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The KL-VS sequence variant of *Klotho* and cancer risk in *BRCA1* and *BRCA2* mutation carriers

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Abstract

Klotho (KL) is a putative tumor suppressor gene in breast and pancreatic cancers located at chromosome 13q12. A functional sequence variant of *Klotho* (KL-VS) was previously reported to modify breast cancer risk in Jewish *BRCA1* mutation carriers. The effect of this variant on breast and ovarian cancer risks in non-Jewish *BRCA1/BRCA2* mutation carriers has not been reported. The KL-VS variant was genotyped in women of European ancestry carrying a *BRCA* mutation: 5,741 *BRCA1* mutation carriers (2,997 with breast cancer, 705 with ovarian cancer, and 2,039 cancer free women) and 3,339 *BRCA2* mutation carriers (1,846 with breast cancer, 207 with ovarian cancer, and 1,286 cancer free women) from 16 centers. Genotyping was accomplished using TaqMan® allelic discrimination or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Data were analyzed within a retrospective cohort approach, stratified by country of origin and Ashkenazi Jewish origin. The per-allele hazard ratio (HR) for breast cancer was 1.02 (95% CI 0.93–1.12, $P = 0.66$) for *BRCA1* mutation carriers and 0.92 (95% CI 0.82–1.04, $P = 0.17$) for *BRCA2* mutation carriers. Results remained unaltered when analysis excluded prevalent breast cancer cases. Similarly, the per-allele HR for ovarian cancer was 1.01 (95% CI 0.84–1.20, $P = 0.95$) for *BRCA1* mutation carriers and 0.9 (95% CI 0.66–1.22, $P = 0.45$) for *BRCA2* mutation carriers. The risk did not change when carriers of the 6174delT mutation were excluded. There was a lack of association of the KL-VS *Klotho* variant with either breast or ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers.

Keywords

Breast cancer; Ovarian cancer-*Klotho*; BRCA; Modifier gene

Introduction

The *Klotho* (KL) gene (MIM # 604824) encodes a 1,014 amino acid transmembrane protein [1]. The Klotho protein has pleiotropic activities, including potent inhibition of the insulin receptor (IR) and the IGF-1 receptor (IGF-1R) [2]. This pathway plays a prominent role in breast cancer pathogenesis [3, 4] and specifically in *BRCA1–BRCA2*-associated breast cancers [5, 6] and epithelial ovarian cancer [7]. These functional activities, taken together with the in vitro evidence of its tumor suppressor activity in breast cancer cell lines and altered expression somatically in breast cancer tissues [8], combined with the chromosomal location of the gene at 13q12, a frequent site of allelic loss in breast and ovarian tumors [9], make KL a putative tumor suppressor gene in breast and ovarian cancer. Since the lifetime risk for developing these tumor types is substantially increased in women who carry germline mutations in *BRCA1* or *BRCA2* [10, 11], it seemed plausible that KL could affect cancer penetrance in *BRCA1* and/or *BRCA2* mutation carriers. Indeed, we have previously shown that loss of function sequence variants in the *Klotho* gene [amino acid substitutions F352V (rs9536314) and C370S (rs9527025) both in linkage disequilibrium—coined KL-

VS] that can be evaluated by genotyping the rs9536314 SNP are associated with an altered breast cancer risk in Jewish Ashkenazi *BRCA1* mutation carriers [12]. As the penetrance of mutated *BRCA1* and *BRCA2* alleles may in part be determined by the location and the type of mutation, and by other genetic factors, at times ethnically restricted, and since the mutational spectrum in Ashkenazi Jews in *BRCA1* is limited [13, 14], this study aimed to evaluate the putative modifier effect of the KL-VS sequence variants on mutant *BRCA1* and *BRCA2* allele penetrance in a larger cohort of ethnically diverse mutation carriers.

