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Obtaining DNA in the mail from a national sample of children with a chronic non-fatal illness

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Post-marketing studies could be ideal *post hoc* sources of biologic materials. The US Food and Drug Administration (FDA) mandates post-approval/marketing studies that focus on the exploration of safety signals associated with new therapeutics. These studies are often disease-specific, are large, and are national in scope thereby improving the generalizability of their findings. Several post-marketing studies are currently being conducted evaluating treatments for psoriasis, atopic dermatitis (AD), and acne. We demonstrate the feasibility of obtaining DNA from a large national community-based population of children (PEER, www.thepeerprogram.com) who have AD (Kapoor *et al*., 2008). In addition, we assess the effectiveness of using a pre-notification letter and the use of monetary incentives to improve participation rates for obtaining buccal-DNA through the mail.

Children with AD are being recruited nationwide (by dermatologists, pediatric dermatologists, pediatricians, allergists, and/or other treating physician's offices) for the PEER study. After enrollment, subjects are contacted twice yearly, via mailed surveys, to inquire about their general health and the status of their AD. In order to obtain DNA from these subjects, we assessed the use of a pre-notification letter. All eligible subjects were contacted using a modification of the Dillman Tailored Design Method (Dillman, 2000) with a pre-notification letter describing our new, not PEER participation related, request for DNA. This process is detailed in the Figure S1 with supplemental Text.

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Conflict of Interest
The authors state no conflicts of interest.

All subjects (whether they returned a pre-notification postcard indicating interest or not) were sent a study participation package. It included an Oragene DNA Self-Collection Kit; an incentive ($1, $2, or $5 bill, which was randomly allocated); return postage; and IRB approved consent and assent forms that described the study and use of DNA in future studies.

Responses were evaluated with respect to the full PEER population, the sub-cohort that agreed to participate via the pre-notification letter (responders), those who did not respond to our pre-notification (non-responders), and then those who ultimately provided a sample (these individuals could have been part of either of the two preceding groups). Odds ratios with 95% confidence intervals (CI) estimated from unadjusted and adjusted logistic regression models are presented (Table 1).

Overall, there were 3,264 eligible participants of whom 46.4% were male, 48.7% African American, the average age was 7.1(SD: 4.0) years, and 732 (22.4%) provided sputum. Basic demographics and other variables of interest differed little between the overall sample, those who responded to our pre-notification letter **agreeing** to provide a saliva sample, those who did not respond to our request, and those who provided a sample (see Table S1).

Actual **sample providers** included 402 of the 674 from the pre-notification group (402/674 or 59.6%) and 330 who did not respond to our pre-notification mailings (330/2590 or 12.7%)(Table 1). The odds ratio was 10.1 (8.4, 12.3) favoring individuals who returned their pre-notification postcard versus those who did not. Adjusting for sex, age at enrollment, African American, asthma, seasonal allergy, region of the country, severity of dermatitis, and family income had little effect (9.79 (8.04, 11.93)). Once a subject was mailed a DNA self-collection kit, the average time to the submission of a sample did not vary based on whether a subject had agreed to submit a sample (61.5 days, SD=80.5) or not (64.3 days, SD=78.8) (p=0.64). After processing the saliva specimens, on average 30 μg of DNA (SD 28 μg, median 20 μg, 25% percentile 12 μg, 75% percentile 38 μg) was obtained. Age (p<0.01) but no other factors influenced DNA quantity. By age category we obtained 20 μg (SD 14 μg) for those 3 to less than 6 years of age, 30 μg (26 μg) for those greater than 6 but less than 9 years of age, and 31 μg (31 μg) for those greater than 9 years.

The incentive also influenced the response. As compared to the $1 incentive, the odds ratios were 1.01 (0.82, 1.24) for $2 and 1.26 (1.03, 1.51) for $5 (test for trend p=0.016). The improved response due to the $5 incentive was most pronounced for those who did not respond to the pre-notification letter (1.46 [1.10, 1.92]) (Table 1).

