

Report

Cerumen phenotype and proliferative epithelium in breast fluids of U.S.-born vs. immigrant Asian women: a possible genetic-environmental interaction

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Summary

To determine whether a genetic-environmental interaction exists between the breast, a modified apocrine gland, its secretions, and the genetic polymorphic phenotypes of wet and dry cerumen, we examined nipple aspirate fluid (NAF) for proliferative disease in 172 U.S.-born and immigrant Chinese and Japanese women.

Cytologic evidence of proliferative epithelial cells (benign hyperplasia and/or atypical hyperplasia) was found in the NAF of 36 women (20.9%). A significantly higher incidence of proliferative epithelial cells was present in the NAF of U.S.-born than in immigrant Asian women (28.6% vs. 16.5%) ($p = 0.05$).

A higher proportion of U.S.-born Asian women with wet cerumen than women with dry cerumen had proliferative epithelial cells in NAF: 39.3% vs. 20.0% ($p = 0.08$). No significant difference in NAF proliferative cells was found between immigrant women with wet and dry cerumen: 15.8% vs. 17.3%; $p = 0.50$. A strong association of proliferative epithelial cells and cerumen phenotype was found in parous U.S.-born women (wet = 47.6% vs. dry = 16.0%; $p = 0.002$). No significant association with wet and dry cerumen phenotype was found in parous immigrant women (wet = 12.2% vs. dry = 20%).

These findings support the hypothesis that an apocrine genetic polymorphic trait differentially influences susceptibility of the breast to proliferative disease in Asian women born in environments presumed to be of high risk for breast cancer compared to women from low risk environments.

Introduction

An important objective in cancer epidemiology is to detect individuals who are likely to develop malignancy because of genetic susceptibility to environmental factors. If we can identify genetic factors that influence susceptibility, we may be able to determine how environmental factors interact with genetic factors to increase risk of cancer and to suggest leads to develop preventive measures [1].

A familial component in breast cancer risk has been recognized for many years [2]. However, no specific genetic factor has been identified as being responsible for the two- to threefold increased risk to women who have a first-degree relative with breast cancer. That environmental factors may be important in the etiology of breast cancer is demonstrated by the increased incidence of breast cancer in Japanese and Chinese immigrants and their off-

spring in the U.S. compared to the incidence in their countries of origin [3].

Petrakis and associates [4, 5] reported an association of a polymorphic apocrine genetic trait, detectable by cerumen phenotype, with worldwide variation in breast cancer rates. Such an association is biologically plausible because the cerumenous, mammary, and certain axillary and genital sweat glands are histologically of the apocrine type and their secretions have many biochemical similarities [6]. Examinations of NAF have shown a variety of biochemical substances, including mutagens, carcinogens, and promoters of endogenous and exogenous origin, to be secreted and concentrated in the ducts of the nonpregnant breast gland and to be in intimate contact with breast epithelium [6, 7]. For examining breast secretions, nipple aspirate fluid (NAF) cytology provides a useful clinical technique for evaluation of the status of the breast epithelium in apparently normal women in whom breast biopsy is not clinically indicated [8].

Secretion by the breast and other apocrine glands, such as the cerumenous glands, has been shown to be influenced by a pair of alleles (W,w) which are inherited in a simple Mendelian fashion [9]. Women with the dominantly inherited wet-type (WW, Ww) cerumen yield NAF more frequently and in greater quantities than women with the homozygous recessive dry-type (ww) cerumen [10]. We have hypothesized that this genetic polymorphic trait, for which cerumen phenotype serves as a clinical marker, through its physiologic effects on apocrine gland secretory activity, differentially influences exposure of the epithelium to endogenous and exogenous carcinogens and promoters, and thus increases susceptibility to proliferative disease and breast cancer [5, 6]. If breast glands of women with wet-type cerumen secrete greater quantities of these mutagenic and carcinogenic substances than do breast glands of women with dry-type cerumen, then a higher proportion of women with wet-type cerumen will experience proliferative changes in breast epithelial cells than will women with dry-type cerumen. In an earlier test of this hypothesis in Caucasian women in San Francisco, a positive association of cerumen type and NAF atypical hyperplasia was found [11].

Asian populations have very high frequencies of the dry cerumen phenotype, ranging from 40% to 80% in Chinese and Japanese. In contrast, wet-type cerumen is found in 93% to 100% of U.S. Caucasian and black populations [5, 9]. The established disparity in rates of breast cancer and precursor lesions between Asian and white populations might be partly related to genetically influenced differences in apocrine gland secretion, as well as to factors in the environment. The present study sought to determine whether proliferative epithelial hyperplasia (benign hyperplasia and/or atypical hyperplasia) in NAF is differentially associated with cerumen phenotype in U.S.-born vs. immigrant Chinese and Japanese women.