Methods

Study participants—recruitment and data collection

All study participants were women, aged 18 years or above, who carried a deleterious germline mutation in either *BRCA1* or *BRCA2*. Study participant genotype and phenotype data were submitted from 16 centers participating in the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) [15]. The recruitment strategy and the type of clinical, demographic, and phenotypic data collected from each participant, as well as the means used to ensure that there are no overlaps and no duplicate genotyping, have previously been reported [15, 16]. All study participants took part in research studies at the parent institutions under ethically approved protocols as previously detailed [15, 16].

Genetic analysis

All participating centers genotyped the KL-VS variant (rs9536314) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [16], or TaqMan® allelic discrimination with limited sequencing, as previously described [12]. Genotyping quality control procedures were carried out as reported elsewhere (http://www.srl.cam.ac.uk/consortia/cimba/eligibility/eligibility.html).

Statistical analysis

Data were analyzed within a retrospective likelihood approach [17], assuming a proportional hazards model for the effect of the genotype on cancer incidence. For the breast cancer analysis, carriers were censored at the age of the first of the following events: breast cancer diagnosis, ovarian cancer diagnosis, bilateral prophylactic mastectomy, or age at last observation. Only those censored at a breast cancer diagnosis were assumed to be affected. To evaluate the association with ovarian cancer risk, data were analyzed within a competing risks model framework, which evaluates the associations with breast and ovarian cancer risk simultaneously [18]. For this analysis, mutation carriers were followed up to the age of the first breast or ovarian cancer diagnosis and were considered to have developed the corresponding disease. Individuals were censored for breast cancer at the age of bilateral prophylactic mastectomy and for ovarian cancer at the age of bilateral oophorectomy and were assumed to be unaffected for the corresponding disease. The remaining individuals were censored at the age at last observation and were assumed to be unaffected for both diseases. For all models, the effect of each SNP was modeled either as a per-allele hazard ratio (HR) (multiplicative model) or as separate HRs for heterozygotes and homozygotes, and these were estimated on the log scale (i.e., β_j). Analyses were carried out using the pedigree analysis software MENDEL [19]. A robust variance-estimation approach was used to allow for the non-independence among related carriers [20]. Analyses were stratified by country of residence and used calendar-year- and cohort-specific cancer incidences for *BRCA1* and *BRCA2* [21]. Because of the previously reported linkage disequilibrium between the KL-VS variant and *BRCA2* 6174delT mutation [12], the US American *BRCA2* carriers, who contributed the majority of the 6174delT mutation carriers, were further stratified according to whether they carried this particular mutation or not.

Results

A total of 9,080 mutation carriers (5,741 *BRCA1* and 3,339 *BRCA2* mutation carriers) from 16 centers were included in the current analysis (Table 1, Supplementary Table 1). In the breast cancer analysis there were 4,870 subjects with breast cancer (3,020 *BRCA1* mutation carriers, 1,850 *BRCA2* mutation carriers), and 4,210 unaffected carriers (2,721 *BRCA1* carriers, 1,489 *BRCA2* carriers) (Tables 1, 2). The genotype frequencies and estimated HRs by mutation and disease status for breast and ovarian cancer are shown in Tables 2, 3, and 4. There was no evidence of association between breast cancer risk and the KL-VS genotype for either *BRCA1* or *BRCA2* mutation carriers (per-allele HR = 1.02, 95% CI 0.93–1.12, P = 0.66 and 0.92, 95% CI 0.82–1.04, P = 0.17 for *BRCA1* and *BRCA2*, respectively). There was no evidence of heterogeneity in the HRs across studies: p-het = 0.91 for *BRCA1* and p-het = *0.78* for *BRCA2* (Fig. 1a, b). There was also no evidence of association of the *Klotho* sequence variant with ovarian cancer risk for *BRCA1* mutation carriers (per-allele HR = 1.01, 95% CI 0.84–1.20, P = 0.95) or *BRCA2* mutation carriers (per-allele HR = 0.90, 95% CI 0.66–1.22, P = 0.49) (Table 4). The results were unchanged when long-term cancer survivors (prevalent cases) were excluded from the analyses, or when carriers of the 6174delT mutation were excluded. Furthermore, the KL-VS variant was not significantly associated with breast cancer risk in an analysis of self-reported Ashkenazi Jewish participants (HR = 1.22, 95% CI 0.91–1.64, P = 0.18 for 374 *BRCA1* mutation carriers and HR = 0.97, 95% CI 0.56–1.69, P = 0.93 for 209 *BRCA2* mutation carriers).

