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Inherited Chromosomally Integrated Human Herpesvirus 6 and Breast Cancer

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Abstract

Background: Inherited chromosomally integrated human herpesvirus 6 (iciHHV-6) is a condition observed in approximately 1% of the population. Whether such a genetic alteration predisposes to cancer development in currently unknown. Two studies were conducted to determine whether iciHHV-6 is associated with cancer development.

Methods: First, a screen of 19,597 people from the province of Quebec (Canada) was conducted. A replication test, using data from a population-based case–control study of 1,090 women with incident breast cancer and 1,053 controls from British Columbia and Ontario (Canada) was conducted. DNA samples were analyzed by qPCR and droplet digital PCR to identify iciHHV-6$^+$ carriers.

Results: In the initial study, a potential association between iciHHV-6 positivity and breast cancer was identified [OR = 2.66; 95% confidence interval (CI), 0.95–7.44]. In the replication dataset, no association was found between iciHHV-6 positivity in women and breast cancer (OR = 0.87; 95% CI, 0.35–2.15).

Conclusions: We found no statistically significant associations between inherited chromosomally integrated HHV-6 and breast cancer in women.

Impact: These results do not provide evidence to suggest that iciHHV-6 is a risk factor for breast cancer. *Cancer Epidemiol Biomarkers Prev; 26(3); 425–7. ©2016 AACR.*

Introduction

Human herpesvirus-6 (HHV-6) is unique among human herpesviruses in its ability to integrate its genome in the telomeric region of host chromosomes (reviewed in ref. 1). When HHV-6 infection and integration occur in gametes, germline transmission of the viral genome occurs according to the Mendel's law of chromosome segregation, meaning that 50% of children will inherit the integrated HHV-6 (2). Consequently, individuals with inherited ciHHV-6 carry one copy of the viral genome in every somatic cell. It is estimated that approximately 1% of the world population (70 million individuals) has inherited chromosomally integrated HHV-6 (iciHHV-6). Considering that the viral genome is relatively large (*circa* 160 kbp), insertion within the telomeric region may affect telomere integrity and contribute to disease development. Interestingly, integration of Marek disease virus (a chicken herpesvirus) into the telomeric region of chicken chromosomes is linked with the development of lymphomas (3). Using samples from the CARTaGENE cohort (19,597 subjects from the province of Quebec, Canada), we recently reported that iciHHV-6$^+$ subjects are at three times greater risk of developing angina than iciHHV-6$^-$ subjects (4). Whether iciHHV-6 contributes to other diseases, such as cancer, is currently unknown. We were therefore interested in determining whether iciHHV-6$^+$ subjects are at a greater risk of developing cancer.

Materials and Methods

The study was performed in two stages. The first used DNA samples from men and women ($N = 19,597$) from the province of Quebec between the ages of 40 and 69 years. Details on the CARTaGENE cohort were previously described (5). The second stage utilized DNA samples from the Canadian Breast Cancer Study (CBCS) in Vancouver, British Columbia, and Kingston, Ontario (6). Cases were women, ages 40 to 80 years, with a diagnosis of either *in situ* or invasive breast cancer with no previous cancer history (except nonmelanoma skin cancer; $n = 1,090$). Controls were cancer-free age–frequency matched women from breast screening clinics in the same geographic areas who consented to participate in research ($n = 1,053$). Detailed pathology information was available for most cases. DNA samples were screened using qPCR, and the results are validated by ddPCR as described previously (4). The prevalence of iciHHV-6 at the time of blood sampling was determined. ORs and 95% confidence intervals (CI) were used to compare the prevalence of iciHHV-6 among women with or without a diagnosis of breast cancer. Breast cancer types (ER$^{+/-}$, PR$^{+/-}$, Her2$^{+/-}$) were also examined in relation to iciHHV-6.

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Table 1. Prevalence of iciHHV-6 status according to sex and cancer prevalence from CARTaGENE

Sex	*n* (%)			iciHHV-6$^-$*N* (%)			iciHHV-6$^+$*N* (%; 95% CI)			
Males	9,560 (48.78)			9,496 (48.74)			64 (0.33; 0.47–0.67)			
Females	10,037 (51.22)			9,988 (51.26)			49 (0.25; 0.34–0.53)			
Total	19,597 (100)			19,484 (99.42)			113 (0.58)			
Cancer type	**iciHHV-6$^-$**	**iciHHV-6$^+$**		**iciHHV-6$^-$ males**	**iciHHV-6$^+$ males**		**iciHHV-6$^-$ females**	**iciHHV-6$^+$ females**		
	***N* = 19,484 (%)**	***N* = 113 (%)**	***P*[a]**	***n* = 9,496 (%)**	***n* = 64 (%)**	***P*[a]**	***n* = 9,988 (%)**	***n* = 49 (%)**	***P*[a]**	**OR (95% CI)**
Cancer (all)	1,553 (7.97)	12 (10.62)	0.38	621 (6.54)	5 (7.81)	0.86	932 (9.33)	7 (14.29)	0.34	1.63 (0.73–3.62)
Breast	326 (1.67)	4 (3.54)	0.24	3 (0.03)	0 (0.00)	ND	323 (3.23)	4 (8.16)	0.12	2.66 (0.95–7.44)
Skin	327 (1.68)	0 (0.00)	0.31	152 (1.60)	0 (0.00)	0.60	175 (1.75)	0 (0.00)	0.70	0.56 (0.03–9.20)
Prostate	177 (0.91)	1 (0.88)	0.64	177 (1.86)	1 (1.56)	0.84	N/A	N/A	N/A	N/A
Cervix	124 (0.64)	0 (0.00)	0.80	N/A	N/A	N/A	124 (1.24)	0 (0.00)	0.80	0.80 (0.04–13.06)

Abbreviations: N/A, not applicable; ND, not determined (too few cases).
[a]Fisher exact test.

