INHERITED CHROMOSOMALLY-INTEGRATED HUMAN HERPESVIRUS 6 AND BREAST CANCER

Running title: iciHHV-6 and 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 was conducted. A replication test, using data from a population-based case-control study of 1090 women with incident breast cancer and 1053 controls from British Columbia and Ontario was conducted. DNA samples were analyzed by Q-PCR 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).

Conclusion

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.

Introduction

Human herpesviruses-6 (HHV-6) is unique among human herpesviruses in its ability to integrate its genome in the telomeric region of host chromosomes (reviewed in (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) have 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's 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 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 aged between 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, aged 40–80 years, with a diagnosis of either *in situ* or invasive

breast cancer with no previous cancer history (except non-melanoma skin cancer) (n=1090). Controls were cancer-free age-frequency matched women from breast screening clinics in the same geographic areas who consented to participate in research (n=1053). Detailed pathology information was available for most cases. DNA samples were screened using Q-PCR and the results are validated by ddPCR as previously described (4). The prevalence of iciHHV-6 at the time of blood sampling was determined. Odds ratios and 95% confidence intervals 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.

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)(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 (4/330, 1.22%) than in women without breast cancer (45/9988, 0.45%) (odds ratio of 2.66, 95% confidence interval 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 (9/1090, 0.82%) or without (10/1053, 0.95%) breast cancer (OR=0.87, 95%CI=0.35-2.15). No differences in iciHHV-6 prevalence was observed between breast cancer subtypes, or by menopausal status at 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). While 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.

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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	9560 (48.78)	9496 (48.74)	64 (0.33)[0.47-0.67]
Females	10037 (51.22)	9988 (51.26)	49 (0.25)[0.34-0.53]
Total	19597 (100)	19484 (99.42)	113 (0.58)

Cancer type	iciHHV-6- N=19484 (%)	iciHHV-6+ N=113 (%)	p Value*	iciHHV-6- males N=9496 (%)	iciHHV-6+ males N=64 (%)	p-value*	iciHHV-6- females N=9988 (%)	iciHHV-6+ females N=49 (%)	p Value*	OR	95% CI
Cancer (all)	1553(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	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

*Fisher's exact test
ND=not determined (too few cases).
N/A= not applicable.

Table 2: Prevalence of iciHHV-6 according to case-control status in the Canadian Breast Cancer Study.

	Controls (N=1053)	Cases (N=1090)			
	Mean age (years ±SD) at enrollment/diagnosis		p-value		
	56.90 ±10.10	57.30 ±10.30	0.21*		
	iciHHV-6 prevalence			OR	95% CI
iciHHV-6-	1043 (99.05)	1081 (99.18)			
iciHHV-6+	10 (0.95)	9 (0.82)	0.94**	0.87	0.35-2.15

*t-test
**Fisher's exact test

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