Twins as Willing Research Participants: Successes From Studies Nested Within the California Twin Program

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The California Twin Program (CTP) is a population-based sample of over 52,000 twins in which a number of nested studies are ongoing. We outline our experience to date, providing estimates of crude response rates for a variety of different study designs and protocols. We have experienced very high response rates in our studies to date, even in studies with demanding protocols. Lowest response rates have occurred in studies among afflicted individuals, and in one with an unusual protocol. We have experienced some difficulty in locating original members of the cohort, despite efforts to trace individuals using a variety of sources of information. However, in most analyses, the participating sample of twins does not differ substantially from the underlying sample from the CTP. Future work will focus on improving methods of recontacting cohort members.

The California Twin Program (CTP) was established in 1991, with the intent of generating an unbiased sample of a sufficiently large number of twins to aid in the study of chronic disease etiology. We have reported elsewhere on the genesis of the cohort (Cockburn et al., 2001, 2002), and the extent to which it represents a truly population-based sample (Cockburn et al., 2001). Subsequently we have initiated a number of studies nested within the cohort, using similar methods of recruitment, retention, and data collection, and we report here on our experiences to date.

The CTP consists of records of 256,616 live twin births in the state of California occurring between 1908 and 1982, obtained from the California Department of Vital Statistics. This roster has been linked periodically to the records of the California Department of Motor Vehicles to obtain current addresses. Addresses linked to the names of 166,610 individual twins have been identified to date. Verification of addresses using the National Change of Address Index reduced this number to 136,147, and a capture–recapture sampling of current valid addresses reduced the estimate further to 115,733. We sent a letter of introduction, and subsequently a 16-page baseline questionnaire (twins.usc.edu/questionnaire), to each twin with a valid address. The questionnaire was designed to identify pairs suitable for a range of chronic disease studies, and covers basic demographic characteristics (age, sex, education, occupation, and marital status), zygosity, growth and development, reproductive history, use of medical services, dietary preference, and disease history. The questionnaire emphasizes cancer precursors and known lifestyle determinants of cancer (smoking, alcohol consumption, exercise, and sun exposure). Some questions request that a participant provides information about the co-twin as well as themselves, and both parametric (e.g., 'How tall are you?' or 'How much taller or shorter than you is your twin?') and nonparametric (e.g., 'Which of you was taller at age 10?') questions are included.

To date, completed questionnaires have been returned from 51,609 individuals, for a crude response rate of 44.6%; among those more than 40 years of age in 1990, the response rate was over 52%. For a survey of healthy males and females of all social classes, solicited 'out of the blue', and with no prior relation to our institution and no common affiliation, we consider this response rate to be high. The California Behavioral Risk Factor Surveillance Survey (BRFSS) telephone interview has 20,000 responses for a response rate of 58% but only with the removal from the denominator of people who didn't answer the phone. The crude response rate in the California BRFSS is 26%.

We have initiated a number of studies nested in the CTP, each of which uses baseline data to select twins for further study. In each nested study the process involves re-contact of the twins to verify and update baseline information, collect new information, and usually to collect some form of biospecimen. While the CTP baseline information represents a valuable source of cross-sectional information in a large population-based study (Cockburn et al., in press; Cockburn et al., 2002; Hamilton et al., 2006; Hawkins et al., 2004;

Received 1 August, 2006; accepted 2 September, 2006.

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Ringold et al., 2002), the true value of the cohort lies in ongoing studies incorporating biospecimen data, longitudinal analyses, linkage with cancer registries, and the collection of more detailed risk factor information for specific diseases. We report here on our experiences to date carrying out nested studies within the cohort, focusing on response rates and the representativeness of the nested study participants in comparison with the overall CTP dataset.

Methods

Maintaining Contact Information for Twins

Contact information for twins obtained at baseline included their address, home and work telephone numbers, and e-mail addresses. These were provided by 84.6%, 48.0% and 60.4% of twins respectively. We also asked twins to provide contact information for someone who might know where they are if we lose contact with them (provided by 51.9% of twins). Since 1997, we have maintained a website (twins.usc.edu) for twins to update their contact information — in order to ensure confidentiality, before making any changes, twins need to first enter their unique study ID (from the baseline questionnaire) and their name or date of birth. To date 1324 twins have updated some or all of their contact information using this method.