Table 2. Prevalence of iciHHV-6 according to case–control status in the CBCS

	Controls (*n* = 1,053)	**Cases (*n* = 1,090)**		
	Mean age (years ± SD) at enrollment/diagnosis		***P***	
	56.90 ± 10.10	57.30 ± 10.30	0.21[a]	
	iciHHV-6 prevalence			OR (95% CI)
iciHHV-6$^-$	1,043 (99.05)	1,081 (99.18)		
iciHHV-6$^+$	10 (0.95)	9 (0.82)	0.94[b]	0.87 (0.35–2.15)

[a]*t* test.
[b]Fisher exact test.

Results

In the population screen of the CARTaGENE cohort, prevalence of iciHHV-6 in men and women was 0.58% (113/19,597; Table 1). The overall prevalence of cancer was similar between participants with or without iciHHV-6 (Table 1; ref. 4). For individual cancers, the prevalence of skin cancer, prostate cancer in males, and cervical cancer in females was similar between iciHHV-6$^+$ and iciHHV-6$^-$ participants. The prevalence of other cancer types was too low to analyze. A finding of interest was that the prevalence of iciHHV-6 was greater among women with breast cancer (4/327, 1.22%) than in women without breast cancer (45/9,710, 0.46%; OR = 2.66; 95% CI, 0.95–7.44). This suggested that iciHHV-6 may be a risk factor for breast cancer development. We therefore sought to test this finding in the CBCS study (6).

In the CBCS study (Table 2), the prevalence of iciHHV-6$^+$ was similar in women with (9/1,090, 0.82%) or without (10/1,053, 0.95%) breast cancer (OR = 0.87; 95% CI, 0.35–2.15). No differences in iciHHV-6 prevalence were observed between breast cancer subtypes or by menopausal status at the time of breast cancer diagnosis (data not shown).

Discussion

Genome-wide and large-scale candidate gene association studies have identified more than 75 common susceptibility loci. Thus, more than one third of the genetic variance in breast cancer risk can now be explained by known loci (7–9). Although additional important high-risk loci are unlikely to exist, the remaining proportion of the genetic variance in breast cancer risk could be explained by a combination of intermediate- and low-risk alleles (10). Despite initial results in the CARTaGENE cohort that suggested higher prevalence of iciHHV-6$^+$ among women with breast cancer, an independent investigation did not confirm the association.

A limitation of these analyses lies in the small number of iciHHV-6$^+$ subjects in these populations, which limits power to detect associations between iciHHV-6 positivity and disease. Furthermore, depending on the chromosome targeted for integration, different disease outcome may occur. Cytogenetic analyses on a large number of iciHHV-6$^+$ subjects would enable determination of whether telomeric integration into specific chromosomes represents a risk factor for malignancy development. In conclusion, our data suggest that iciHHV-6$^+$ women are at no greater risk of developing breast cancer than women without iciHHV-6.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Other (co-conception, design, and conduct along with J.J. Spinelli of the original breast cancer case–control study on which part of the current research is based): K.J. Aronson

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References

1. Kaufer BB, Flamand L. Chromosomally integrated HHV-6: impact on virus, cell and organismal biology. Curr Opin Virol 2014;9C:111–8.
2. Daibata M, Taguchi T, Sawada T, Taguchi H, Miyoshi I. Chromosomal transmission of human herpesvirus 6 DNA in acute lymphoblastic leukaemia. Lancet 1998;352:543–4.
3. Kaufer BB, Jarosinski KW, Osterrieder N. Herpesvirus telomeric repeats facilitate genomic integration into host telomeres and mobilization of viral DNA during reactivation. J Exp Med 2011;208:605–15.
4. Gravel A, Dubuc I, Morissette G, Sedlak RH, Jerome KR, Flamand L. Inherited chromosomally integrated human herpesvirus 6 as a predisposing risk factor for the development of angina pectoris. Proc Natl Acad Sci USA 2015;112:8058–63.
5. Awadalla P, Boileau C, Payette Y, Idaghdour Y, Goulet JP, Knoppers B, et al. Cohort profile of the CARTaGENE study: Quebec's population-based biobank for public health and personalized genomics. Int J Epidemiol 2013;42:1285–99.
6. Grundy A, Richardson H, Burstyn I, Lohrisch C, SenGupta SK, Lai AS, et al. Increased risk of breast cancer associated with long-term shift work in Canada. Occup Environ Med 2013;70:831–8.
7. Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet 2015;47:373–80.
8. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45:353–61.
9. Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet 2013;45:371–84.
10. Couch FJ, Nathanson KL, Offit K. Two decades after BRCA: setting paradigms in personalized cancer care and prevention. Science 2014;343:1466–70.