyping

DNA was isolated from blood leukocytes, and *CHI3L1* rs4950928 genotyping was performed using a TaqMan (Applied Biosystems by Life Technologies Corporation, Carlsbad, USA) assay as described earlier [20].

2.6. Statistical analyses

We used STATA, version 13, (Stata Corp LP, College Station, TX). We used the χ^2 test, analysis of variance test, and log-rank test of equalities of survival functions. Hardy-Weinberg equilibrium was investigated using a χ^2 test.

The primary end-point was overall survival. Survival probability was computed using the Kaplan-Meier method, which estimates the unadjusted survival function as a function of time. Hazard ratios and corresponding 95% confidence intervals for death were computed using proportional hazards Cox regression analysis adjusted for sex and age at blood sampling. In the multifactorially adjusted model, we adjusted additionally for AJCC stage (stages I-II versus stages III-IV). Unknown stage was multiple imputed using age at blood sampling and sex. Some patients with melanoma at hospital had the blood sampling performed more than 6 months after their melanoma diagnosis, and the multifactorially adjusted Cox regression analysis was also adjusted for the time gap between the date of diagnosis and the date of blood sampling, using delayed entry. Thus, the underlying time scale was left-truncated time since diagnosis. However, for patients for whom blood samples were obtained after the date of melanoma diagnosis, follow-up began at the date of blood sampling to avoid immortal time bias. For the general population melanoma cohort, individuals were included at the time of diagnosis, accounting for the preceding time since blood sampling.

Patients were followed up until 19th April 2018, date of death or emigration ($n = 16$), whichever came first.

Because plasma YKL-40 is age-dependent, the YKL-40 percentile as a function of age in years and plasma YKL-40 in μg/L was calculated: YKL-40 percentile $= 100/[1+(\text{YKL-40}^{-3} \times 1.062^{\text{age}} \times 5000)]$ [24]. In the analysis of measured plasma YKL-40, risk in the 96–100th percentiles was estimated versus the 1–95th percentiles as previously done [9,10] and continuously per 10-percentile increase.

The association between *CHI3L1* rs4950928 and survival was estimated with the GG genotype as the reference group.

In the meta-analysis, estimates from Cox regression analyses from the hospital melanoma cohort and the general population melanoma cohort were compiled using measured and genetically predicted YKL-40 levels. The change of plasma YKL-40 per C-allele was calculated by a linear regression, modelling the relationship between *CHI3L1* rs4950928 and the natural logarithm of plasma YKL-40. This estimate was used in the Cox regression analysis of measured YKL-40 levels to facilitate comparison with results for genetically predicted YKL-40 levels. Both analyses were multifactorially adjusted. The meta-analysis was performed using the 'metan' command in STATA with the random-effects model.

All P-values were two-sided.

3. Results

A total of 4031 patients from hospital clinics ($n = 2618$) and general population ($n = 1413$) were genotyped for *CHI3L1* rs4950928, and 2165 had plasma YKL-40 measured. *CHI3L1* rs4950928 genotype distribution did not differ from the Hardy-Weinberg equilibrium in the hospital melanoma cohort ($p = 0.52$) or in the general population melanoma cohort ($p = 0.35$).

In the hospital melanoma cohort, patients with AJCC stage III-IV metastatic melanoma and unknown

melanoma stage versus AJCC stage I-II localised melanoma had reduced survival (Supplementary Fig. 2), as expected.

3.1. Measured plasma YKL-40

In the hospital melanoma cohort of 1893 patients, those with plasma YKL-40 levels in the 96–100th percentile versus 1–95th percentile had a 10-year survival of 65% and 84%, respectively (p-value for the log-rank test 8×10^{-6}; Fig. 1). The corresponding age- and sex-adjusted hazard ratio for death was 1.52 (95% confidence interval, 1.07–2.16) and 1.26 (0.88–1.80), respectively, in the multifactorially adjusted analysis. Using 10-percentile increase of YKL-40 gave equivalent estimates of 1.07 (1.02–1.11) and 1.04 (1.00–1.09). Among 272 individuals from the general population melanoma cohort, the equivalent estimates were 1.05 (0.61–1.82, age- and sex-adjusted) and 1.11 (0.63–1.96, multifactorially adjusted) for those with plasma YKL-40 levels in the 96–100th percentile versus 1–95th percentile and 0.98 (0.93–1.05) and 1.02 (0.96–1.09) per 10-percentile increase of YKL-40 levels.