Materials and methods

Participants were Asian women seen at the Breast Screening Clinic at the University of California, San Francisco, between 1975 and 1989, in an ongoing epidemiologic study of NAF composition and breast disease [12]. The procedures used were approved by the University Committee on Human Research. Of 522 Chinese and Japanese women, the cerumen type had been recorded according to the criteria of Matsunaga [9] as wet, dry, or unclassifiable. Less than 2% of women were unclassifiable and were eliminated from the analysis. Nipple aspiration of breast fluid employing a breast pump was attempted in all women by the technique of Sartorius [13]. NAF was obtained in 203 (38.9%) of the 522 women, but the present analysis is based on 172 women, of whom 63 are U.S.-born and 109 immigrants, whose breast fluids were considered by the cytopathologist (EBK) as satisfactory for cytologic evaluation and whose place of birth had been recorded. Data on age at migration and duration of residence in the U.S. had been obtained from only a minority of the immigrant Asian women and were therefore not included in the present analysis.

All NAF samples were prepared for cytologic examination according to the methods described by King *et al.* [14, 15]. The criterion for a satisfactory NAF specimen was the presence of at least

10 epithelial cells on a slide [16]. Epithelial cells were classified as normal, or as demonstrating benign hyperplasia or atypical hyperplasia according to the criteria of King *et al.* [16]. *Hyperplasia* was defined as: minimal changes from normal ductal epithelium; slight cell and nuclear enlargement; cell distribution predominately in groups; papillary grouping – a subcategory; apocrine type – a subcategory. *Atypical hyperplasia* was defined as: moderate to severe abnormality; distinct nuclear enlargement; increasing nuclear to cytoplasmic ratio alteration and chromatin change; cell distribution in groups and papillary formations; single cells increase in numbers with increasing degree of abnormality; apocrine type – a subcategory.

For statistical analyses, the diagnoses of benign and atypical hyperplasia were combined as proliferative epithelium because of the small numbers in these diagnostic categories. Because a significantly greater proportion of women who yielded fluid were parous compared to nonyielders of fluid, analyses were conducted on: a) the entire sample of 172 women who yielded NAF and b) on the 140 who were parous. There were too few nulliparous women for analysis.

The strength of association of a diagnosis of proliferative epithelial cytology compared with normal cytology by cerumen phenotype, stratified by place of birth, was determined by nonparametric statistical tests [17] including chisquare for heterogeneity

and Fisher's exact test. Age-adjusted odds ratios and their 95% confidence intervals were determined by the Mantel-Haenszel chi-square test employing the immigrant women with dry cerumen as the referent group.

Results

Characteristics of the Asian women in the study are shown in Table 1. Except for differences in the proportions of parous women and women with wet-type cerumen, other characteristics of yielders and nonyielders of breast fluid were not statistically significantly different.

The proportions of Asian women yielding NAF by place of birth subdivided by cerumen type are shown in Table 2. Among U.S.-born Asian women, no significant differences were found by cerumen type in proportions yielding NAF and proportions with satisfactory NAF preparations (10 or more epithelial cells). In contrast, among immigrant Asian women, a significantly lower proportion of women with dry-type than with wet-type cerumen yielded NAF ($p = 0.001$), but no differences were found in the proportions of NAF containing epithelial cells. Compared with immigrant women, U.S.-born Asian women with wet and dry cerumen had lower proportions of satisfactory NAF preparations.

Table 1. Demographic and other characteristics of 522 Asian women according to nipple aspirate fluid (NAF) status: nonyielders, yielders, and yielders with satisfactory cytology

Characteristic	NAF nonyielders (N = 319)	NAF Yielders	
		Total (N = 203)	Satisfactory cytology (N = 172)
Age (mean years)	43.9 ± 13.2	41.1 ± 11.4	40.0 ± 10.5
Age at menarche (mean years)	13.4 ± 2.1	13.4 ± 1.6	13.4 ± 1.6
Education (mean years)	13.2 ± 4.5	13.3 ± 4.0	13.1 ± 4.1
% Parous	60.4 ^a	80.0 ^a	82.9 ^a
Age at first fullterm birth (mean years)	25.4 ± 4.9	25.8 ± 5.3	25.8 ± 5.2
% Oral contraceptive users	38.3	40.2	43.0
% Menopausal estrogen use	10.4	8.9	7.1
% Family history of breast cancer	5.7	5.6	5.1
% Wet-type cerumen	31.7 ^a	48.8 ^a	50.7 ^a
% U.S.-born	42.6	43.8	38.9

^aNonyielders compared with total or with satisfactory cytology: $p < 0.001$; other comparisons not statistically significant.