In conclusion, we demonstrated that it is possible to obtain DNA non-invasively through the mail from a cohort of children previously enrolled in a mandated post-marketing study that was not initially designed to request or obtain a biosample. We observed that an affirmative response to a pre-notification letter significantly improves the likelihood of obtaining DNA, that very few subjects complain about a request for DNA, and that it is still possible to obtain DNA from individuals who had not indicated interest in participation (non-responder). The likelihood of obtaining DNA from the PEER children was associated with the severity of their atopic illness, as well as other socioeconomic and demographic factors

(Table 1). However, the clinical significance of these differences is not known. Using a pre-notification strategy likely minimizes the cost per retrieved sample (i.e., only send collection kits and incentives to those who indicate interest in participation). However, when reviewed from a study perspective the overall number of specimens received from study participants (e.g., the overall rate of response) is not remarkably different based on a subject's response to a pre-notification letter. From this perspective, the rate of response when a pre-notification letter was returned was about 12.2% (20.5% of the full sample agreed to provide DNA via the pre-notification letter times 59.6% of these subjects who provided DNA) and was 12.7% for those who did not respond to the initial letter. Ultimately, this cost difference (i.e., sending $30 of materials to the 674 responders versus sending materials to all 3,264 PEER subjects) needs to be considered with respect to the sample size needed to conduct a study. Our ultimate goal is to combine longitudinal data currently collected from the PEER study with data from our *post hoc* DNA collection in order to better understand the influence of genetic factors on the natural history of AD(Sandilands *et al*., 2006;Sandilands *et al*., 2007). We encourage others to use this technique to obtain biologic samples from the many other FDA mandate phase IV studies of dermatologic illnesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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Table 1

The rate of submission of a sample and unadjusted odds ratio of a DNA sample based on basic demographics of the initial study population, those that agreed to participate via a postcard response, and those who did not respond to the pre-notification letter.

Variable	Rate of response			Odds ratio of response		
	Full population	Responder	Non responder	Full population	Responder	Non responder
Overall	732/3264 (22.4%)	402/674 (59.6%)	330/2590 (12.7%)			
Male	340/1513 (22.5%)	184/302 (60.9%)	156/1211(12.9%)	1.00(0.85, 1.18)	1.10(0.80, 1.50)	1.02(0.81, 1.29)
African American	311/1590(22.4%)	158/293 (53.9%)	153/1297(11.8%)	0.72 (0.61, 0.85)	0.65 (0.48, 0.90)	0.84(0.67, 1.06)
Asthma	388/1889 (24.6%)	228/371 (61.4%)	160/1207 (13.3%)	1.26(1.07, 1.50)^	1.18(0.87, 1.61)	1.08 (0.86, 1.37)
Seasonal Allergy	473/2415 (23.4%)	268/446(60.1%)	205/1572 (13.0%)	1.16(0.98, 1.38)	1.06(0.76, 1.46)	1.07 (0.85, 1.36)
AD Control						
Controlled	21/139(15.1%)	13/25 (52.0%)	8/114(7.0%)	Reference	Reference	Reference
Good	327/1544(21.2%)	173/284 (60.9%)	154/1260 (12.2%)	1.51 (0.93, 2.44)	1.44(0.63,3.27)	1.84(0.88,3.86)
Limited	305/1230 (24.80%)	177/291 (59.2%)	128/939 (13.6%)	1.85(1.14, 3.00)^	1.43 (0.63, 3.25)	2.09(1.00,4.39)*
Uncontrolled	79/342 (24.8%)	39/73 (53.4%)	40/269 (14.9%)	1.68 (1.00, 2.86)*	1.06(0.42,2.63)	2.31(1.04,5.11)*
Dollar						
1	229/1087 (21.1%)	127/225 (56.4%)	102/862(11.8%)	Reference	Reference	Reference
2	240/1130(21.2%)	150/246 (61.0%)	90/884 (10.2%)	1.01 (0.82, 1.24)	1.20(0.83, 1.74)	0.84(0.62, 1.14)
5	262/1047 (25.1%)	125/203 (61.6%)	138/844 (16.4%)	1.26(1.03, 1.54)^	1.24(0.84, 1.82)	1.46(1.10, 1.92)^
Region						
New England	21/58 (36.2%)	13/19 (68.4%)	8/39 (20.5%)	Reference	Reference	Reference
Middle Atlantic	71/274(25.9%)	47/73 (64.4%)	24/201 (11.9%)	0.62(0.34, 1.12)	0.83 (0.28, 2.46)	0.53 (0.22, 1.27)
East North Central	136/621 (21.9%)	72/115 (62.6%)	64/506 (12.6%)	0.49 (0.28, 0.87)^	0.77(0.27,2.18)	0.56(0.25, 1.27)
West North Central	56/187 (30.0%)	31/51 (60.8%)	25/136 (18.4%)	0.75 (0.40, 1.40)	0.72(0.23,2.19)	0.87 (0.36, 2.12)
South Atlantic	184/869 (21.2%)	98/178(55.1%)	86/691 (12.4%)	0.47 (0.27, 0.83)^	0.56 (0.20, 1.55)	0.55 (0.24, 1.24)
East South Central	120/626 (19.2%)	67/115(58.3%)	53/511(10.4%)	0.42 (0.24, 0.74)^	0.64 (0.23, 1.82)	0.44(0.20, 1.02)
	89/424(21.0%)	38/78 (48.7%)	51/346(14.7%	0.47 (0.26, 0.84)^	0.44(0.15, 1.27)	0.67 (0.29, 1.54)

