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The Twin Research Registry at SRI International

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The Twin Research Registry (TRR) at SRI International is a community-based registry of twins established in 1995 by advertising in local media, mainly on radio stations and in newspapers. As of August 2012, there are 3,120 same- and opposite-sex twins enrolled; 86% are 18 years of age or older (mean age 44.9 years, *SD* 16.9 years) and 14% less than 18 years of age (mean age 8.9 years, *SD* 4.5); 67% are female, and 62% are self-reported monozygotic (MZ). More than 1,375 twins have participated in studies over the last 15 years in collaboration with the University of California Medical Center in San Francisco, the University of Texas MD Anderson Cancer Center, and the Stanford University School of Medicine. Each twin completes a registration form with basic demographic information either online at the TRR Web site or during a telephone interview. Contact is maintained with members by means of annual newsletters and birthday cards. The managers of the TRR protect the confidentiality of twin data with established policies; no information is given to other researchers without prior permission from the twins; and all methods and procedures are reviewed by an Institutional Review Board. Phenotypes studied thus far include those related to nicotine metabolism, mutagen sensitivity, pain response before and after administration of an opioid, and a variety of immunological responses to environmental exposures, including second-hand smoke and vaccination for seasonal influenza virus and *Varicella zoster* virus. Twins in the TRR have participated in studies of complex, clinically relevant phenotypes that would not be feasible to measure in larger samples.

■ **Keywords:** twins, genetics, metabolism, vaccines, immunology

Overview

Investigators within the Center for Health Sciences at SRI International (formerly known as the Stanford Research Institute), an independent, not-for-profit research organization founded in 1946 with headquarters in Menlo Park, California, have a substantial track record of utilizing the twin design to determine the relative proportion of environmental and genetic variance in survey-based and clinically measured phenotypes. Utilizing twin pairs from the National Academy of Sciences/National Research Council (NAS/NRC) World War II Twin Registry (Hrubec & Neel, 1978) and a more intensively studied subset of twins known as the NHLBI Twin Study (Feinleib et al., 1977), these investigators and their collaborators published biometric studies of the heritability of tobacco smoking behaviors (Carmelli et al., 1992), the co-occurrence of substance use including tobacco, alcohol, and caffeine (Swan et al., 1996, 1997), brain morphology as determined by magnetic resonance imaging (Carmelli et al., 2002; Lessov-Schlaggar et al., 2012; Pfefferbaum et al., 2001; Sullivan et al., 2001) and cognitive functioning as assessed by neuropsychological testing (Lessov-Schlaggar et al., 2007; Swan & Carmelli, 2002; Swan et al., 1999).

Creation, Growth, and Maintenance of the Twin Research Registry at SRI International

As a follow-up to our 1992 *New England Journal of Medicine* paper on the genetics of ever smoking, smoking quantity, and smoking cessation (Carmelli et al., 1992), the Twin Research Registry (TRR) at SRI International was created to develop a study sample of adult twins to support more in-depth studies of nicotine dependence and related phenotypes including nicotine metabolism. California laws pertaining to the privacy of birth records and Department of Motor Vehicles files precluded the development of a population-based registry of adult twins. Therefore, we instead elected to create a community-based registry in the San Francisco Bay Area and in 1995 initiated an extensive advertising campaign that included 19 newspapers, San

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TABLE 1
Basic Demographics of Participants on the Twin Research Registry at SRI International

	Registered twins	Self-reported zygosity			Gender	
		Monozygotic	Dizygotic	Unknown	Male	Female
Adults	2,683 (86%)	1,769 (66%)	798 (30%)	116 (4%)	844 (30%)	1,839 (70%)
Under 18	437 (14%)	159 (36%)	239 (55%)	39 (9%)	199 (46%)	238 (54%)
Total	3,120	1,928 (62%)	1,037 (33%)	155 (5%)	1,043 (33%)	2,077 (67%)

Francisco Bay Area-wide movie theaters, and AM/FM radio stations. Within 2.5 years, this campaign resulted in the enrollment of a total of 1,054 individual twins. A 5-year anniversary celebration party, held in July 2000, increased enrollment to 1,765 individual twins. In 2001, the TRR was expanded to include twins under the age of 18 years. By 2009, as a result of sustained and intensive advertising in local media and TRR member referrals, membership in the TRR increased to 2,700 twins. Table 1 provides a description of the basic demographics of the sample of over 3,000 twins currently enrolled in the TRR.