After stratifying the hospital melanoma cohort according to disease stage (AJCC stage I-II localised melanoma, AJCC stage III-IV metastatic melanoma and unknown stage), plasma YKL-40 levels in the 96–100th versus 1–95th percentile were associated with reduced survival in patients with AJCC stage I-II (p-value for the log-rank test 0.001) and unknown stage (p-value for the log-rank test 0.0002). After multifactorial adjustment, plasma YKL-40 levels in the 96–100th versus 1–95th percentile were associated with increased mortality among patients with AJCC stage I-II melanoma with a hazard ratio of 1.52 (95% confidence interval, 0.94–2.46). Equivalent estimate using 10-percentile increase of YKL-40 levels was 1.08 (1.01–1.14) (Fig. 2).

3.2. Genetically predicted plasma YKL-40

Per C-allele of *CHI3L1* rs4950928, mean plasma YKL-40 level increased by 32% in the hospital melanoma cohort (Fig. 3, upper panel), by 43% in the general population melanoma cohort (Fig. 3, middle panel) and by 35% in individuals without cancer from the general population (Fig. 3, lower panel) (all three p-values below 7×10^{-13}).

In both cohorts, there was no association between *CHI3L1* rs4950928 genotype and survival using log-rank statistics (p-values, 0.09 and 0.15) or in Cox regression analyses (Fig. 4).

3.3. Measured and genetically predicted plasma YKL-40 levels

We compared the estimates from analyses of the measured and genetically predicted YKL-40 levels. Because one *CHI3L1* rs4950928 C-allele increase