Prior to initiating a nested study, we take a snapshot of all these sources of information and produce our best estimate of the current contact information for each twin. Most studies have then taken advantage of commercially available tracing services, which use various public and credit record sources to identify updated contact information for individuals. These sources either verify the existing contact data, or provide updated data. Linkage with tracing sources is achieved using social security number (provided at baseline by 79.3% of respondents), or, where this is unavailable, combination of name and date of birth information, with manual review of results to ensure they match the original twin respondent.

Recruitment Procedures

All follow-up studies within the cohort receive separate Institutional Review Board (Ethics Committee) approval. Most nested studies initiate contact with twins using direct telephone recruitment or telephone recruitment after an introductory letter. Where no response is received, an introductory letter is usually posted to the twin, providing either a postage-paid mailer for the return of materials, or information on how to contact us by phone. If the contact telephone number for a twin is no longer valid, we attempt to contact the twin using other information provided, including their additional contact person noted above. For some twins, we are able to contact their co-twin, and ask for updated contact information from them. Each nested study maintains active contact information on twins for the duration of the study, and then that information is updated into the CTP database for future use.

When we have valid contact information for twins (i.e., no evidence that the contact information is incorrect), we will usually make a minimum of five calls at varying times of the day, and days of the week, until we contact twins. Because we have found an increasing number of individuals use voicemail to screen their calls, we will usually leave a message asking twins to call our toll-free number if they are interested in participating in the nested study.

Some twins ask whether or not their co-twin is participating before deciding themselves. In these cases we coordinate calling both members of the twin pair, in order to increase our chances of obtaining the participation of either twin. In some cases we find that one twin may be hesitant, but their co-twin convinces them to participate.

Response Rate Calculations

We have calculated response rates for nested studies conducted to date. Most studies have multiple components (e.g., informed consent, questionnaire, and biospecimen collection), and not all participants complete every component. However, every twin completing at least one component of a study provides us with information relevant to the nested study, so for the numerators of response rates, we included all twins who completed any part of the study requirements. For denominators, we included all twins for whom contact information was verifiable, and who were eligible to participate (some studies have strict eligibility criteria, so where a twin was ineligible, we excluded them from the denominator). Therefore, the analyses of response rates do not take into account twins who are lost to follow-up. The impact of the exclusion of those twins we consider by comparing the nested study population with the underlying sample (CTP or a relevant subsample) from which they were drawn.

Comparisons of Study Sample Populations With the Underlying CTP Cohort

For every nested study completed, we have compared the respondents with the appropriate subsample of CTP members from which they were drawn on the basis of demographic and appropriate risk factor data obtained from the baseline questionnaires. For example, in our mammogram study, we compared respondents to all female monozygotic (MZ) and dizygotic (DZ) twin pairs who originally provided a baseline questionnaire. We evaluate differences using chi-square tests and means/standard deviations for continuous variables, and are able to report on subsequent potential for selection bias in the nested study. We provide two examples here, one from our largest nested study with a high response rate, and one from a collaborative study with a low response rate (among twins with self-reported bipolar disorder). These comparisons with baseline data can be assessed, along with our previous publications on the representativeness of

https://doi.org/10.1375/twin.9.6.927 Published online by Cambridge University Press

the CTP as a population-based study (Cockburn et al., 2001), to determine the extent to which the nested studies reflect a population-based sample.

Results

Response Rates in Nested Studies Conducted to Date

Table 1 provides data on the nested studies conducted to date in the CTP cohort. The response rates for most studies exceed those of all other population-based studies we are conducting (in nontwin samples), which are typically in the range of 40% to 60% (Langholz et al., 2000). However, response rates vary among the nested studies. Response rates were lower in some studies with intense study regimens: for example, in one study requiring participants to go to a local laboratory and provide a blood specimen (Cozen, Diaz-Sanchez, et al., 2004; Cozen, Gill, et al., 2004; Table 1, Study 1), the overall response rate was 60%, whereas in the study of mammographic density (Study 2) which required only a buccal swab (and interview), the response rate exceeded 85%. One study requiring 30 consecutive days of exercise log and saliva specimens (Study 3) also had among the lower response rates. Response rates also appear to be highest in studies focusing on female twins: the highest response rate was in our mammogram study (Study 2).

