Short Communication

Factors Associated with Refusal to Provide a Buccal Cell Sample in the Agricultural Health Study

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Abstract

Epidemiological studies are increasingly collecting buccal cells and other sources of DNA for genetic analysis. However, high refusal rates raise concerns about possible selection bias. This study examines the subject characteristics associated with refusal or failure to provide a buccal cell sample. Subjects were male farmers in the Agricultural Health Study, which is being conducted in Iowa and North Carolina. As part of a 5-year follow-up, cohort members were contacted by telephone and asked to participate in a telephone interview and to consent to providing a buccal cell sample using a kit that was mailed to them. Demographic, lifestyle, disease, and occupational characteristics were compared between consenters who returned a sample ("compliers"), nonconsenters ("refusers"), and consenters who failed to return a sample ("noncompliers"). Compliers ($n = 8794$), refusers ($n = 3178$), and noncompliers ($n = 3008$) were quite similar, although compliers tended to be slightly older. Although some significant differences between these groups were observed, the magnitude of these differences was generally small, usually no more than a few percentage points. In conclusion, this study found little difference between male farmers who agreed to provide buccal cell samples *versus* those who either refused to provide a sample or who agreed but failed to return the sample. Observed differences were typically small and would be unlikely to compromise etiologic associations identified in such a prospective study. In short, there appears to be little selection bias in the Agricultural Health Study buccal cell collection process, further supporting the use of such mailed collection kits in epidemiological research.

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Introduction

Growing interest in genetic risk factors for disease, including direct gene effects and gene-environment interactions, has resulted in an increase in the collection of genetic material from subjects in epidemiological studies. Buccal cells are an increasingly common source of genetic material, in part because of the noninvasive means by which they can be obtained. Certain techniques of buccal cell collection also make possible the collection and shipment of the material via telephone and mail, substantially reducing study costs. This is especially important for many large prospective studies among dispersed populations. In such cases, an increasingly frequent method involves mailing subjects a kit containing all of the necessary materials to store and mail back a sample.

In all epidemiological studies, some eligible subjects will refuse to participate in some or all aspects of the study, potentially resulting in selection bias. In most studies involving mailed buccal cell collection, nonparticipation can occur either when a subject refuses the request to provide a sample or when a subject who consents to provide a sample fails to return the sample. These two forms of nonparticipation may be caused by different factors and may introduce different biases. It is important for the design of epidemiological studies and the analysis and interpretation of their results to identify such factors.

The present study examines subject characteristics associated with refusal or failure to provide a buccal cell sample among participants in the Agricultural Health Study.

Materials and Methods

Subjects. Subjects consisted of male private pesticide applicators (primarily farmers) participating in the Agricultural Health Study, a prospective cohort study that has been described in detail elsewhere (1). The study cohort includes ~52,400 farmers from Iowa and North Carolina, who were enrolled between 1993 and 1997. We are currently attempting telephone contact with all of the subjects as part of a 5-year follow-up. On contact, subjects are first asked if they will participate in a CATI.[2] If the subject agrees, then after the interview, the interviewer briefly explains to the subject the reason for collecting buccal cells, describes the "swish and spit" buccal cell collection technique (2), and asks if the subject is willing to participate. The subjects are encouraged by the interviewer to ask any questions they may have. Of the 22,079 farmers for whom contact was attempted by mid-July 2000, 72% agreed to the CATI, and 15% refused. The remaining subjects either could not be reached (12%) or were too ill to participate in the interview (1%).

Buccal Cell Collection Technique. Subjects who consented to provide a buccal cell sample were mailed a kit containing a consent form, instructions for collecting and returning the sam-

[2] The abbreviation used is: CATI, computer-assisted telephone interview.

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Table 1 Age at interview distribution (percentage) among male farmers who completed the CATI by July 2000

Age at CATI (yr)	Iowa			North Carolina		
	Consent, sample returned (complier) $n = 6846$	No consent (refuser) $n = 1445$	Consent, sample not returned (noncomplier) $n = 1960$	Consent, sample returned (complier) $n = 1948$)	No consent (refuser) $n = 1733$	Consent, sample not returned (noncomplier) $n = 1048$
≤28	74 (1.1)	36 (2.5)	58 (3.0)	19 (1.0)	31 (1.8)	23 (2.2)
29–38	635 (9.3)	240 (16.6)	360 (18.4)	116 (6.0)	153 (8.8)	124 (11.8)
39–48	1833 (26.8)	432 (29.9)	734 (37.4)	348 (17.9)	330 (19.0)	264 (25.2)
49–58	1715 (25.1)	292 (20.2)	480 (24.5)	469 (24.1)	378 (21.8)	274 (26.1)
59–68	1726 (25.2)	277 (19.2)	253 (12.9)	514 (26.4)	433 (25.0)	208 (19.8)
≥69	863 (12.6)	168 (11.6)[a]	75 (3.8)[a]	482 (24.7)	408 (23.5)	155 (14.8)[a]

[a] Significant difference within state between distributions for this group (either refusers or noncompliers) and compliers, with $P < 0.0005$.

ple, a bottle of Scope mouthwash, a collection container, and a preaddressed stamped mailer for returning the sample. They were asked to swish the mouthwash around in their mouth vigorously for 45 s, spit it into the collection container, and return the sample along with the signed consent form in the mailer.

