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## Design and Sampling Considerations, Response Rates, and Representativeness in a Finnish Twin Family Study

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**Abstract.** Kinships composed of twin parents, their spouses and children, offer a robust and flexible sampling design for research in genetic epidemiology. Families-of-twins designs circumvent some of the sampling problems that arise when independent data sets are combined, and these designs provide unique evaluations of maternal influences, assortative mating and X-linkage. Unfortunately, empirical studies of families of twin parents have been limited by relatively small samples and by the self-selection biases intrinsic in ascertainment of families from volunteer twin registries.

A large and representative cohort of monozygotic and dizygotic twin parents, drawn from a population-based twin registry, provides the optimal sampling frame for twin-family research. This paper reviews the sampling considerations underlying the initial family study based on the Finnish Twin Cohort and evaluates the representativeness of the sampled twins. Spouses and adult children (over 18 years) of 236 pairs of twins, about equally divided by gender and zygosity, were evaluated by a postal questionnaire. Individual response rates exceeded 86% and in 464 of the 472 nuclear families (98.3%), at least one member of the twin's family completed the questionnaire. The sampled twins, selected for fecundity to maximize statistical power of the obtained data, were broadly representative of non-selected twins drawn from the Cohort, with whom they were matched on age, gender, and zygosity. Such results suggest that the Finnish Cohort has excellent potential for extended twin-family research designs.

**Key words:** Twins, Registries, Sampling, Questionnaire, Substance exposure, Personality

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## INTRODUCTION

While twin studies are a powerful method for estimating the degree of genetic influence on traits, they cannot answer questions on the mode of trait inheritance. For human traits, cultural as well as biological transmission occurs [3]. The effect of assortative mating also cannot be studied by conventional twin comparisons. While the aforementioned can be studied using nuclear family and adoption data, an alternative design with some unique features is the study of twin families.

Nearly 25 years ago, extended twin-family designs were advocated for incisive and efficient analyses of multifactorial traits. At a conference directed to problems and methods in human genetics, Franz Kallman reviewed the methodology of twin studies and advocated that the classical twin approach be extended to include full, half- and step-sibs of a representative sample of monozygotic (MZ) and dizygotic (DZ) twin pairs, because such a data set “extends the number of genotypically dissimilar sibship groups which can be compared under similar conditions of culture and home milieu” [12; p.160]. Kallman called this design a “twin-family” method, and he advocated its use when “an integrated analysis” of traits is required “by the apparent interdependence of multiple factors of causation”.

Kallman had suggested studying twins and their siblings within a single generation. He did not recognize that genetic half-siblings were nested within the sibships of MZ twin parents, nor did he fully appreciate the multiple genetic and social relationships to be found within these kinships. But in the early 1970s, a twin-research group at Indiana University made those relationship explicit [1,2,23,30] and in subsequent studies of the Indiana University Human Genetics Center, then directed by W.E. Nance, over 100 kinships of identical twin parents were ascertained, and the “MZ half-sib” model [21] was applied to a variety of polygenic and multifactorial traits ranging from fingerprint ridge counts [24] and lipid levels [4] to blood pressure [28] and non verbal IQ scores [26]. Further theoretical work on the model was carried out by Haley et al [8].

These Indiana studies suggested the flexibility of families-of-twins designs and offered initial opportunities to employ twin-family data for evaluating maternal influences [25], assortative mating [22], and X-linkage [27]. The design was subsequently extended to the kinships of DZ twin parents [9] and to the combination of twin parents and their children with younger twins and their parents [9], and analytic methods have been simulated requiring twin-family samples far more extensive than any thus far empirically realized [10]. To date, the potential of extended families-of-twins designs has been constrained by practical problems in recruiting adequate samples and by self-selection biases that appear inevitable when sampling from volunteer twin panels. As Kallman had recognized, the index twins, whose family members form the study sample, must be representative of the population. And, for adequate statistical power, the samples must be quite large [10]. Neither requirement is easily met in volunteer twin research, where participating twins and their families are demonstrably unrepresentative of socioeconomic variation [28] and biased by self-selection associated with gender and zygosity [18]. For such reasons, the population-based twin registries of the Nordic countries possess unique advantages. The excellent compliance of adult twin in the Finnish Twin Cohort suggested that a twin-family study based on the Cohort would achieve excellent results. This paper presents initial data from the first Finnish effort to ascertain families of twin parents for epidemiological research.