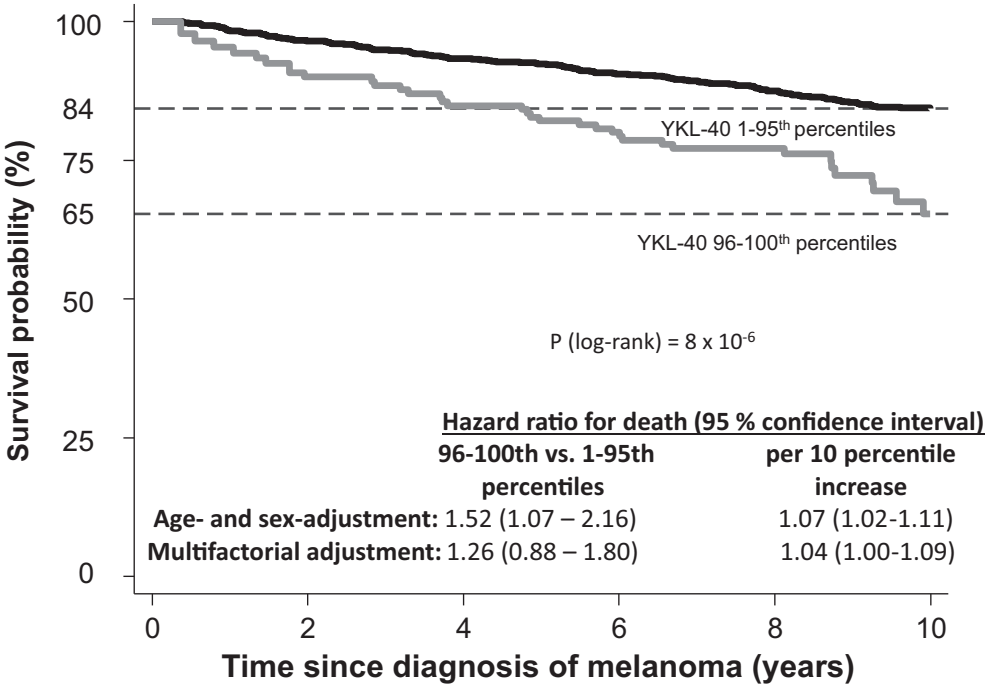
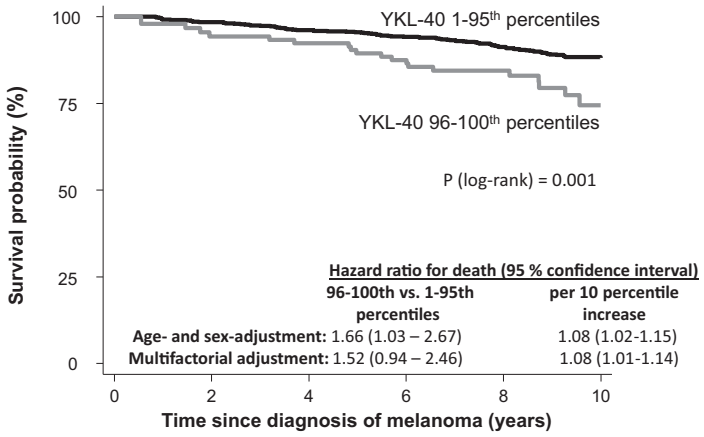
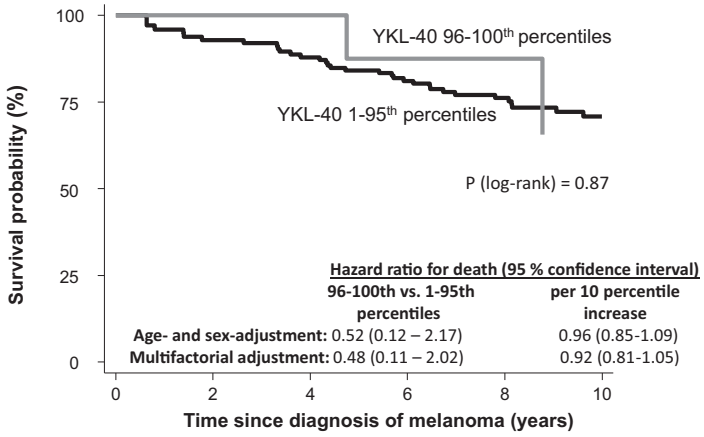


Fig. 1. Survival probability as a function of left-truncated time since diagnosis in the hospital melanoma cohort. For patients for whom blood samples were obtained after the date of melanoma diagnosis, follow-up began at the date of blood sampling instead of the date of diagnosis. Adjustment was for age, sex, disease stage, comorbidity and the time gap between the date of diagnosis and the date of blood sampling.

AJCC stage I-II localised melanoma (n = 1309)



AJCC stage III-IV metastatic melanoma (n = 144)