Discussion

In this study, functional *KL* gene variants had no discernable effect on either breast or ovarian cancer risk in a large group of ethnically diverse *BRCA1* and *BRCA2* mutation carriers. These findings are in contrast with our previous report which showed that in Jewish *BRCA1* mutation carriers there was an association of the same sequence variant on breast cancer risk [12]. The estimated HRs based on the complete sample as well as the restricted sample of Ashkenazi Jewish mutation carriers was considerably smaller than the relative risk reported previously. Several reasons could account for the lack of replication in this study: the number of self-reported Ashkenazi Jewish individuals in this study was relatively small (*BRCA1* n = 374, *BRCA2* n = 209). Therefore, if the association is restricted to Ashkenazi Jewish mutation carriers only, the present analysis may be under-powered. Alternatively, given the sample size of this study, the original finding may have been a false positive. It is well established that the mutation location and mutation type affect cancer risk in *BRCA1* and *BRCA2* mutation carriers [22, 23]. As the mutational spectrum locations and types in the present analysis was more diverse than in the Ashkenazi population, it is also possible that these inconsistencies in the results between this study and the previous one may relate to the effect of the *Klotho* variant on the specific Ashkenazi mutations based on their location and effect on protein.

Although this particular polymorphism did not show any effect on breast/ovarian cancer risk in *BRCA1/BRCA2* mutation carriers, *Klotho* gene may still modify these risks by another sequence alteration that may be in linkage equilibrium in the Ashkenazi Jewish population or by other variants not or only in weak linkage disequilibrium with the current polymorphism, by epigenetic alterations in gene expression (e.g., methylation, miRNA effects) or in combination and interaction with other polymorphisms that in concert have an overall effect on cancer risk.

In conclusion, the KL-VS sequence variant in the *Klotho* gene has no major effect on breast or ovarian cancer risk in genetically susceptible, ethnically diverse individuals who carry either a deleterious *BRCA1* or a *BRCA2* mutation. However, the effect of this gene and its

