Differences in Smoking Habits of MZ and DZ Twins: A Commentary on Tishler and Carey

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Tishler and Carey (this issue, 2007) have analyzed the difference in ever smoking prevalence between monozygotic (MZ) and dizygotic (DZ) twins in 8 published twin studies. They conclude that the lower prevalence in MZ twins might invalidate conclusions from classic twin models. I critique the conducted meta-analysis and suggest additional analyses to be conducted. A reanalysis of the Finnish Twin Cohort 1975 questionnaire data (included in Tisher and Carey) indicates that when intrapair correlations are correctly taken into account, the MZ–DZ difference in that study is no longer statistically significant. Even if the Tisher/Carey finding holds up in further analyses, it pertains only to the observed trait, the effect is small in size, and the mechanism underlying it needs to be explored.

When we use family and twin data to estimate heritability, we assume that all family members, irrespective of their status in the family, come from the same base population. This base population is then the one to which the heritability estimate is applied. Thus, the trait under study is presumed to be distributed similarly among parents and children, or MZ and DZ twins, at least after correction for obvious confounders such as age and sex. Thus, in a twin study, one test for this assumption is that the means and variances of continuous traits, or the prevalence of binary traits, is the same among MZ and DZ twin individuals.

Tishler and Carey remind us of the need to test this assumption, and to document it carefully. They have examined the prevalence of ever smoking in eight large twin studies from Australia, the Nordic countries, the United Kingdom (UK) and the United States (US) after observing a very large difference in MZ versus DZ twins in their own study. In all but the Danish study, the prevalence of ever smoking was higher in DZ than MZ twins. This difference was found in all age groups in a pooled analysis, but the effect was not statistically significant for subjects aged 26 to 35, or those 60 and over. Likewise, a significant overall effect was found for men and women; for women there was significant heterogeneity over samples.

Is this effect real? The pooled analysis is quite convincing, and the sample sizes, with the exception of Tishler and Carey's own, are large. Tishler and Carey have analyzed the data based on meta-analytic techniques, using published tabular data, and they did not have access to individual level raw data for pooling. The prevalence differences in the individual studies are modest, again with the exception of the Tishler study. Their definition of a never smoker is 'less than 4 pack-years', that is, a person can have smoked a pack a day for 4 years (a total of nearly 30,000 cigarettes) and still be considered a nonsmoker. The Tishler study is clearly problematic, and should have been excluded from the meta-analysis. Because of small sample size, it is nonetheless unlikely to have had a major effect on the overall results.

The other studies have used varying definitions for smoking status, and Tishler and Carey have selected from among these. The UK study is the strictest, and defines a smoker to be a person who has ever smoked a whole cigarette; the prevalence difference between MZs and DZs is among the largest (5.9 percentage points). One definition for an ever smoker has been to use a maximum number of cigarettes smoked lifetime; this being 100 cigarettes in the US WWII and Vietnam Veteran studies, while the Swedish and Finnish studies used a limit of 5 to 10 packs of cigarettes (100–200 cigarettes). Given the highly addictive nature of smoking, these two definitions are very similar in their implication of who becomes a regular smoker. Currently, having smoked 100 cigarettes ever is the one most often used in epidemiological studies of adults. The Danish study is the oldest, from 1960, and is relatively small; never smokers were persons who 'denied smoking on the questionnaire without

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further comment'. The Australian and Virginian definitions are closer to definitions of regular, rather than ever smoking. A certain fraction of persons report in studies that they have smoked more than the most common definition of ever smoking (100 cigarettes lifetime), but have not been regular smokers. In our Finnish studies, after asking whether a twin had smoked more than 5 to 10 packs ever, we then asked whether they have ever smoked regularly, that is, daily or almost daily. Those ever smokers who were never regular smokers were defined as occasional smokers; these can be persons who smoke, for example, only at parties or other special occasions. In a reanalysis of our 1975 questionnaire data, 3.4% of DZ twins and 3.2% of MZ twins were occasional smokers. While it is understandable that Tishler and Carey have focused on one aspect of smoking, which they were able to derive from published data, a full analysis would utilize all the information on smoking status, including whether a regular smoker was still a current smoker or not, and the amount smoked.

Relying on published data, Tishler and Carey have carried out the analyses under the assumption that the individual prevalence data come from samples of unrelated individuals, rather then having been sampled from twin pairs. They recognize this limitation, but state that 'correcting for any intra-twinship correlation is unlikely to eliminate the effect we have reported'. I reanalysed our 1975 questionnaire data, the numbers being somewhat different from the published 1978 figures, one reason being updated information on zygosity over the years for our cohort. The overall MZ–DZ prevalence difference was 2.3%, which was significant (p = .045) in an age–sex adjusted logistic model, which did not correct for the sampling procedure. After adjusting for sampling, that is, the intra-twinship correlation, the difference was no longer statistically significant (p = .104). Thus, in testing this key assumption for this trait in our cohort, we have concluded that there is no significant difference, and that the MZ and DZ twins represent the same base population with respect to ever smoking. From the perspective of the individual study and researcher, there is no problem in estimating heritability, nor in answering to a question raised at a congress about whether smoking differs in prevalence between MZ and DZ twins. Given that the Finnish cohort is among the largest (> 21,000 twins) included in the Tishler/Carey analysis, the other individual studies probably have not observed significant differences in MZ–DZ prevalence.