Unknown melanoma stage (n = 440)

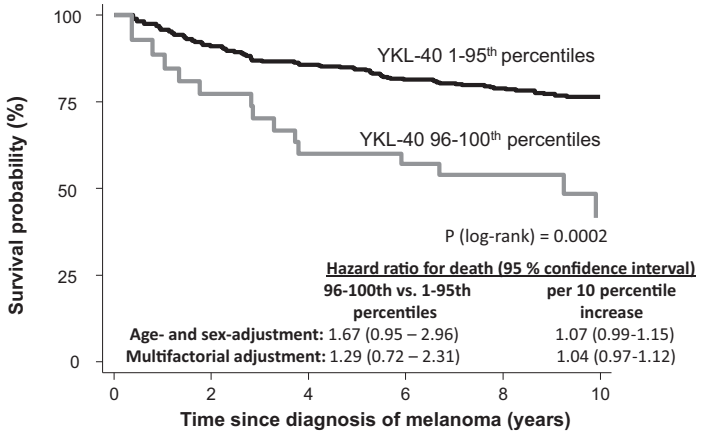


Fig. 2. Survival probability as a function of left-truncated time since diagnosis in the hospital melanoma cohort with AJCC stage I-II localised melanoma, AJCC stage III-IV metastatic melanoma and with unknown melanoma stage. For patients for whom blood samples were obtained after the date of melanoma diagnosis, follow-up began at the date of blood sampling instead of the date of diagnosis. Adjustment was for age, sex, disease stage, comorbidity and the time gap between the date of diagnosis and the date of blood sampling. AJCC: American Joint Committee on Cancer.