Contact with twins in the TRR is maintained via annual newsletters and birthday cards. The TRR Web site (<http://www.sri.com/twin>) is updated periodically and referrals to the TRR by registered twins are encouraged with a \$25 incentive. The TRR also has a presence on Facebook and Twitter. All activities related to recruitment, advertising, and ongoing contact with the twins are reviewed and approved by the Institutional Review Board of SRI International.

Zygosity Assessment

Originally, twins were asked to complete a short registration form consisting of the following questions (Cederlof et al., 1961; Sarna et al., 1978): (1) 'As far as you know, are you and your twin: fraternal, identical or don't know?'; (2) 'During your entire life, how close do you feel that you and your twin have been compared with your impression of closeness between ordinary siblings: less close, as close as, somewhat closer, or much closer than ordinary siblings?'; (3) 'How far in miles do you live from your twin now?' (4) 'How frequently do you and your twin get together now: almost daily, 1–4 times per week, 1–3 times per month, occasionally during the year, less than once per year?' Responses to the initial questions were then used to assign preliminary zygosity status. More recently, a revised questionnaire was mailed to all registered twins to collect responses to a series of questions developed at Washington University in St. Louis to estimate twin zygosity. The classification algorithm assigns weights to responses to items concerning physical similarity, whether parents, teachers, or strangers ever mistook one twin for the other, whether blood test verification was ever obtained, and self-reported zygosity (Heath et al., 2003).

For those twins participating in one of our studies, self-reported zygosity is confirmed based on genotyping of short tandem repeat (STR) regions of genomic DNA (Edwards et al., 1991) and the twins are notified of the results. We have learned that this information is an important motivator for twins to participate in research studies. For those twins who have indicated in surveys that they have had their zygosity confirmed by laboratory testing, we have asked that they send in their results so we can add them to our database. So far, we have received 39 such reports.

Projects Involving the Twin Research Registry at SRI International

Since 1998, the TRR at SRI International has supported at least 10 distinct, funded studies. These studies fall into two broad categories: those that are focused on drug metabolism and related phenotypes such as subjective effects and dependence and those that are focused on the effects of a variety of agents that influence the human immunological response repertoire. Figure 1 provides a timeline for each study and Table 2 summarizes the phenotypes described in published papers that have been examined in twin participants recruited from the TRR.

Studies Focused on Drug Metabolism and Related Phenotypes

The study 'Pharmacokinetics of Nicotine in Twins' (DA011170) involved 139 twin pairs (110 MZ and 29 dizygotic [DZ] pairs) who participated in an in-hospital fixed dose infusion of radio-labeled nicotine and cotinine protocol. The pharmacokinetics of nicotine metabolism in both plasma and urine were subsequently determined using techniques developed by Benowitz, Jacob, and colleagues (Benowitz & Jacob, 1994; Jacob et al., 1988, 1991, 2002). Data from this study, the world's largest existing twin study of nicotine metabolism, have resulted in several reports on biometric estimates of genetic and environmental contributions to different aspects of nicotine metabolism (Benowitz et al., 2008; Conti et al., 2009; Lessov-Schlaggar et al., 2009; Swan & Lessov-Schlaggar, 2009; Swan et al., 2004, 2005, 2009), as well as measured genetic and environmental influences on metabolism itself (Al Koudsi et al., 2006; Benowitz, Lessov-Schlaggar et al., 2006; Benowitz, Swan et al., 2006; Mwenifumbo et al., 2008).