Data Analysis. We examined the effects of demographic, lifestyle, disease, and occupational factors on participation rates. Data obtained in the CATI were used when available because they were the most current; otherwise, data from the first phase of the study were used.

Subjects were compared according to whether they had (*a*) refused to provide a sample when asked immediately after the interview ("refusers"); (*b*) agreed to return a sample and had done so within 3 months or by the end of follow-up for the present study, whichever came later ("compliers"); or (*c*) agreed to return a sample, but had not done so within that time period ("noncompliers"). The distributions for each factor were compared using the Pearson χ^2 statistic. A χ^2 trend statistic was used for ordinal factors. To account for possible confounding by age, comparisons of all factors except age were age-adjusted using stratified χ^2 analyses (3), with the following age strata (in years): ≤28, 29–38, 39–48, 49–58, 59–68, and ≥69. All frequency distributions, except of age, were directly standardized to the age distribution of all of the subjects who completed the CATI.

Although potential bias cannot be assessed via statistical testing, statistical significance of intergroup differences can be informative and is, therefore, presented. Approximately 170 comparisons were made in each state, although many were highly correlated. To adjust, but not overadjust, for multiple comparisons, we used a Bonferroni adjustment (4) assuming 100 comparisons per state, with a resulting α of 0.0005. Significant findings at this level are indicated in Tables 1 and 2. Differences that were significant at the conventional level (*i.e.*, $\alpha = 0.05$) in one state, and that supported differences at the more stringent significance level in the other state, are reported in the text.

Results

This study examined a total of 14,980 farmers, including 10,251 interviewed in Iowa through July 14, 2000, and 4,729 interviewed in North Carolina through July 1, 2000. There were 11,802 (78.8%) subjects who agreed to provide buccal samples and 3,178 (21.2%) who refused. Of the 11,802 who consented, 3,008 (25.5%) failed to return their samples. Iowa subjects consisted of 66.8% compliers, 14.1% refusers, and 19.1% noncompliers. North Carolina subjects consisted of 41.2% compliers, 36.6% refusers, and 22.2% noncompliers.

Table 1 compares compliers, refusers, and noncompliers by their ages at the time of the CATI. In Iowa, compliers were significantly older than refusers and noncompliers (average age = 53.8 years *versus* 51.0 and 47.4, respectively). In North Carolina, the average age of compliers was similar to that of refusers (58.2 and 57.0 years, respectively) but was significantly higher than for noncompliers (53.4 years).

Education played a modest, but statistically significant, role in a subject's likelihood of complying (Table 2). In Iowa, compliers tended to have more years of education than refusers, whereas in North Carolina, compliers reported more education than both refusers and noncompliers. Marital status distributions were only slightly, but significantly, different between compliers and refusers in Iowa, with compliers more likely than refusers to be married. The likelihood of consenting and returning the sample was unrelated to the number of children among married subjects. In North Carolina, compliers were slightly, but significantly, more likely than noncompliers to be Caucasian (95.3% *versus* 91.2%, respectively); in Iowa, there were too few non-Caucasian subjects to assess differences by race.

Within each state, the proportion of ever smokers and the number of cigarettes that they smoked per day were similar across respondent groups (Table 2). However, among ever smokers, compliers were more likely than noncompliers to have quit smoking. Alcohol use did not differ significantly between groups.

Comparison of occupational factors in this agricultural cohort also indicated little variation across groups (data not shown). Consent status was unrelated to most measures of crop, livestock, or pesticide exposure, and the differences that were observed were small.

The patterns of self-reported previous disease diagnoses among subjects and diseases diagnosed in first-degree relatives were similar across groups (data not shown), with the exception of melanoma in first-degree relatives, which was more commonly reported by compliers than refusers in both states (8.7% *versus* 4.8% in Iowa; 8.1% *versus* 6.1% in North Carolina; $P = 0.018$).

Discussion

The purpose of this study was to identify characteristics associated with subject refusal or failure to provide buccal cell samples to public health researchers. The results indicate few important differences among compliers, refusers, and noncom-

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Table 2 Percentage distribution[a] of demographic and lifestyle characteristics among male farmers who completed the CATI by July 2000