## METHODS

### Subjects

The Finnish Twin Cohort is a population-based panel of adult like-sexed twin pairs born before 1958 with both twins alive in 1967 [14]. The Cohort contains information on over 17,000 pairs. Two questionnaire surveys of the entire cohort have been carried out in 1975 and 1981 to collect data for genetic epidemiological studies. Zygosity has been determined by a validated questionnaire method [29]. Morbidity and mortality data has also been collected from nationwide computerized registries. The Finnish Twin Cohort was extended to include spouses and first degree relatives in 1984.

### *Civil registration in Finland*

Finland has a long tradition of civil registration with compulsory registration of live births, deaths, stillbirths and marriage uninterrupted since 1628 [17]. The local church officials (clergy) were responsible for collecting the information on all parishioners and were obliged to give annual reports to the state. A summary of this manual record-keeping is given by Eriksson et al [7].

In the 1960s, the transfer of these population data to a central, computerized population data to a central, computerized population register was begun. Personal identification numbers (PIN), based on the date of birth and a unique four-digit identification sequence, were assigned initially to working aged persons under the national pensions and sickness insurance legislation of 1964. Two years later, the Central Population Register (CPR) was established and all Finnish citizens were assigned PINs. By law, the local parishes were required to report to the CPR data on the persons in their parish. This transfer of data occurred during 1966-69. Among the data reported was that on the spouse and minor children. The reporting of other family data, such as information on parents of adults or adult children, was not required by law, but was left to the discretion of the local registration officials. If the relatives were deceased or had migrated, they were not generally reported.

After the basic transfer of population data, the local parish officials remain responsible for the primary collection of data on births, deaths, marriages and migration. They regularly report these events to the CPR. Thus, family data on parents and offspring in the CPR can be considered rather complete, for children who were under age 18 in 1969.

### *Linkage of family data to the Finnish Twin Cohort*

The Finnish Twin Cohort was compiled in 1974 from the CPR. The family data were not used in the process; the selection procedures have been described in detail elsewhere [14]. In 1984, the Finnish Twin Cohort was expanded to include data on first-degree relatives using the CPR information. All data on spouses, children, parents and sibs were gathered for all pairs belonging to the following groups:

- 1) All MZ twin pairs.
- 2) A random selection of the DZ pairs equal in number to the MZ pairs, except for the inclusion of all pairs with cancer diagnosed in one or both twins prior to 1979. As there are about twice as many like-sex DZ pairs as MZ pairs in the Twin Cohort, essentially one-half of eligible pairs were selected at random.

3) All twin pairs with cancer diagnosed in one or both twins prior to 1979 of unknown zygosity, since one of the purposes of the collection of family data was to permit studies of family cancer [13].

Technically the linkage of family records is done using the PIN. In the CPR, the record of each person contains the PIN of the parents, spouse and children as well as a code reporting details of the relationship. Thus, for parent-offspring relationships there are data on whether the child was born in or out of wedlock, is adopted, or a foster-child. For spouses, the date of marriage is recorded, former spouses are included, their dates of marriage, as well as the cause and date of the end of the marriage.

For the twins, the current spouses, parents and children were first linked. Using the parents' information on their children, the sibs of the twins could be identified. After the identification data on the relatives were obtained, their basic records were produced, with information on name, current address, occupation, marital status, and date of death if deceased. For 8,181 twin pairs (16,362 twins) the records of 59,968 first-degree relatives were found. For 3,922 pairs the mothers were identified, and for 3,444 pairs the fathers were found. If either the mother or father was found, sibs could be identified ( $N = 12,994$ ). A total of 13,570 spouses were identified as well as 25,988 children. Fifty adoptive parents were also found.