putative involvement in penetrance of mutant *BRCA1* or *BRCA2* alleles by other genetic or epigenetic mechanisms cannot be ruled out, especially in Ashkenazi Jews.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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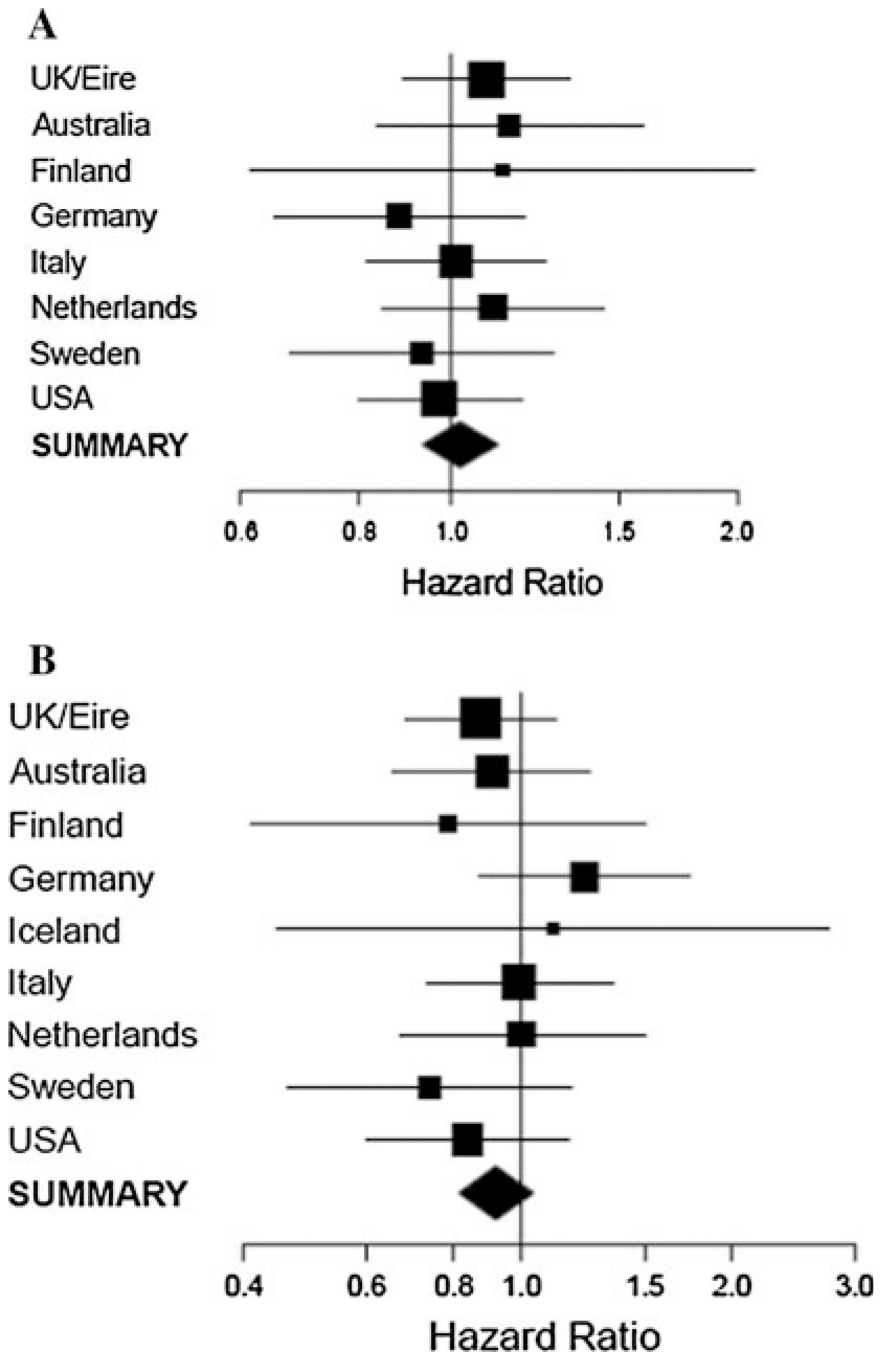

Fig. 1.

a Per-allele stratum-specific HRs for breast cancer risk in *BRCA1* carriers and **b** Per-allele stratum-specific HRs for breast cancer risk in *BRCA2* carriers

Table 1

Summary characteristics for the 9080 eligible *BRCA1* and *BRCA2* mutation carriers used in the analysis