The Tishler and Carey meta-analysis does raise the possibility that overall, in larger pooled studies, a true difference may be observable. One issue to be addressed is the use of different definitions for the trait, which may have contributed to the observed heterogeneity between studies, at least for women. Instead of using published data, a way forward would be to conduct pooled studies of individual data. As such data sets can be fully anonymized, data protection would not be an issue. Such pooling has been done in the GenomEutwin collaboration. Even after such an analysis, the observed difference is likely to be small, and of little practical importance in heritability estimation.

But would the finding of a difference in prevalence obviously invalidate the estimation of heritability from twin data? For that, we need to ask why such a relatively minor difference in prevalence has arisen. Tishler and Carey offer as an explanation, differences in dependence and interaction between twins, which have recently been studied within our Finntwin16 cohort by Varpu Penninkilampi-Kerola and colleagues (2005a, 2005b). She demonstrated that MZ pairs are more often dependent on each other in adolescence than DZ pairs, female pairs more so than DZ pairs. Within dependent pairs, genetic effects on alcohol related variables were smaller than for independent pairs (Penninkilampi-Kerola et al., 2005). This association does not, however, resolve the issue of causality — are the dependent twins spending time together and drinking together, irrespective of zygosity, or does drinking together cause the twins to become more dependent on each other. After all, at this age they are engaged in an illicit habit, which they may be jointly hiding from their parents. Without longitudinal analyses, this cannot be resolved. It should be noted that even in mid-adolescence, at most, one third of twins characterized themselves as dependent on their cotwin. As noted by Tishler and Carey, for some MZ pairs, the emotional ties with one's co-twin may indeed be very intense. Despite this, it would appear that for most pairs, individuation and forming one's own peer network develops in the same way as for siblings overall. Varpu Penninkilampi-Kerola presented smoking related results at the Twin Congress in Ghent this June. She reported a 6 to 7 percentage point difference at age 16 in smoking initiation (having smoked at least one cigarette) between MZ and DZ twins (n = 2676 twins), but among those who smoked, there was no difference by zygosity in either sex of the lifetime number of cigarettes smoked, or their current (age 16) smoking status. Moreover, within zygosity groups, there was no difference in the distribution of smoking between dependent and independent twins. By age 25, the difference in smoking initiation had disappeared, and the amount smoked daily among smokers was the same in MZ and DZ twins. Thus, in our cohort, smoking experimentation appears to occur later in MZ twins than DZ twins, but this is unrelated to dependence status, and furthermore, by adulthood the difference disappears. Obviously, studies from other large, population-based cohorts are needed.

Other reasons for the difference in prevalence need to be explored. One is to use measures of intrapair personal relationships other than dependence/attachment, and to assess the peer networks of MZ versus DZ

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twins in adolescence. A remote possibility is that MZ or DZ twinning is associated genetically (or epigenetically) with a predisposition to smoking.

It is more likely that characteristics differentially associated with MZ or DZ twinning may also be associated with smoking, such as social class and/or parental age, and hence, affect the probability of smoking initiation in adolescence. Given that smoking rates vary by country, calendar time, sex, smoking restrictions and legislation, and many other social factors, unraveling the contributing factors is not a trivial task. Furthermore, all the studies reported by Tishler and Carey have been surveys with varying response rates. Non-smokers generally participate in surveys more actively than smokers, possibly due to the psychiatric and substance use comorbidity associated with smoking. Because MZ twins have higher participation rates in twin studies, the effect of smoking on response rate should be studied by zygosity.

Even if a real MZ–DZ difference in smoking initiation can be demonstrated after these further analyses, it is not likely to apply to traits other than some aspects of substance use and abuse. It certainly does not invalidate the vast body of twin research, in which the lack of MZ/DZ differences in means and distributions have been carefully documented.

I can concur with Tishler and Carey that measures of the social environment of MZ and DZ twins should be included and assessed in all twin studies. We have demonstrated that measures of parenting modify the genetic effects on smoking frequency in adolescence (Dick et al., 2007a; Dick et al., 2007b). By including information on siblings, offspring and parents of twins, twin-specific effects can be estimated more accurately (Maes et al., 2006). Such extended family studies also provide more confidence in our heritability estimates. Identification of specific genetic loci will provide important confirmatory evidence for genetic effects, and in this area rapid progress is now being made, both with large family studies specifically designed to study smoking behavior and nicotine dependence (Saccone et al., 2007b; Loukola et al., 2007), and with genome-wide association studies (Bierut et al., 2007; Saccone et al., 2007a; Uhl et al., 2007).

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