Among the 3,919 MZ pairs linked to the family data, there were 1,736 pairs in which both parents of the twins were identified. There were 2,362 MZ pairs in which both were married at the time of data collection, and 2,211 MZ pairs, including divorced and widowed persons, in which both twins had at least one child. In short, there are sufficient numbers of twin pairs with identification data on their relatives for families-of-twins studies of various designs.

#### *Selection of twin families for the 1985 questionnaire study*

For the twin family study, those kinships were selected in which both twins had replied to the health questionnaire mailed in 1981 to all twins of the cohort. In both twins families there were to be a spouse and at least two adult children alive and with known addresses. The principles of the sampling procedure are summarized in Figure 1.

A total of 236 families were selected. That selection included all families with three or more children in both nuclear families of the twin kinship, as well as a random 50% of those with two in one family and three or more in the other family of the kinship. The selection occurred so that all the largest kinships would be studied; these yield the most information per subject studied. The objective of the initial study was to obtain, after loss due to non-response, some 50 kinships of each sex and zygosity combination. The final sample consisted of 118 male twin kinships (61 MZ, 57 DZ) and 118 female twin kinships (63 MZ, 55 DZ).

## **Procedures**

### *Questionnaire content*

Both twin parents, within each kinship, had already returned a health questionnaire in 1981. A similar questionnaire, identical except for deletion of questions assessing zygosity and social contact between twins, was mailed in 1985 to the 1,575 adult children and 472 spouses of the 236 selected twin pairs. Two mailing rounds in May and August 1985

REPRESENTATIVENESS OF TWIN-FAMILY DATA SETS

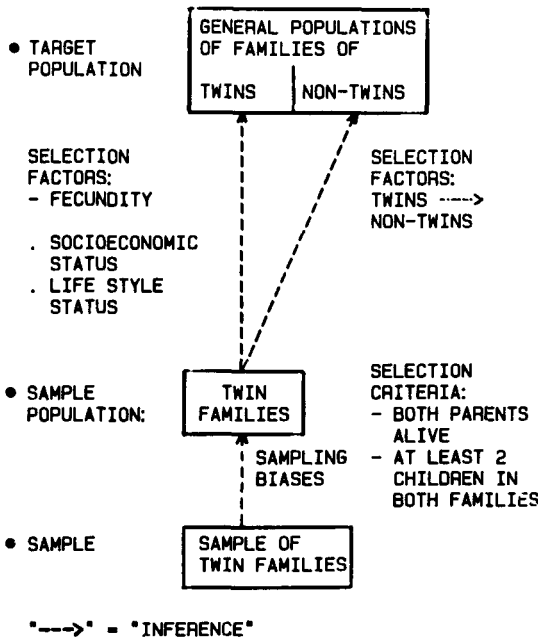


Fig. 1 - Sampling scheme for twin family sets and path of inference to sample and general population.

yielded a response rate among individuals of 86.7% . In all, 1,372/1,575 offspring and 403/472 spouses complied. Accordingly, the data set for the initial Finnish Twin-Family study includes N = 2,207 individuals who comprise 224 twin fathers, 208 twin mothers, 403 non-twin parents and 1,372 adult offspring. A check of the family data in the Central Population Registry revealed that two of the children were biologically unrelated to either rearing parent, while 55 others were not genetically related to the non-twin parent. These relationships were confirmed by inquiries to the local parish registries in order to exclude possible errors in the CPR data. Excluding these cases left 1,315 biological children in the sample.

Table 1 shows the distribution of family size and of the respondent children in the 236 sampled kinships. Complete data were obtained from one or more adult children on both sides of each kinship in 224 of the 236 twin pairs. Table 2 gives the kinship response rates from which we can assess the number of units of analysis that will be available. The mean ages (and ranges) for the twin family members is given in Table 3.