Characteristic	*BRCA1*		*BRCA2*	
	Unaffected	**Breast Cancer**	**Unaffected**	**Breast Cancer**
Number	2,721	3,020	1,489	1,850
Person-years follow-up	116,795	122,809	66,559	81,486
Median age at censure (IQR)	42 (33–51)	39 (34–46)	43 (35–53)	43 (37–50)
Age at censure, *N*(%)				
<30	567 (20.8)	392 (13.0)	288 (19.3)	169 (9.1)
30–39	707 (26.0)	1205 (39.9)	359 (24.1)	546 (29.5)
40–49	727 (26.7)	968 (32.1)	391 (26.3)	678 (36.7)
50–59	463 (17.0)	350 (11.6)	242 (16.3)	324 (17.5)
60–69	178 (6.5)	89 (3.0)	137 (9.2)	100 (5.4)
70+	79 (2.9)	16 (0.5)	72 (4.8)	33 (1.8)
Year of birth, *N*(%)				
<1920	9 (0.3)	13 (0.4)	11 (0.7)	23 (1.2)
1920–1929	62 (2.3)	105 (3.5)	46 (3.0)	85 (4.6)
1930–1939	196 (7.2)	279 (9.2)	115 (7.0)	214 (11.6)
1940–1949	413 (15.2)	651 (21.6)	206 (13.8)	420 (22.7)
1950–1959	603 (22.2)	890 (29.5)	315 (20.8)	584 (31.6)
1960–1969	755 (27.8)	794 (26.3)	429 (27.2)	458 (23.1)
1970+	683 (25.1)	288 (9.5)	367 (27.6)	96 (5.2)
Ethnicity, *N*(%)				
Ashkenazi Jewish	172 (6.3)	202 (6.7)	105 (7.1)	104 (5.6)
Not Ashkenazi Jewish	2,549 (93.7)	2,818 (93.3)	1,384 (92.9)	1,746 (94.4)

IQR interquartile range Carriers of European or Ashkenazi Jewish ancestry only

Table 2

SNP genotype distribution and association with breast cancer risk. Analysis restricted to mutation carriers of European ancestry

Mutation	Genotype	Unaffected, *N* (%)	Affected[a], *N* (%)	HR	95% CI	*P* value
BRCA1	GG	1,941 (71.3)	2,160 (71.5)	1		
	AG	701 (25.8)	775 (25.7)	1.02	0.91–1.14	
	AA	79 (2.9)	85 (2.8)	1.06	0.79–1.41	
	2-df test					0.90
	Per-allele			1.02	0.93–1.12	0.66
BRCA2	GG	909 (61.1)	1,201 (64.9)	1		
	AG	504 (33.9)	589 (31.8)	0.96	0.83–1.10	
	AA	76 (5.1)	60 (3.2)	0.75	0.53–1.05	
	2-df test					0.24
	Per-allele			0.92	0.82–1.04	0.17

[a]Breast cancer

Table 3

Association with breast cancer risk, after excluding prevalent breast cancer cases

	Unaffected, *N*	Affected, *N*	HR	95% CI	*P* value
Excluding prevalent breast cancer cases					
BRCA1	2,721	1,513	0.99	0.89–1.11	0.86
BRCA2	1,489	908	0.92	0.79–1.06	0.26

Analysis restricted to mutation carriers of European ancestry

Table 4

Competing risk analysis

	Unaffected, *N* (%)	Breast cancer, *N* (%)	Ovarian cancer, *N* (%)	Breast cancer			Ovarian cancer		
				HR	95% CI	*P* value	HR	95% CI	*P* value
BRCA1									
GG	1,447 (71.0)	2,142 (71.5)	512 (72.6)	1			1		
AG	536 (26.3)	770 (25.7)	170 (24.1)	1.01	0.90–1.13		0.94	0.76–1.16	
AA	56 (2.8)	85 (2.8)	23 (3.3)	1.12	0.83–1.52		1.29	0.76–2.19	
2-df test						0.76			0.51
Per-allele				1.03	0.93–1.13	0.60	1.01	0.84–1.20	0.95
BRCA2									
GG	787 (61.2)	1198 (64.9)	125 (60.4)	1			1		
AG	435 (33.8)	589 (31.9)	69 (33.3)	0.94	0.81–1.09		0.82	0.58–1.14	
AA	64 (5.0)	59 (3.2)	13 (6.3)	0.73	0.51–1.04		0.97	0.45–2.08	
2-df test						0.19			0.48
Per-allele				0.91	0.80–1.02	0.11	0.90	0.66–1.22	0.49

Association with breast and ovarian cancer risk for *BRCA1* and *BRCA2* mutation carriers. Analysis restricted to mutation carriers of European ancestry

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