		Rate of response			Odds ratio of response		
Variable	**Full population**	**Responder**	**Non responder**	**Full population**	**Responder**	**Non responder**
West South Central	19/80 (23.8%)	14/16 (87.5%)	5/64 (7.87%)	0.55 (0.26, 1.15)	3.23 (0.55, 19.0)	0.33(0.10, 1.09)
Mountain	36/125 (28.8%)	22/29 (75.9%)	14/96 (15.6%)	0.71 (0.37, 1.38)	1.45 (0.40, 5.26)	0.66(0.25, 1.73)
Pacific						
Age at enrollment						
<3	93/513 (15.8%)	47/93 (50.5%)	46/420(11.0%)	Reference	Reference	Reference
3 to 6	234/1063 (32.6%)	125/219(57.1%)	109/844 (12.9%)	1.27 (0.98, 1.66)	1.30(0.80,2.11)	1.20(0.84, 1.74)
>6 to 9	184/713 (21.8%)	105/162 (64.8%)	79/551 (14.3%)	1.57(1.19, 2.08)^	1.80(1.07, 3.03)^	1.36(0.92,2.00)
>9	221/975 (29.9%)	125/200 (62.5%)	96/775 (12.4%)	1.32(1.01, 1.73)*	1.63 (0.99, 2.68)	1.14(0.79, 1.67)
Income ($)						
0 to 24,999	204/1117(18.3%)	97/197 (49.2%)	107/920(11.6%)	Reference	Reference	Reference
25,000 to 49,999	141/624 (22.6%)	67/139 (48.2%)	74/485 (15.3%)	1.31 (1.02.1.66)*	1.37 (0.99, 1.88)	0.96(0.62, 1.48)
50,000 to 74,999	100/382(11.7%)	59/93 (63.4%)	41/289 (14.2%)	1.58 (1.21.2.09)^	1.26(0.85, 1.84)	1.79 (1.08, 2.97)*
75,000 to 99,999	79/231 (34.2%)	49/66 (74.2%)	30/165 (18.2%)	2.33 (1.70. 3.18)^	1.69 (1.08, 2.63)^	2.97 (1.60, 5.51)*
>100,000	93/269 (34.6%)	67/78 (85.9%)	26/191 (13.6%)	2.36(1.76, 3.17)^	1.20(0.75, 1.90)	6.27(3.13, 12.60)*
Unknown	113/637(17.7%)	61/99 (61.6%)	52/538 (9.7%)	0.95 (0.74, 1.24)	0.81 (0.57, 1.15)	1.65(1.01,2.71)*

*
$p<0.05$,

^
$p<0.001$.