The lowest response rates were in two unusual studies: the first aimed to validate self-report of bipolar disorder (Study 11) and it was followed by a study validating self-report of depression (Study 12). Both studies involved answering questions on a 14-item Mood Disorder Questionnaire in a mailed instrument. While the poor response rates in these studies could be due to the fact that they focused on afflicted twins (noting that the lowest response rate was in twins reporting depression), these are the only two studies that used mail recruitment alone, and both had only one follow-up mailing, with no attempt to telephone twins (due to confidentiality restrictions).

Another low response rate was found in a study testing the acceptability of a breath test for determining infection with *Helicobacter pylori* infection (involving blowing in a bag, swallowing a pill and then blowing in another bag 10 minutes later). This study (Study 5) was conducted in unlike sex twin pairs (i.e., DZ male/female), and notably, the only other study we have done in unlike sex twins (Study 9) also had a low response rate. Study 5 required following a complex set of instructions, and 56% of refusals or failures to complete the protocol were because the participant couldn't understand the protocol or thought it was too unusual. Study 9 involved completing two food consumption questionnaires, and only 11% of refusals were observed because the materials were too time consuming.

In the one major study of chronic disease conducted so far (Study 6) we observed an excellent response rate (almost 90%). Despite the high response rates in this and other nested studies, the impact of selection bias in each study can only be assessed by comparing the respondent sample with the CTP population from which they were drawn. This is because our response rate calculations do not take into account the numbers of twins we were unable to contact for whatever reason (they never returned our calls, or they are deceased). We next provide an example of the potential impact of this 'loss to follow-up' in our largest nested study (Study 2).

Detailed Example of Recruitment and Retention of Twins in a Nested Study — Case Study: Mammographic Density (Study 1)

We selected, and attempted to contact, 3246 women from CTP baseline questionnaires. Of these, 981 (30.2%) were not reached (166 had an incorrect telephone number, 186 did not answer after five attempts, and 629 had insufficient contact information). We successfully contacted the households of 2265 individuals. Of these, 1726 (806 MZ and 920 DZ) were eligible, agreed to participate and completed our telephone interview. Of the individuals that were not eligible, 30 were deceased, 213 had had no mammograms, 191 had no interest in the study, and 51 cited a specific problem in not wanting to participate. There were 54 additional individuals whose twin did not agree to participate, who were therefore not included in the study. Thirty-five pairs were excluded because of missing information regarding their medical histories, and the remaining 1656 individuals consisted of 828 twin pairs who participated in the study. The overall response rate refers to respondents who completed any of the study requirements — in addition, we received mammographic films covering a 5-year span for 91.8% of these participants.

The comparison of selected variables among the participating twins and those selected for the study is provided in Table 2. We obtained participation among a greater proportion of MZ than DZ twins, but among those factors likely to impact observable outcomes in the study due to selection bias (i.e., other potential risk factors for mammographic density), none differed substantially between the participant group and the twins selected from CTP. The exception was hip-to-waist ratio for MZ twins, where there were a greater proportion of participants reporting that their waist was narrower than their hips (a measure of central fat mass) than in the original CTP selection. However, this difference was not reflected in overall body mass index (BMI), presumably because the difference, although statistically significant, was small. Parity and age at menarche did not differ substantially between the participant and CTP groups, although DZ twins were slightly more likely than participants to be nulliparous.