Characteristic	Iowa			North Carolina		
	Consent, sample returned (complier) $n = 6846$	No consent (refuser) $n = 1445$	Consent, sample not returned (noncomplier) $n = 1960$	Consent, sample returned (complier) $n = 1948$	No consent, (refuser) $n = 1733$	Consent, sample not returned (noncomplier) $n = 1048$
Highest educational level[b]						
1–8 years	3.2	4.8	3.0	4.6	5.9	8.3
Some high school	2.3	3.3	3.1	6.7	11.4	10.2
High school graduate	48.9	58.3	48.1	41.9	47.8	40.6
Beyond high school	26.8	20.5	26.4	22.5	18.3	21.8
College graduate	15.7	11.3	17.0	18.1	13.9	14.9
Beyond college	3.1	1.7[c]	2.5	6.2	2.7[c]	4.2[c]
Marital status						
Never married	8.5	11.8	7.5	9.3	10.3	9.0
Married	87.6	84.3	86.4	85.5	84.8	84.0
Divorced	3.1	2.9	4.8	4.3	3.8	5.9
Widowed	0.9	1.0[c]	1.3	1.0	1.1	1.0
No. of children[b,d]						
0	5.6	6.1	5.1	8.9	8.9	8.1
1	6.8	8.5	7.2	15.9	18.0	14.7
2–3	59.3	56.2	58.4	62.0	60.8	60.6
4–5	22.4	22.7	24.4	11.8	10.8	12.5
≥6	5.9	6.5	4.9	1.5	1.5	4.1
Cigarette smoking						
Ever smoked[b]	39.6	40.4	42.3	58.5	58.6	59.9
Quit smoking[b,e]	78.2	73.8	71.0[c]	67.5	60.4	55.2[c]
Number of cigarettes/day[b,e]						
≤10	29.3	28.3	35.5	24.5	20.4	22.9
11–20	41.0	45.0	34.7	39.0	42.9	35.8
21–40	25.1	23.6	25.5	30.1	31.2	33.2
>40	4.6	3.2	4.3	6.4	5.5	8.1
Frequency of alcohol consumption[b]						
Never	25.8	25.4	26.6	54.7	60.2	55.8
<1 time/mo	17.9	18.0	18.0	15.6	12.7	14.3
1–3 times/mo	20.6	17.4	18.8	10.6	9.6	10.2
1–4 times/wk	29.4	32.7	29.7	14.7	13.4	14.8
≥5 times/wk	6.2	6.5	6.9	4.5	4.0	5.0

[a] Relative frequency distributions age-adjusted by direct standardization to the combined study population in both states.
[b] Data from phase I.
[c] Significant difference within state between distributions for this group (either refusers or noncompliers) and compliers, with $P < 0.0005$.
[d] Analysis restricted to married farmers.
[e] Analysis restricted to ever smokers.

pliers. Although this study focused on a farming population, such findings support the use of mailed buccal cell collection kits in epidemiological research.

Subjects who agreed to provide samples were, in general, very similar to subjects who either refused to provide a sample or who agreed but failed to return the sample. Although some significant differences between these groups were observed, the magnitude of these differences was generally small, usually no more than a few percentage points. This is particularly important for known or suspected disease risk factors, such as certain lifestyle characteristics and family history of disease. These findings are similar to those reported by Tarone *et al.* (5), who found little difference between subjects in the Agricultural Health Study who completed and returned a take-home questionnaire in addition to the enrollment questionnaire and subjects who completed only the enrollment questionnaire. The most notable difference between groups in that study was the older age of the responders, which was comparable with our finding of the older age of compliers.

We have been unable to locate other studies reporting the characteristics of subjects who refuse to provide buccal cells or other biological specimens for research purposes in non-high-risk populations. Although several studies have examined willingness to undergo genetic testing, they have given little attention to subject characteristics associated with that willingness and have tended to focus on either prenatal screening or testing for known disease-susceptibility genes (6–10). These studies indicate that there is widespread support for genetic testing of specific diseases, but that a person's level of interest is associated with his or her perceived risk of the disease (6). However, such findings are not readily generalized to etiologic studies such as the Agricultural Health Study in which (*a*) the genetic information obtained is often of unknown clinical importance (and, consequently, is not typically provided to subjects) and (*b*) subjects may not perceive themselves to be at high risk of disease. In other words, such studies ask subjects to make a contribution that is unlikely to provide them any direct benefit.

A limitation of this study is that some comparisons involved data that had been collected 5 years earlier and might have changed in the interim. However, given the average age of these subjects (53.6 years), many of the demographic characteristics are unlikely to have changed appreciably. Most occu-

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pational characteristics were current, having been obtained from the follow-up CATI.

This study benefited from its large sample size, which allowed a wide variety of comparisons, including those of rare characteristics. Given the large sample size, many small differences achieved statistical significance. However, the stability of these estimates based on large numbers increases confidence that the true differences between these response groups are quite small and are unlikely to introduce appreciable bias in etiologic analyses.

In conclusion, this study found little difference in the characteristics of subjects who agreed to provide buccal cell samples *versus* those who either refused to provide a sample or who agreed but failed to return the sample. Although some statistically significant differences were observed, most were small and would be unlikely to compromise etiologic associations ascertained in such a prospective study. In short, there appears to be little selection bias resulting from the collection procedure in this study, further supporting the use of mailed buccal cell collection kits in epidemiological research.

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