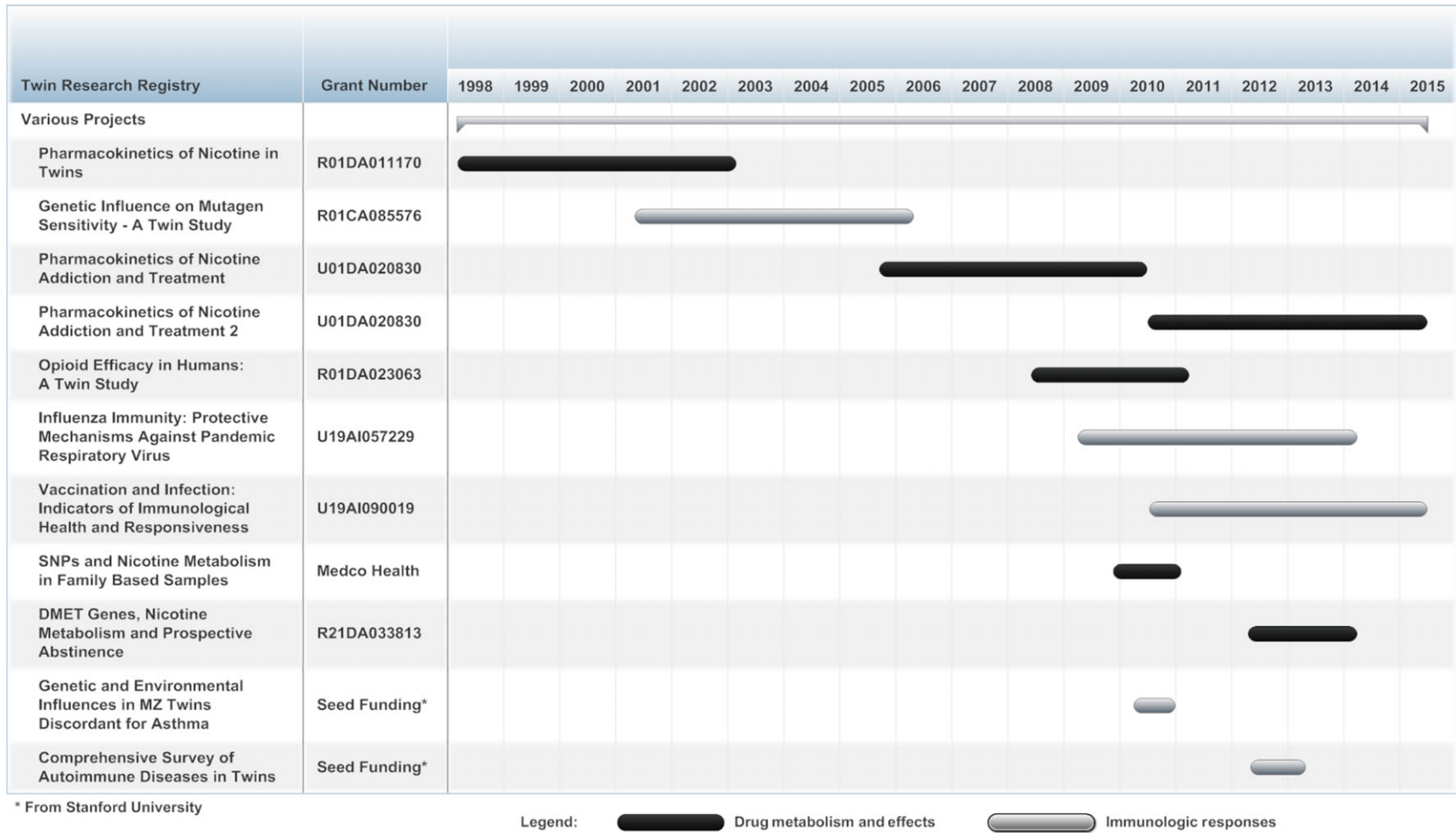


FIGURE 1
 (Colour online) A timeline of funded studies that have utilized data from participants in the Twin Research Registry at SRI International.

TABLE 2**Representative Phenotypes Examined in the Twin Research Registry at SRI International (Published Only)**

Project/phenotype
Pharmacokinetics of nicotine in twins
Hepatic — nicotine and cotinine clearance, the conversion of nicotine to cotinine, nicotine metabolic ratio in plasma and urine Glucuronidation — nicotine-glucuronide/nicotine, cotinine-glucuronide/cotinine, 3 hydroxycotinine-glucuronide/3 hydroxycotinine, nicotine-glucuronide/nicotine area under the curve, cotinine-glucuronide/cotinine area under the curve Renal — total clearance nicotine and cotinine, glomerular filtration rate, net secretory/reabsorptive clearance of nicotine and cotinine Pharmacodynamic — heart rate acceleration and deceleration during and after infusion of a fixed dose of nicotine
Genetic influence on mutagen sensitivity
Cancer susceptibility markers — mutagen sensitivity (mean breaks per cell) in response to bleomycin, benzo(alpha)pyrene dioloxide, gamma radiation, 4-nitroquinoline-1-oxide; mitochondrial DNA content
Opioid efficacy in humans
Experimental pain — threshold and tolerance to heat and cold pain before and after infusion of Alfentanil Nonanalgesic effects — sedation, respiratory depression, nausea, pruritis, positive affective response before and after infusion of Alfentanil