The health questionnaire covered a wide range of risk factors and other health related variables. Weight and height serve as useful benchmark variables in the analyses, and the study of obesity is of interest, in itself. Smoking (type smoked, duration, quantity, inhalation, tar, nicotine and carbon monoxide content of cigarettes), alcohol use (quantity, frequency, density and black-outs), use of coffee and tea, diet (milk and fat use, salt use,

**Table 1 - A Finnish family of twins study: distribution of respondent children in families of 236 twin pairs**

No. of respondent children	No. of families
0	12
1	26
2	156
3	145
4	90
5	26
6	12
7	4
8	1

**Table 2 - A Finnish family of twins study: individual and kinship response rates by gender and zygosity of the twin parents**

Sample population	Individual response rate	Kinship response rate
61 MMZ kinships; 527 family members	465/527 88.2%	58/61 95.1%
63 FMZ kinships; 517 family members	452/517 87.4%	60/63 95.2%
57 MDZ kinships; 517 family members	461/517 89.2%	55/57 96.5%
55 FDZ kinships; 487 family members	397/487 81.5%	51/55 92.7%
216 twin kinships; 2048 members	1775/2048 86.7%	224/236 94.9%

Kinship response rate is here defined as those kinships in which at least one offspring from each side of the kinship returned the questionnaire. A total of 1,575 adult offspring were sent questionnaires; 1,372 (87.1%) returned them. Of the 472 spouses of the twins, 403 (85.4%) responded.

egg, vegetable and fruit consumption), physical activity (at work and during leisure – intensity and duration), are all factors that have been studied in relation to cancer and ischemic heart disease risk. Other variables of interest are those related to sleep (sleep length, quality, snoring and diurnal type), personality (extraversion, neuroticism, life satisfaction, Type A coronary-prone behavior, hostility), socioeconomic status (education, social class, occupation) and medical history (blood pressure, use of certain common drugs, cardiovascular symptoms, disease history).

#### **Methods of data analysis**

Familial aggregation of disease may be due to the contribution of both genetic and environmental factors that are correlated between relatives. Various mechanisms for environmental transmission from parent to offspring have been proposed [11]:

- a) direct effect of parental genotypes on offspring environment, that is not mediated through the parental phenotype.

Table 3 - Age of twin parents and respondent offspring in the Finnish Twin Family Study

Parents					
Sex	Zygoty	Age		Range	
		mean	sd		
M	MZ	58.1	6.8	45–75	
M	DZ	59.0	6.8	48–75	
F	MZ	54.1	6.7	42–72	
F	DZ	54.7	6.1	44–68	

Offspring					
Sex of offspring	Sex of twin	Zygoty	Age		Range
			mean	sd	
Male	M	MZ	28.6	5.7	18–43
	M	DZ	27.8	5.4	18–43
	F	MZ	28.2	5.8	18–46
	F	DZ	27.3	5.3	18–44
Female	M	MZ	27.3	5.6	18–44
	M	DZ	28.3	5.4	18–43
	F	MZ	26.7	5.1	18–38
	F	DZ	27.5	5.5	18–39

b) direct effect of parental childhood environment on offspring environment, that is not mediated through the parental phenotype.

c) direct effect of parental phenotype on offspring environment.

In the twin family design, comparison of parent-offspring and cotwin-offspring correlations in kinships of MZ and DZ twin parents permit powerful resolution of these competing hypotheses. An alternative to the study of twin parents and their offspring is the study of MZ and DZ pairs and their parents together with a biological uncle or aunt. This is another very powerful design [10]. The number of such extended nuclear families identifiable from the Finnish Twin Cohort is at least 500 families of each zygosity type.