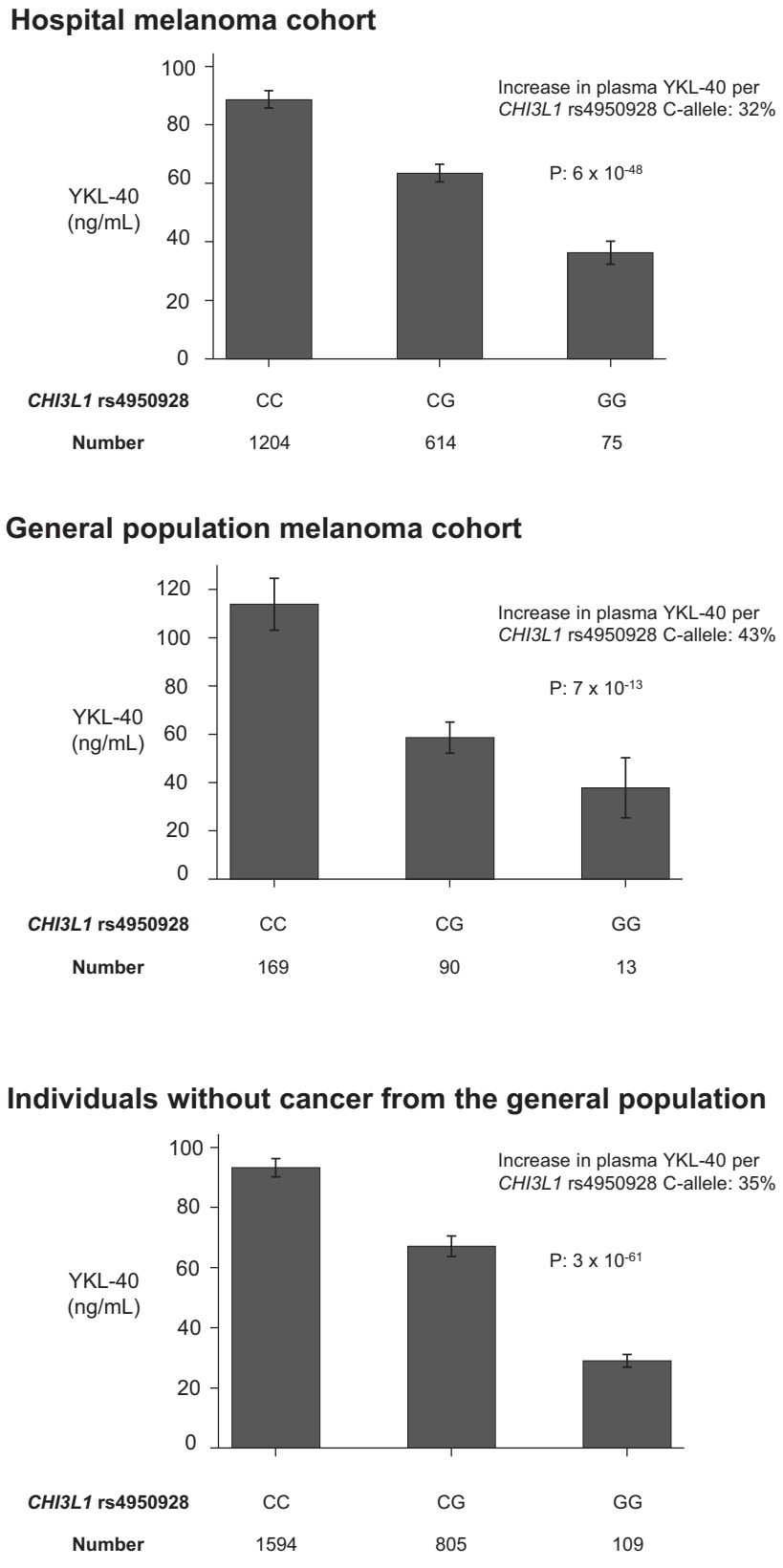


Fig. 3. Geometric mean of plasma YKL-40 according to *CHI3L1* rs4950928 genotype and the change in percent per *CHI3L1* rs4950928 C-allele in the hospital melanoma cohort, the general population melanoma cohort and in age- and sex-matched individuals from the Danish general population without cancer. The P-value is for the linear regression of the number of C-allele on the natural logarithm of measured YKL-40.