Comparisons of Study Respondents With the Underlying Cohort — Case Study: Twins Participating in a Study of Coronary Heart Disease Risk Factors

Compared to all participants in the California Twin Program, the twins in a study of coronary heart disease risk factors (Zhang et al., 2004; Study 10)

https://doi.org/10.1375/twin.9.6.927 Published online by Cambridge University Press

Table 1

Details of Studies Nested in the California Twin Program Cohort, 1995–Present

Study number	Study name	Twins targeted	Contact/recruitment method	Interview/ questionnaire method	Type of biospecimens collected	Additional data collected	*N*	% Female	Response rate[1]
1	Smoking behavior and cytokine polymorphisms	Healthy MZ	Telephone	Telephone, 45-minute interview	Blood		178	59%	60.0%
2	Mammographic density and exogenous hormone (HRT) use	Healthy MZ and DZ	Telephone and mail	Telephone, 45-minute interview	Buccal swabs	Last 5 years of annual mammograms, medical records for HRT use	603	100%	85.4%
3	Exercise and endogenous hormone levels	Healthy MZ	Telephone	Telephone, 60-minute interview	Saliva for measuring hormone levels (daily for 30 days)	Exercise diary (daily for 30 days)	78	100%	64.2%
4	Compliance with skin self-examination in the prevention of skin cancers	Healthy MZ and DZ	Telephone and mail	Mail, 16-page questionnaire	None	Skin self-examination procedure results (describing 5 largest nevi)	355	54%	70.4%
5	Pilot of a mailed procedure for measuring *Helicobacter pylori*	Healthy unlike-sex twins	Telephone and mail	Mail, 2-page questionnaire	Urea breath test for *Helicobater pylori* infection		22	40%	39.3%
6	Environmental and genetic factors in multiple sclerosis	MZ and DZ twins where at least one has multiple sclerosis	Telephone	Telephone or mail	Buccal swabs (DNA) and/or blood		75	64%	89.3%
7	Gene prevalence in twins at high risk for melanoma and other skin cancers	Healthy MZ twins	Telephone	Telephone (60-minute interview)/Mail (skin self-examination kit)	Saliva	Lifetime residential history, skin self-examination kit (validated by clinical exam)	248	62%	71.9%
8	Determinants of height and developmental differences in twins	Healthy DZ twins	Telephone	Mail or online	None		154	56%	77.0%
9	Validation of food frequency and food preference questionnaires	Healthy unlike-sex twins	Telephone	Mail (two 16-page questionnaires)	None	Change in weight and hip/waist ratio since baseline questionnaire	100	45%	74.0%
10	Genetic determinants of coronary disease	Healthy MZ and DZ twins	Telephone and mail	Mail (12-page questionnaire)	Blood		112	72%	70.1%
11	Validity of self-reported bipolar disorder	MZ and DZ twin pairs in which at least one twin reported bipolar disorder	Mail (introductory letter with one reminder)	Mail (14-item questionnaire)	None	Willingness to take part in 3-day comprehensive psychological testing	69	74%	31.9%
12	Validity of self-reported depression	MZ and DZ twin pairs in which at least one twin reported unipolar disorder (depression)	Mail (introductory letter with one reminder)	Mail (14-item questionnaire)	None	Willingness to take part in 3-day comprehensive psychological testing	166	64%	6.6%

Note: [1]See methods for calculation of response rates.

Table 2

Comparison of Selected Risk Factors Between Monozygotic and Dizygotic Twins Who Participated in the Mammographic Density Study Compared to Twins Selected for the Study from the California Twin Program

	Participated in mammogram study		All selected from CTP		MZ	DZ
	MZ (n = 806)	DZ (n = 920)	MZ (n = 1636)	DZ (n = 1610)	p	p
Body mass index						
≤ 25	55.3%	49.2%	52.2%	49.3%		
26–29	4.4%	3.6%	6.5%	3.3%		
30 and over	15.3%	9.4%	12.4%	8.9%	.24	.95
Hip/waist ratio						
Hips > waist	83.1%	81.9%	75.5%	75.0%		
Hips = waist or hips < waist	3.1%	2.1%	6.3%	2.7%	.02	.48
Age of menarche						
≤ 11	15.3%	8.5%	10.8%	6.8%		
12	14.6%	8.2%	14.6%	8.9%		
13	16.6%	12.7%	15.8%	10.0%		
≥ 14	11.2%	4.2%	10.7%	5.6%	.53	.4
Number of live-births						
0	10.5%	6.0%	12.4%	9.7%		
1–2	33.2%	33.2%	32.0%	27.5%		
3 or more	5.0%	7.0%	6.9%	6.9%	.47	.05