Nipple aspirate cytology

Proliferative epithelial cells were more frequently diagnosed in NAF from U.S.-born than from immigrant Asian women: 18/63 (28.6%) vs. 18/109 (16.5%) (Fisher $p = 0.047$).

Nipple aspirate cytology by cerumen type and birthplace

Total sample. The numbers and percentages of women with proliferative epithelium in NAF grouped by birth place and by cerumen phenotype are shown in Table 3. Chi-square test for homogeneity was 3.12, $p = 0.080$, suggesting that the relationship among U.S.-born differed from that among immigrant Asian women. A higher proportion of NAF with proliferative epithelial cells was found in U.S.-born women with wet cerumen than in U.S.-born women with dry cerumen: 11/28 (39.3%) vs. 7/35 (20.0%) (Fisher $p = 0.080$). Among immigrants, no significant differences were found in the frequencies of proliferative epithelium in NAF from women with wet and dry cerumen: wet = 9/57 (15.8%) vs. dry = 9/52 (17.3%) (Fisher $p = 0.499$; NS).

Age-adjusted odds ratios for the various groups were calculated using the immigrant women with dry cerumen as the referent group (Table 4). A significantly increased age-adjusted odds ratio, 3.11, was found for U.S.-born women with wet cerumen (95% C.I.: 1.2–9.9). Odds ratios for U.S.-born women with dry cerumen and for immigrant women with wet cerumen compared to the referent group did not significantly differ from unity.

Parous women. Analyses of parous women yielded more significant differences. The chisquare for homogeneity was 6.9, $p = 0.01$. When parous women were grouped by birth place and cerumen type, even greater differences in the proportions with proliferative epithelium were found: U.S.-born, wet cerumen 10/21 (47.6%) vs. dry cerumen 4/25 (16.7%) (Fisher $p = 0.022$); immigrant, wet cerumen 6/49 (12.2%) vs. dry cerumen 9/45 (20.0%) (Fisher $p = 0.228$) (Table 3).

The age-adjusted odds ratio of U.S.-born women with wet cerumen compared to the immigrant women with dry cerumen increased from an O.R. = 3.11 in the total sample to O.R. = 4.75 (95% C.I.: 1.4–16.4) in parous women. No significant differences from unity were found for the remaining groups (Table 4).

Discussion

It is well established that epithelial hyperplasia and atypical hyperplasia in breast biopsies are associated with an increased risk of breast cancer [18–23]. However, few studies have been reported on the frequency of these precursor lesions comparing populations at differing risks of breast cancer. One study of benign breast disease in Tokyo and New York found that proliferative lesions were less frequent in breast biopsies from Japanese women in Tokyo than from white women in New York [24]. In an autopsy study in Hawaii and Japan, intraductal hyperplasia was found more often in breasts of Hawaiian-Japanese women than of women in Japan [25, 26]. A forensic study in New Mexico showed a gradient in the frequency of proliferative

Table 2. Proportion of Asian women yielding NAF containing breast epithelial cells satisfactory for evaluation

Birthplace	Cerumen type	NAF obtained		Epithelial cells	
		No./total	Percent	No./total	Percent
U.S.-born	wet	38/90	(42.2)	28/38	(73.7)
	dry	50/134	(37.3)	35/50	(70.0)
Immigrant	wet	61/110	(55.5) ^a	57/61	(93.4)
	dry	54/188	(28.7) ^a	52/54	(96.3)

^a $p < 0.001$.

Table 3. Proportion of Asian women yielding NAF containing proliferative epithelial cells by birthplace and cerumen phenotype

Birthplace	Cerumen type	Total sample (N = 172)		Parous women (N = 140)	
		Prolif. epithelium		Prolif. epithelium	
		No./total	Percent	No./total	Percent
U.S.-born	wet	11/28	(39.3)	10/21	(47.6)
	dry	7/35	(20.0)	4/25	(16.7)
		Fisher p = 0.080		Fisher p = 0.022	
Immigrant	wet	9/57	(15.8)	6/49	(12.2)
	dry	9/52	(17.3)	9/45	(20.0)
		Fisher p = 0.499		Fisher p = 0.228	
		χ^2 homog. 3.12; p = 0.080		χ^2 homog. 6.19; p = 0.01	

and atypical hyperplasia: highest in Anglo, intermediate in Hispanic, and low in American Indian women [27]. The findings in these studies suggest that, similar to breast cancer itself, environmental factors may play a role in the pathogenesis of the epithelial hyperplastic lesions predisposing to breast cancer. Our study in Asian women suggests that an apocrine genetic factor influencing breast secretion may interact with environmental factors in the pathogenesis of these proliferative lesions.