Data from the study described above contributed to the first and second generations of the project 'Pharmacokinetics of Nicotine Addiction and Treatment' (U01DA020830). In the first generation of this project, DNA samples from the twin study as well as those from a family study of nicotine metabolism (University of California Tobacco-Related Disease Research Program, 7PT2000-1004) were integrated with a collection of DNA samples from eight randomized clinical trials of various smoking cessation treatments for genotyping or sequencing and analysis of selected pharmacodynamic and pharmacokinetic loci. Analysis of pharmacodynamic candidate genes resulted in the identification of genetic associations (both common and rare variants) with responsiveness to treatment (Conti et al., 2008; Lee et al., 2011, 2012; Swan et al., 2011) and with nicotine dependence (Bergen et al., 2009; Falcone et al., 2011; Wessel et al., 2010). The second generation of this project (ongoing) will examine the extent to which individual variation in the nicotine metabolic ratio (NMR; 3'hydroxycotinine/cotinine ratio) with known heritability (Swan et al., 2009) mediates responsiveness to medications commonly used to treat nicotine dependence in a randomized trial design (Lerman et al., 2006; Swan et al., 2010).

The most recent studies involving DNA samples collected from the original twin study of nicotine metabolism utilize data from the DMET™ Plus assay from Affymetrix that was used to interrogate 1,936 markers at 236 drug metabolizing and transporter genes for their association with the NMR in the twin dataset and in the previously mentioned family study of nicotine metabolism dataset. The initial work of collecting and analyzing this genotype data, 'Metabolic SNPs and Nicotine and Cotinine Metabolism in Two Family Based Samples', was funded by a Collaboration Agreement among Medco Health Solutions, Affymetrix, and SRI International (Bergen et al., 2010), and the work will be extended using funding from National Institutes of Health (NIH) in the project 'DMET Genes, Nicotine Metabolism, and

Prospective Abstinence' (DA033813). The initial analysis of genotype data identified variation at several pharmacokinetic genes associated with nicotine metabolism, including classical genes and genes not previously associated with nicotine metabolism (Bergen et al., 2010). Future work will take a unique approach to gene discovery by using analysis of the two family-based datasets, where associations identified in the twin dataset (Bergen et al., 2010) are being confirmed in the family study dataset (Bergen, Javitz et al., 2012; Bergen, Wacholder et al., 2012). Those single-nucleotide polymorphism (SNP) associations common to both datasets will then be examined for their significance in relation to abstinence outcomes in the previously described collection of eight clinical trials. This approach should determine in a more rapid fashion those SNPs that have potential for translation to the clinical setting as biomarkers for association with nicotine metabolism-related phenotypes such as cigarettes per day and prospective abstinence.

The latest project in the group of studies focused on drug effects, 'Opioid Efficacy in Humans' (DA023063), recruited 81 MZ pairs and 31 DZ pairs from the TRR to participate in a computer-controlled infusion of the mu opioid agonist Alfentanil or placebo in a single occasion, randomized cross-over study of pain sensitivity and analgesic effects (Angst et al., 2010). In this unique study, experimental heat and cold pressor pain models were examined both before and after administration of Alfentanil. Analyses determined the relative proportion of genetic and environmental factors in pain tolerance and threshold (Angst et al., 2012) and, in a separate analysis, subsequent analgesic and drug side effects (Angst et al., 2012). Ruau et al. (2012) recently used a bioinformatics approach to identify candidate genes associated with pain ratings of several medical conditions. A number of identified candidates were subsequently genotyped using DNA from twin participants who had been prospectively assessed for experimentally induced pain phenotypes as a step in the validation process.

Studies Focused on the Human Immunological Response Repertoire

Mutagen sensitivity is an in-vitro short-term lymphocyte culture assay that gauges host susceptibility by measuring induced chromatid breaks following exposure to an array of mutagens. A series of previous studies indicated that mutagen sensitivity is a promising environmentally related cancer risk predictor. The assay was expanded by replacing the initial test mutagen bleomycin with 4-nitroquinoline-1-oxide (4-NQO, an ultraviolet light [UV] mimetic agent), gamma-radiation, and benzo(alpha)pyrene diolepoxide (BPDE, a metabolic product of benzo(alpha)pyrene, which is a component of tobacco smoke) to measure risk of other cancers (Wu et al., 2006). At the time this study was conducted ('Genetic Influence on Mutagen Sensitivity', CA085576), the relative contributions of genetic and environmental factors to indicators of mutagen sensitivity were unknown. One hundred and forty-eight pairs of MZ twins, 57 pairs of DZ twins, and 50 siblings were recruited from the TRR to provide peripheral blood lymphocytes for measurement of in-vitro mutagen sensitivity. Subsequent published reports provided estimates for genetic and environmental influences on several markers of mutagen sensitivity (Wu et al., 2006), genotype-phenotype correlations between mutagen sensitivity and genetic variants in the nucleotide excision repair pathway (Lin et al., 2007), and the heritability of mitochondrial DNA content, a risk factor for renal cell carcinoma (Xing et al., 2008).