Within the same sampling frame, the twin family design permits the study of other relationships. These families include individuals who share varying levels of genetic relationships, ranging from zero in the spouses of twins to unity in MZ twins. The spousal correlations can be used to study various aspects of mate selection and assortative mating. The full- and half-sib correlations among the offspring of MZ twins can be used to study nonadditive genetic effects. Compared to half-sibs from nuclear families, who share only one parent in common, MZ half-sibs do not result from break-up of marriages due to divorce or death of a parent [16]. Maternal effects can be studied by comparing half-sib correlations from the families of male and female MZ twins [8,25].

Data analysis can be performed using various approaches. The estimation of the degree of resemblance between family members can be made from correlation and regres-

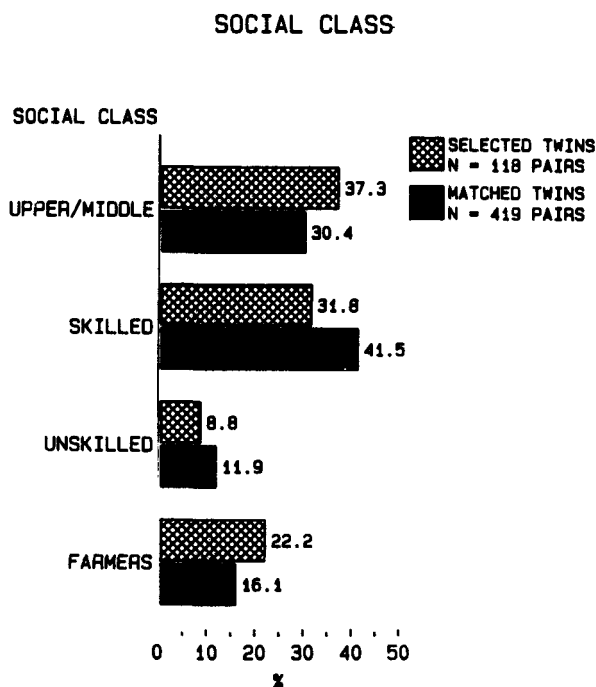
sion techniques, using the CORREL program which adjust for the fact that, within families, sibship size varies [6]. The estimation of components of genetic and environmental variance can be carried out by model-fitting procedures based on correlations or mean-squares [21], and path-analytic methods have been used by McGue et al [19]. An alternative approach is to carry out a pedigree analysis of the raw data by maximum likelihood methods [15].

## RESULTS

The 236 pairs of twin parents were selected on fecundity, which must be expected to correlate with social-economic status and life-style characteristics. To evaluate possible selection effects, we matched the selected twin pairs on gender, age and zygosity with 838 pairs of nonselected twins in the Finnish Twin Cohort. The comparison sample consists of 419 male and 419 female pairs with proportional representation of zygosity.

### Socioeconomic Status

All the selected twin parents were married, while 15% of the matched twins had not been married and 13% were divorced or widowed. Thus only 72% of the matched twins were married. The social class (Fig. 2) distribution was not significantly different in the two



CHI-SQUARE = 6.35, df = 3, P = 0.0956

Fig. 2 - Social class distribution in the twin parents and twins matched for age, sex, and zygosity.



groups, but there were more farmers and upper class (professionals, etc) among the selected twins.

**Psychological Traits**

Twins selected on fecundity are more extravert, less neurotic, and express greater life satisfaction than do age-sex matched controls (Fig. 3). In part, such differences are a direct function of specific item content (eg, "Are you lonely? ") expected to differentiate single adults from age-matched parents.

**Health Behavior**

Selected twin parents reported in their 1981 questionnaires less alcohol use than the sample of matched twins (Figs. 4 and 5). This was true for both males and females, and both MZ and DZ pairs; there was a greater difference for twins of DZ pairs and MZ pairs in the men.

The selected twins were significantly more often nonsmokers or former smokers than the matched twins, 32% of men in the matched sample were current smokers compared to 21% of the selected twin men (Figs. 6 and 7).