were similar in mean age, mean BMI, proportion non-Hispanic white, and proportion with a family history of hypertension. A higher proportion of twins in the nested study were hypertensive (9.5% vs. 5.3%; $p < .0001$), female (76.1% vs. 52.6%; $p < .0001$), while a lower proportion reported ever smoking (29.3% vs. 42.4%; $p < .0001$). There appeared to be no selection bias for subjects with a family history of hypertension, but the nested study sample tended to be biased towards hypertensive individuals. While a lower smoking prevalence in the nested study group could be explained by the increased proportion of females who participated (Zhang et al., 2004), it is likely a reflection of the southern California lifestyle, since we observed no statistically significant difference in the proportion of 'ever smokers' in the twins who participated in the CHD study, compared to the twins within the California Twin Program.

Discussion and Recommendations

Twin registries provide unique opportunities to investigate the combined effects of genes and environment in chronic and other diseases (Heath et al., 2002; Kaprio et al., 2002). Such registries benefit from being truly population-based, to limit to the greatest extent the impact of selection biases on outcomes (Mack et al., 2000). This is particularly important in the use of twin studies to estimate features of heritability that rely on simple comparison of proportions of affected or concordant MZ and DZ twin pairs. Having established the population-based CTP to address these concerns (Cockburn et al., 2001, 2002), we are now in the process of conducting studies nested within the cohort. We have considered here the features impacting the success of these nested studies in terms of overall response rates, and in one large example, the extent to which the nested sample reflects the underlying CTP population from which it was drawn.

We have experienced high response rates for most of our nested studies (Cozen, Diaz-Sanchez, et al., 2004; Cozen, Gill, et al., 2004), especially in comparison to other population-based study designs. These response rates reflect substantial effort in contacting twins using a variety of information sources provided by twins in the baseline questionnaire for the CTP, and using other data sources for tracing. They also indicate that when we can contact twins, they are willing research participants. Additionally, we have successfully enrolled twins in studies with a variety of data collection methods, involving blood (Cozen, Diaz-Sanchez, et al., 2004; Cozen, Gill, et al., 2004) and saliva collection, lengthy questionnaires and interviews, and complex and time-consuming diaries. This success suggests that twin cohorts may also be a good source of subjects for those studies whose protocols are too demanding for other population-based samples.

One area where improvement is required is our ability to maintain follow-up with twins. While we demonstrated that selection bias was likely to be unimportant in our largest nested study, the fact remains that more than 30% of twins responding to the initial baseline assessment were unable to be located in that study. While not affecting the validity of the study, this does result in a reduction in available sample size for studies of rare disorders, and could result in substantial selection bias for some other nested studies. The solution to this problem is unclear — while credit-based tracing services have provided accurate contact information for twins in the CTP, most sources require a substantial amount of existing information on subjects (e.g., valid social security number and previous valid address) in order to attain a match. Other sources of contact information for follow-up need to be identified in order to reduce the number of twins we are unable to contact. Asking twins for additional contact information (work phone numbers and a third party who might be able to locate them) provides some additional information, but for the 30% of twins missing from our mammogram study this information either proved outdated or was itself missing.

As we continue to conduct nested studies within the CTP, we will maintain our practice of passing on updated contact information to the underlying cohort. We will also continue to conduct analyses of the potential impact of selection bias on all nested study outcomes.

There are several major twin studies ongoing at the University of Southern California (USC), each involving different registers developed independently. These include two primary twin registers housed at USC —

https://doi.org/10.1375/twin.9.6.927 Published online by Cambridge University Press

the CTP, and the other more specifically sampling twins in Southern California (Baker et al., 2006). Although the Southern California Twin Register has focused primarily on children and psychological development, it has the potential for substantial expansion to include over 100,000 sets of adult twins in the same geographical area. In addition to these two California-based twin registers, other researchers at USC have ongoing twin studies based on non-US populations, including Sweden and China.

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