The field of immunology is currently in a state of rapid discovery with the advent of increased understanding of the extent to which human immunological processes and mechanisms underlie common and rare conditions. Because the discovery of immunological markers of disease in humans is still in its early stages, the extent to which many of these markers are influenced by genetic and/or environmental sources of variation remains to be determined. The use of the twin design is a cost-effective way to determine the relative proportion of genetic and environmental influences, both on the occurrence of conditions of known or suspected immunologic etiology as well as on biomarkers that are either associated with or predictive of these conditions (Krishnan et al., 2012). A series of twin studies are being conducted with members of Stanford University Medical School's Institute for Immunity, Transplantation, and Infection and the Human Immune Monitoring Core. As a group, these projects utilize MZ and DZ twin pairs from the TRR as a way to determine the relative contribution of genetic and environmental factors to innate and adaptive immunological responses to vaccination for seasonal influenza virus ('Influenza Immunity: Protective Mechanisms Against Pandemic Respiratory Virus', U19AI057229) and for *Varicella zoster* virus ('Vaccination and Infection: Indicators of Immunological Health and Responsiveness', U19AI090019). In the influenza vaccine study, twins of both zygosity are

recruited on a seasonal basis to receive the US Centers for Disease Control-approved vaccine and then followed on a repeated basis to provide blood samples for immunological assay of markers of response. As of the date of this write-up, 74 MZ pairs and 28 DZ pairs of a variety of ages have participated in this protocol. In the second study, the twin design will be used to examine sources of variability in the response to the FDA-approved vaccine for *Varicella zoster*, a viral agent with substantial morbidity and mortality outcomes in older adults (Pickering & Leplege, 2011). For this study, 40 MZ twin pairs over the age of 50 who have had chicken pox in the past will be recruited to participate in a study of the immune response to the Zostavax[®] vaccine. In addition, 10 pairs of MZ twins of age 40–49 who have had chicken pox in the past are being recruited to participate in a cross-sectional study designed to assess immune responses to the naturally acquired *Varicella zoster* virus. These studies are aimed at learning more about age-related differences in immune function. To date three pairs have completed the cross-sectional study and five pairs the vaccine study.

A second series of studies with collaborators and support from Stanford University's Institute for Immunity, Transplantation, and Infection have recruited 27 MZ twin pairs who are either concordant or discordant for asthma. These twin pairs were identified by mailed survey of the entire TRR. Of the approximately 1,350 responses received, 76 pairs were subsequently identified and contacted for possible participation in a clinical study in which medical history, lung function measures, and blood samples were collected for immunological assay. Thus far, 21 MZ twin pairs discordant for asthma have been examined for differences in epigenetic modifications of T cells, mediators of the inflammatory responses linked to asthma and the extent to which such modifications are associated with exposure to second-hand smoke (Runyon et al., 2012). Because of the successful approach taken to identify twin pairs with and without a history of asthma, a second, more comprehensive survey of members of the TRR for a wide variety of conditions of interest to immunologists will be conducted in 2012–2013.

Conclusions

As described above, biometric studies of twins continue to provide clues with regard to genetic and environmental contributors to a wide variety of phenotypes associated with important health outcomes. The inclusion of genotypic status in quantitative models of variation in MZ and DZ twins provides insight into the amount of variation a single gene or set of genes may contribute to the estimate of total additive genetic influence in selected phenotypes (Zaitlen & Kraft, 2012). The study of MZ twins discordant for a particular trait or for an environmental exposure provides insight into epigenetic effects that may explain the twin discordance (Bell & Spector, 2011). The TRR at SRI International, while relatively modest in size compared to

other, much larger population-based registries described in this issue of *Twin Research and Human Genetics*, will continue to contribute to ground-breaking findings concerning the etiologies of complex phenotypes that are not feasible to assess on a larger scale.

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