**Twin Characteristics**

No striking differences were found in age at initial separation (Fig. 8) or current frequency of social contact, once pairs still living together were excluded (Fig. 9). While the dif-

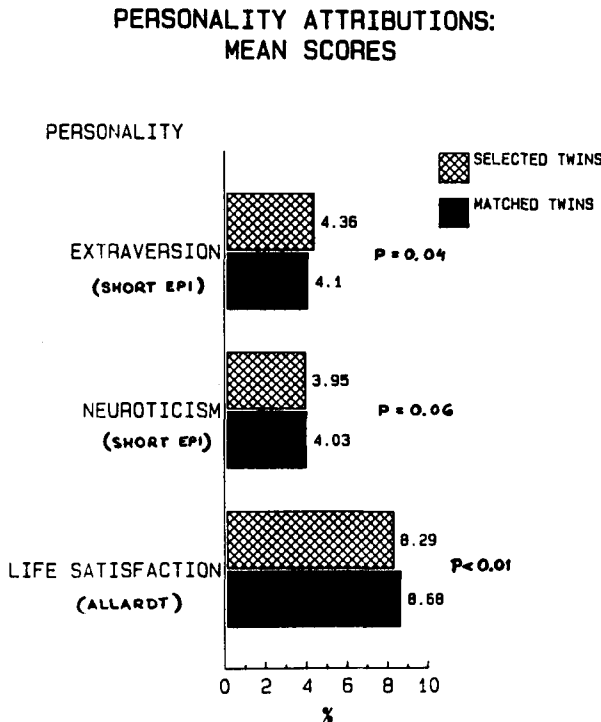


Fig. 3 - Personality attributions in the twin parents and twins matched for age, sex and zygosity.

SELF-REPORTED ALCOHOL USE (MALES)

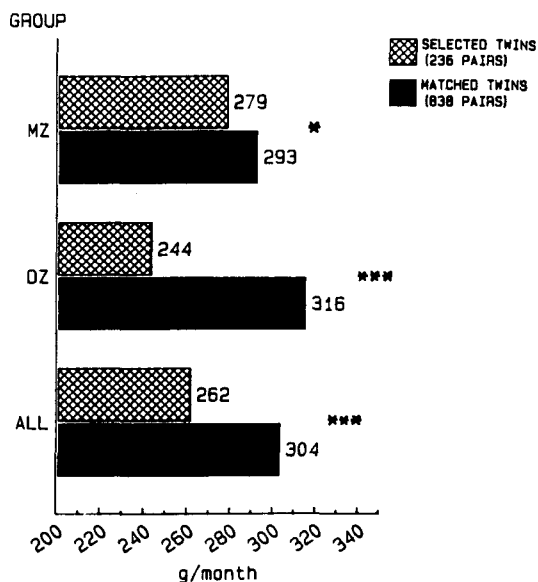


Fig. 4 - Alcohol use among men in the twin parents and twins matched for age, sex, and zygosity.

SELF-REPORTED ALCOHOL USE (FEMALES)