Our findings offer new support for an apocrine genetic-environmental interaction involving the breast epithelium, indicated by the different proportions of proliferative epithelial cells in NAFs in Asian women born in the U.S. versus those born in Asia. This interpretation is supported by the high frequency of proliferative epithelial cells in NAFs in U.S.-born Asian women with wet compared to dry cerumen who have lived in the San Francisco environment where incidence rates for breast can-

cer are high. In women born in environments having low incidence rates for breast cancer, such as Hong Kong and Japan, it can be inferred that less interaction occurs between a low risk environment and the apocrine alleles affecting breast secretion. As a result, no significant difference was found in the proportions of NAFs containing proliferative epithelium in women with wet and dry cerumen. The low frequency of proliferative epithelial cells in NAF of Asian-born women with wet cerumen now living in San Francisco suggests a persisting influence of an Asian-type low risk environment in these women.

As noted in the introduction, Petrakis [28] proposed a possible physiologic association of the genetic phenotype for cerumen and susceptibility to breast cancer. However, a subsequent study in Hong Kong Chinese women did not support this association, and it was concluded that if an association exists between cerumen type and susceptibil-

Table 4. Proliferative epithelium in NAF by birthplace and cerumen type

Birthplace	Cerumen phenotype	Total sample		Parous	
		O.R. ^a	(95% C.I.)	O.R. ^a	(95% C.I.)
Immigrant	dry	1.00	Referent	1.00	Referent
	wet	0.79	(0.32, 2.5)	0.43	(0.17, 1.7)
U.S.-born	dry	1.31	(0.39, 4.1)	0.68	(0.18, 2.9)
	wet	3.11	(1.2, 9.9)	4.75	(1.4, 16.4)

^a Age-adjusted odds ratios.

ity to breast cancer, it must be an indirect one [29]. In view of the present findings, the absence of a relationship of cerumen type and breast cancer risk in Chinese women in the earlier study in Hong Kong raises the possibility that the environment of Hong Kong is less 'carcinogenic' for the breast than that of the U.S. Birth and maturation in a low risk environment such as Hong Kong, where breast cancer rates are low, may result in relatively less exposure of the breast epithelium to hormonal stimuli, secreted ambient carcinogens, and promoters and other factors, irrespective of cerumen type. We defined *environment* as an ecologic complex of geographic, cultural, socioeconomic, chemical, dietary, psychologic, and other factors to which women might be exposed in their country of birth. As such, it lacks specificity. However, differences in hormonal parameters have been reported in Japanese and Chinese women living in different geographic environments [30–32]. Similarly, dietary differences and culturally influenced factors such as age at menarche, parity, age at first full-term pregnancy, and years of education, have all been reported to influence risk of breast cancer [33], and can be considered as 'environmental' in the sense employed here.

The present findings are considered preliminary. Because the number of Asian women in the study is relatively small, the findings, although statistically significant, must be accepted with reservation. Clinical examination of cerumen cannot differentiate the homozygous dominant WW from the heterozygous Ww phenotype, and it is possible that an intermediate physiological influence on secretion and NAF cytology occurs in heterozygous women. The cytologic diagnoses were available only in women who yielded NAF adequate for cytologic classification, and the differences in cytology, if any, between fluid yielders and nonyielders are conjectural. A recent multivariate analysis of NAF secretion by Wensch *et al.* [34] indicates that age and parity status are the two major factors affecting ability to obtain NAF. Because the observed effects in the present study were strongest in parous women who are known to be higher yielders of NAF [34], it is possible that the greater volume of fluid more frequently obtained in women with wet

cerumen is associated with increased likelihood of finding proliferative epithelial cells. This possibility cannot be completely discounted, but is countered by our finding that compared with Asian women, the overall proportion of NAF containing adequate cells was lower for U.S.-born women of both wet and dry cerumen types. In addition, a nonsignificant higher proportion of proliferative cells was found in NAF of parous immigrant women having dry cerumen compared to U.S.-born women with dry cerumen. Because of the relatively small numbers of women, it was not feasible to analyze the data separately for nulliparity or for benign hyperplasia and atypical hyperplasia. Finally, because our previous questionnaire did not request the date and age of migration to the U.S., we were unable to determine what influence the duration of time in the U.S. and its relationship to puberty might have had on the cytologic findings by cerumen type. These considerations are being examined in a larger study of San Francisco Chinese women and Chinese women currently living in an area of Asia characterized by low breast cancer risk.

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