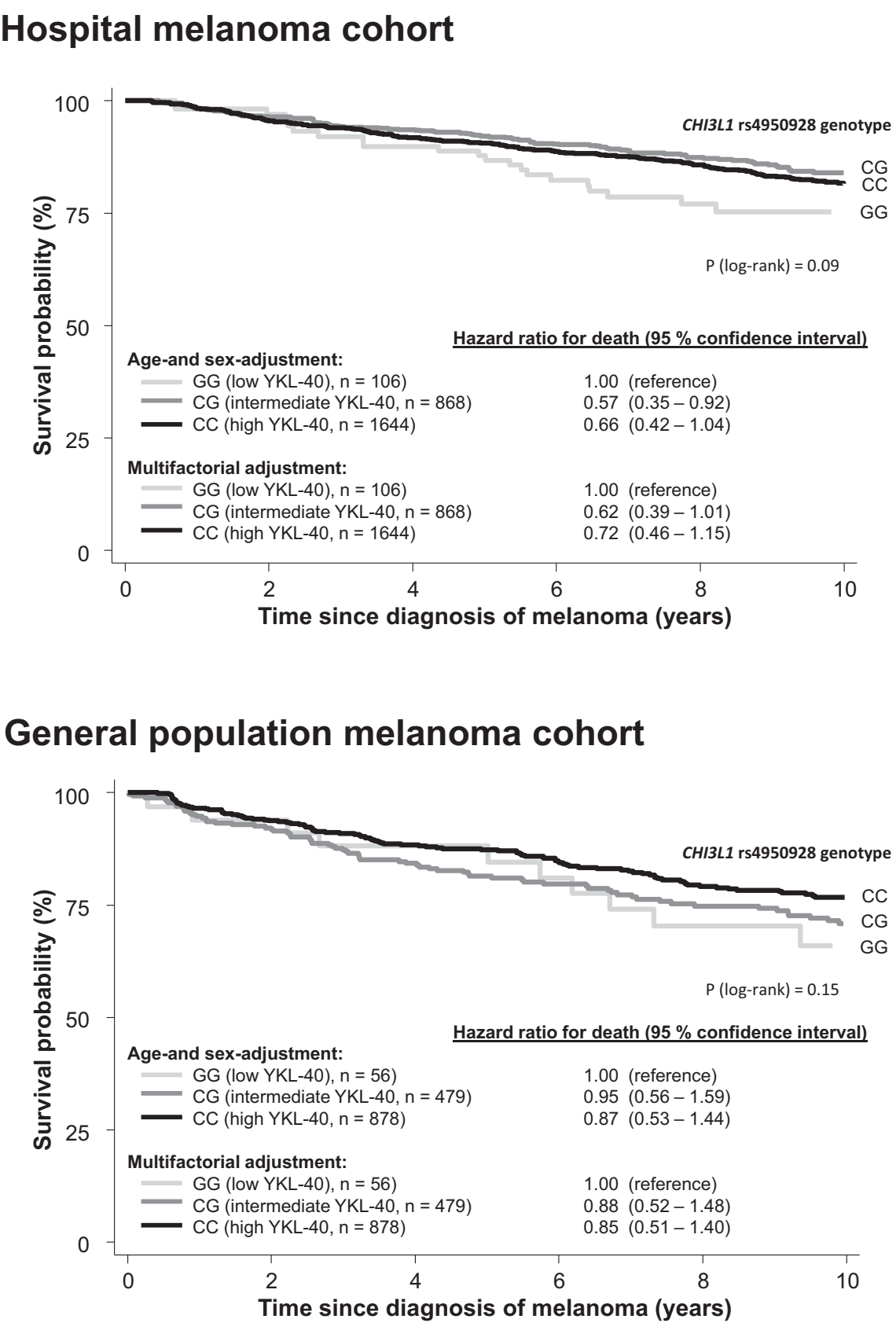


Fig. 4. Survival probability as a function of left-truncated time since diagnosis in the hospital melanoma cohort and general population melanoma cohort. For patients for whom blood samples were obtained after the date of melanoma diagnosis, follow-up began at the date of blood sampling instead of the date of diagnosis. Adjustment was for age, sex, disease stage, comorbidity and the time gap between the date of diagnosis and the date of blood sampling.

corresponds to an increase in plasma YKL-40 of 32% in the hospital melanoma cohort and 43% in the general population melanoma cohort, we used these increases in the calculation of risk associated with measured YKL-40 levels (Fig. 5).

In the hospital melanoma cohort, the multifactorially adjusted hazard ratio was 1.04 (95% confidence interval, 0.99–1.09) per 32% increase of the measured plasma YKL-40 level and 1.01 (0.84–1.21) per *CHI3L1* rs4950928 C-allele increase corresponding to a 32% YKL-40 level increase. In the general population melanoma cohort, the hazard ratio was 1.04 (0.95–1.15) per 43% increase of the measured plasma YKL-40 level and 0.94 (0.78–1.14) per *CHI3L1* rs4950928 C-allele increase corresponding to a 43% YKL-40 level increase. In the meta-analysis in which both cohorts were combined to optimise statistical power, the risk estimate of the measured increased plasma YKL-40 level was 1.04 (1.00–1.09) and 0.98 (0.86–1.12) per corresponding *CHI3L1* rs4950928 C-allele increase corresponding to a 32% or 43% YKL-40 level increase (Table 1).

4. Discussion

We found that measured increasing plasma YKL-40 was associated with increased mortality. In contrast, the *CHI3L1* rs4950928 genotype, highly predictive of lifelong plasma YKL-40 levels, was not associated with altered mortality in either cohort, or the two combined. Thus, our results indicate that measured increasing plasma YKL-40 is a marker and less likely to be a cause of increased mortality in patients with melanoma. These are novel findings.

Mechanistically, the reason for the association between elevated plasma YKL-40 and increased mortality is unknown, but methylation and epigenetic mechanisms might play a role [25] and are likely both caused by other processes, e.g. inflammation. Indeed, inflammation contributes to skin cancer development, growth and progression [26], and high plasma YKL-40 might be caused by the inflammation involved in growth and progression of melanoma. YKL-40 might further play a role in cancer cell proliferation and differentiation, metastasis, protection against apoptosis, stimulation of