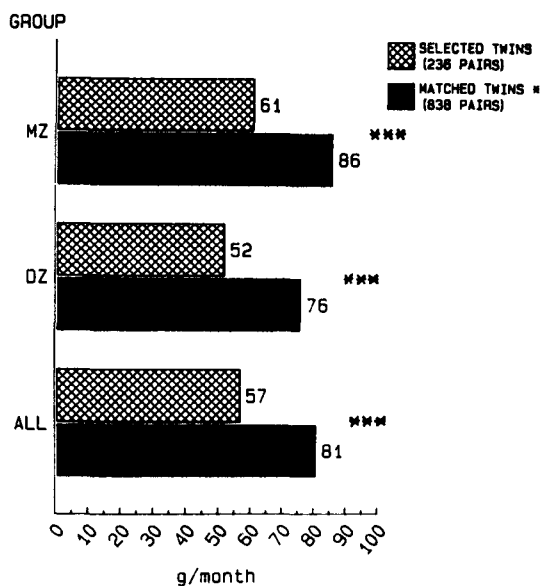
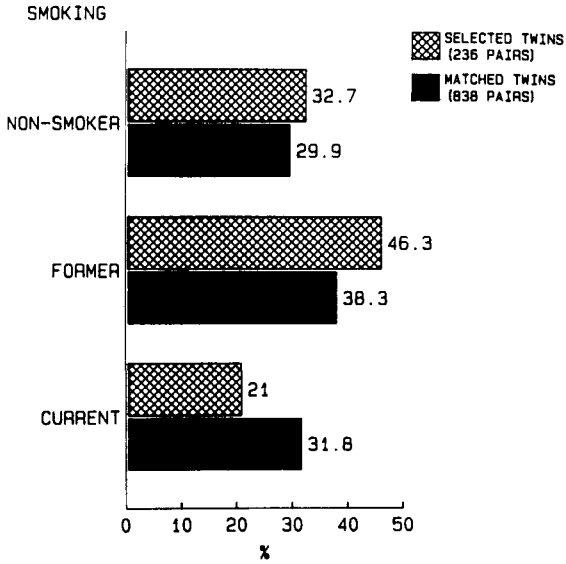


Fig. 5 - Alcohol use among women in twin parents and twin matched for age, sex, and zygosity.

\* ALL OF THE DIFFERENCES ARE SIGNIFICANT, P < 0.001

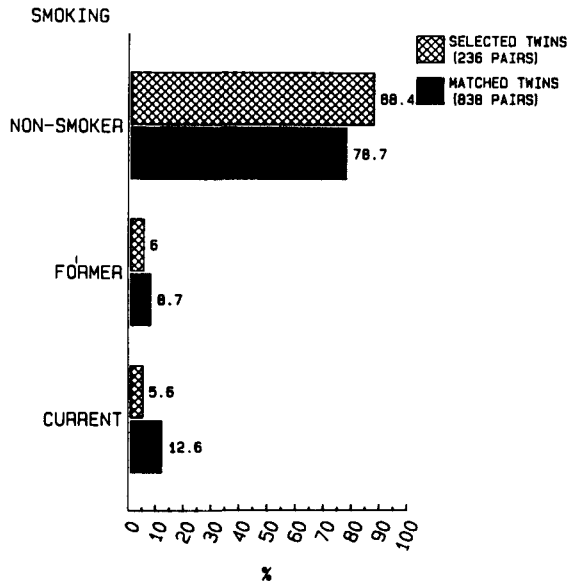
SMOKING STATUS (MALES)



SIGNIFICANCE: P = 0.0059

Fig. 6 - Smoking status among men in the twin parents and twins matched for age, sex, and zygosity.

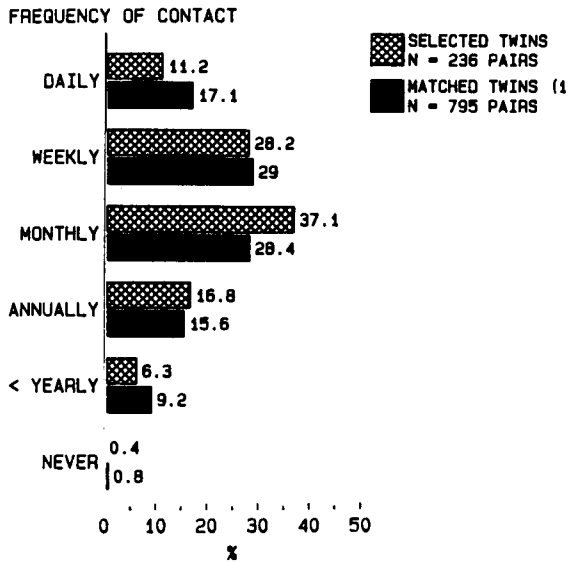
SMOKING STATUS (FEMALES)



SIGNIFICANCE: P = 0.0020

Fig. 7 - Smoking status among women in the twin parents and twins matched for age, sex, and zygosity.

FREQUENCY OF CONTACT



CHI-SQUARE = 21.51, df = 5, P = 0.0006  
 1) 43 PAIRS OF MATCHED TWINS HAS BEEN EXCLUDED, BECAUSE THEY LIVE TOGETHER

Fig. 8 - Intrapair social contact in the twin parents and twins matched for age, sex, and zygosity.

AGE AT SEPARATION

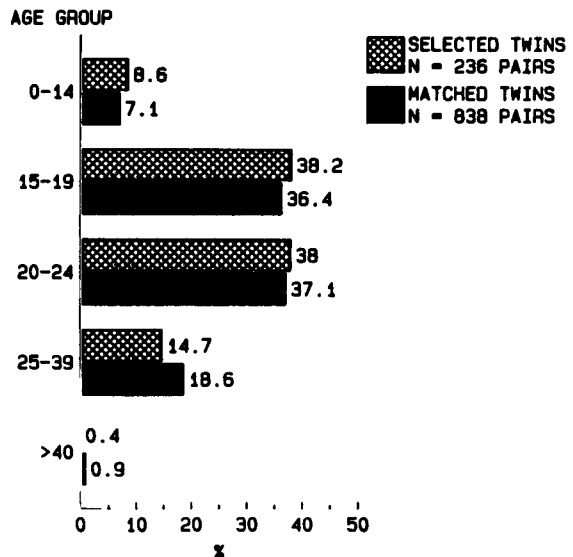


Fig. 9 - Age at separation from cotwin among the twin parents and twin matched for age, sex, and zygosity.

CHI-SQUARE = 5.98, df = 4, P = 0.201

ference in current frequency of social contact is statistically significant, the distribution is very similar for both groups.

## DISCUSSION

The studied sample's size compares very favorably with sample size requirements estimated from computer simulations for adequate detection of cultural and genetic transmission [10]. This twin family sample is larger than other data sets reported in the literature [20], it contains both MZ and DZ twin families, and is a population-based sample. The response rate was very high, lessening the effect of self-selection. In the Scandinavian Twin Registries, the only large twin family study reported to date, is that by Crumpacker et al [5], based on the Swedish Twin Register. In 1975, they sampled kinships of twin pairs living in the two largest cities in Sweden: selection criteria required both twins to have spouses and at least one child over age 18. A sample of 138 twin kinships was ascertained with a total of 548 twins and spouses and 360 adult offspring. The participation rates (approximately 65%) were lower than in the present study; the Swedish study required more of the subject's time and involved travel to the study site. Thus, in contrast to this study, the Swedish study was less restrictive, requiring one child on one side only; it was less representative, sampling from urban areas only; and it was more self-selected, with a 65% compliance rate.

By comparing the characteristics of the twin parents with age-sex-zygosity matched pairs from the entire Finnish Twin Cohort, we have been able to show similarities and differences between the general twin population and the selected study sample. What are the characteristics of the 236 twin pairs chosen for family study? Comparison of the 472 twin parents with 1,676 nonselected twins who are matched on age, sex and zygosity, reveals that the selected twins are less educated and less urban, consume less alcohol and smoke less. Undoubtedly, if the comparison group had been restricted to married pairs, the differences would have been smaller, ie, the differences are correlates of being able to marry and have more than one child. Most of the parents had been married for several decades, though some of the non-twin spouses were not biological parents of the offspring. Both the selected and the matched pairs had participated in the 1981 questionnaire study of the twin cohort.

However, in their age at separation from their cotwin and their current frequency of social contact with their cotwin, selected twins did not differ from the matched pairs. Thus, for analyses of relationships within kinships comparing members of MZ and DZ twin families, the selected twin parents are not distinguishably different from the twins in the entire Finnish cohort. Also the families of the twins can probably be considered representative of families in the population.

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