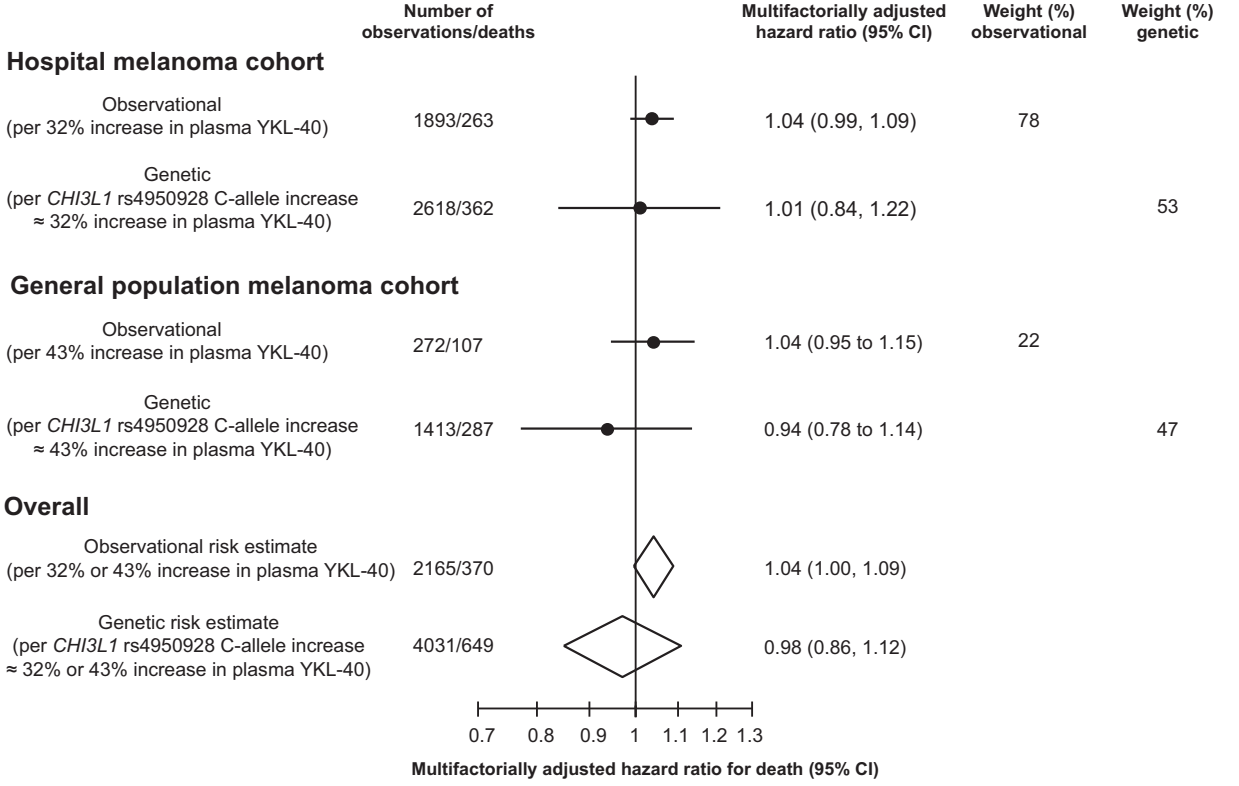


Fig. 5. Meta-analysis of hazard ratios for death as a function of measured plasma YKL-40 and genetically predicted plasma YKL-40 in the hospital melanoma cohort and the general population melanoma cohort. For patients for whom blood samples were obtained after the date of melanoma diagnosis, follow-up began at the date of blood sampling instead of the date of diagnosis. Models were multifactorially adjusted for age, sex, disease stage, comorbidity and the time gap between the date of diagnosis and the date of blood sampling. Overall effects are from meta-analyses using random effects. I-squared = 0% of both observational and genetic risk estimates. CI: Confidence interval.

Table 1
Characteristics of patients with melanoma.

Baseline characteristics	CHI3L1 *rs4950928*	CC	CG	GG	p-value[a]
	Plasma YKL-40	High	Intermediate	Low	
From hospital clinics					
Number (%)		1644 (63)	868 (33)	106 (4)	0.52[b]
Women (% per genotype)		889 (54)	475 (55)	53 (50)	0.65
Age at diagnosis, years (interquartile range)		56 (43–67)	56 (43–67)	51 (39–65)	0.61
American Joint Committee Cancer stage I + II, n (% per genotype)		1229 (69)	585 (67)	67 (63)	
American Joint Committee Cancer stage III + IV, n (% per genotype)		106 (6)	64 (7)	12 (11)	0.65
American Joint Committee Cancer stage missing, n (% per genotype)		409 (25)	219 (25)	27 (25)	
Comorbidity[c] = 0, n (% per genotype)		1118 (68)	583 (67)	66 (62)	
Comorbidity[c] = 1 or 2, n (% per genotype)		283 (17)	155 (18)	18 (17)	
Comorbidity[c]>2, n (% per genotype)		214 (13)	121 (14)	20 (19)	
Comorbidity[c] missing, n (% per genotype)		29 (2)	9 (1)	2 (2)	
From general population					
Number (%)		878 (62)	479 (34)	56 (4)	0.35[b]
Women (% per genotype)		494 (56)	260 (54)	24 (43)	0.14
Age at diagnosis, years (interquartile range)		60 (48–70)	61 (48–70)	63 (50–74)	0.10
Comorbidity[c] = 0, n (% per genotype)		667 (76)	363 (76)	41 (73)	
Comorbidity[c] = 1 or 2, n (% per genotype)		167 (19)	80 (17)	13 (23)	
Comorbidity[c]>2, n (% per genotype)		33 (4)	25 (5)	2 (4)	
Comorbidity[c] missing, n (% per genotype)		11 (1)	11 (2)	0 (0)	

Data are represented as the number and percentage for sex and stage and median and interquartile range for age.
[a] P-values were obtained from Pearson chi-squared test for sex and stage and analysis of variance for age.
[b] P-values for the Hardy-Weinberg equilibrium obtained by Pearson chi-squared test.
[c] Charlson comorbidity index at the date of blood sampling ignoring melanoma diagnoses.

angiogenesis and regulation of extracellular tissue remodelling [27,28]. This plausible causal role has prompted studies of targeting YKL-40 function in cancers as a therapeutic option. In mouse models of xenografted glioblastoma, treatment with a YKL-40–neutralising antibody or ionising irradiation both led to inhibition of tumour growth and increased mouse survival [29]. In addition, in a recent study with mice injected with a melanoma cell line, knock-down of *CHI3L1* reduced the size and number of metastatic melanoma nodules in lung tissues of the mice [30], supported by a recent study [31], but in contrast to another [32].

Our findings on measured plasma YKL-40 are partly supported by a study of 234 patients with AJCC stage I and II localised melanoma in which YKL-40 was a prognostic factor for relapse-free and overall survival [10] and by another study in which high serum YKL-40 level was associated with short overall survival in AJCC stage IIB-III among 1234 patients with melanoma [11]. Furthermore, high serum YKL-40 was an independent prognostic factor for poor survival among 110 patients with AJCC stage IV metastatic melanoma [9]. Finally, among 8899 individuals from the general population, high plasma YKL-40 level was associated with risk of early death from any cancer and other diseases [33].

In contrast, YKL-40 was not associated with survival in two studies of patients with advanced disease [15]. Similarly, in three other studies in which YKL-40 was measured using a different type of ELISA kit, YKL-40 was not associated with survival [12]. However, it should be noted that risk estimates are dependent on the YKL-40 measurement assay.

Increased melanoma mortality was associated with measured, but likely not with genetically predicted increasing plasma YKL-40. This discrepancy has also been found in studies of other diseases and YKL-40: asthma severity [34], cancer risk [20], venous thromboembolism [17] and cardiovascular and liver disease [35]. The SNP *CHI3L1* rs4950928 genotype explains 14% of the variation of plasma YKL-40 concentration [17], which is a very high number compared with that in other genetic studies of plasma markers [36]. In this study, the F statistics for the correlation between plasma YKL-40 and genotype is 438 and thus well above the conventional limit of 10 [37] to be used as an instrument to inform genetic causal analyses.

Strengths of our study include the large sample size of our hospital melanoma cohort, the accuracy of information on date of death and the long follow-up period. It is also a strength that we studied both measured plasma YKL-40 and genetically predicted YKL-40 levels.

A potential limitation of the study is that some patients with melanoma at hospital had blood sampled more than 6 months after melanoma diagnosis. However, because we adjusted for this period, and because higher YKL-40 plasma levels were not associated with increased mortality for patients with higher disease stages, unacknowledged disease progression from localised to metastatic in this period is unlikely to explain our results.

Limitations further include the lack of clinical data and other confounding factors for the population cohort and the long intervals between blood sampling and diagnosis. This might be the cause of the weakened association because the disease was probably not present or at best subclinical at the time of blood sampling for many of these patients. Despite this, however, both the observational and genetic estimates for the association between YKL-40 level and risk of death were similar to the estimates from the hospital melanoma cohort.

In conclusion, measured increasing plasma YKL-40 was associated with reduced survival in patients with melanoma, but YKL-40 is likely not a cause because genetically predicted high plasma YKL-40 does not seem to be associated with reduced melanoma survival.

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Conflict of interest statement

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.08.025.

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