

# Netherlands Twin Register: A Focus on Longitudinal Research

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In 1986 we began The Netherlands Twin Register (NTR) by recruiting young twins and multiples a few weeks or months after birth. Currently we register around 50% of all newborn multiples in The Netherlands. Their parents receive a questionnaire at registration and afterwards when the children are 2, 3, 5, 7, 10 and 12 years of age. Teachers are asked to rate the behavior of the children at ages 7, 10 and 12 years. Adolescent and young-adult twins were recruited through City Councils in the early 1990s. These twins, their parents and siblings participate in longitudinal survey studies that include items about health, fertility, lifestyle, addiction, personality and psychopathology, religion, socioeconomic status, and educational attainment. The total number of twins and multiples registered with the NTR is currently over 60,000. Subgroups of twins and siblings take part in studies of cognitive development, brain function and neuropsychological indices of attention processes, and molecular genetic studies of classical and behavioral cardiovascular risk factors. DNA samples are currently collected in selected twin families for two large linkage studies, which aim to find QTLs for anxious depression and for nicotine addiction. Sisters who are mothers of DZ twins contribute DNA samples for a linkage study of DZ twinning. Large cohorts of phenotyped family members from the general population are very valuable for genetic epidemiological studies and permit selection of informative families for gene finding studies.

The Netherlands Twin Register (NTR)<sup>1</sup> currently consists of two groups of twins and twin families who participate in research projects. The young twins are registered at birth by their parents. Their behavioral development is assessed by rating instruments, which are completed by their parents and teachers, about every two years. The first newborn twins were registered in 1986–1987, but the number of children in the NTR who were born in 1986 is small. The young twins are recruited with the help of a commercial organization (Felicitas B.V.) that visits parents of newborns at home. If, during this home visit, it turns out that there is more than one baby, the parents receive a brochure and a registration form from the NTR. After the parents send in the signed registration card, they receive the first questionnaire. When this questionnaire is returned, the family is registered with the NTR and will be sent a questionnaire every two years, and a newsletter each year.

The adolescent and adult twins, their parents and their siblings, have been recruited through city councils in

1990–1991. They participate in longitudinal survey studies that take place roughly every two years. In 1990 we asked all city councils in The Netherlands for names and addresses of twins aged between 13 and 20 years. Of the 720 city councils that were approached, 252 gave a positive response and supplied 4036 addresses of twin families. Between 1991 and 1993 additional addresses were obtained for 1987 twin families. These included addresses from several of the larger cities in The Netherlands. These addresses still form the majority of addresses available for the adult twins in the NTR. In the past 10 years we have also recruited a few samples of older twins through City Councils, and asked adult twins to register with the NTR in our yearly newsletter (Boomsma et al., 2000). Table 1 gives an overview of the total number of twins (and triplets) and siblings, according to age, who were part of the NTR in January 2002. There are 164 families with 2 twin pairs, 1 family with 3 twin pairs and 3 families with twins and triplets.

In Table 2 detailed information is provided on the number of families (not individuals) with young twins/triplets who have returned the parental and teacher questionnaires. The first part of Table 2 (Table 2A) lists the questionnaires that have been returned by parents at each age of the children. Over the years, there has been an increase in the number of registrations (defined by the return of a completed first questionnaire), which corresponds with the increase in the number of twin and multiple births in The Netherlands. Between 1989 and 2000, the relative number of multiple births in The Netherlands increased from 13.2‰ (a total of 2250 twin and multiple births in 1987) to 17.8‰ (a total of 3639 twin and multiple births in 2000; data CBS). We began collecting teacher data in 1999 for 10-year-old twins and have since also begun collecting data from teachers for 7- and 12-year-olds.

For the adolescent and adult twin families Table 3 lists the number of twins, their parents and siblings who

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**Table 1**

Number of Participants (Twins and Siblings) in the Netherlands Twin Register, According to Age Cohort (January 2002)

Age group	Twins*	Siblings	Total Ss
0–4 year	20,478	1	20,479
5–8 year	14,275	7	14,282
9–12 year	9912	26	9938
13–16 year	3881	92	3973
17–20 year	295	231	526
21–25 year	4493	513	5006
26–30 year	4607	788	5395
31–40 year	1728	1054	2782
41–50 year	547	271	818
51–60 year	306	124	430
61–70 year	109	54	163
> 71 year	39	14	53
Total	60,670	3175	63,845

Note: \*All multiples; there are 413 young triplets (between 0–16 years) and 36 older triplets (> 16 years) and 6 young quadruplets.

returned a questionnaire for every year (1991 through 2000) that a survey was mailed to the available addresses. A total of 4316 families have participated in the study. Parents of twins were asked to participate in the 1991, 1993, and 1995 surveys, and siblings were asked in 1995 (one or two siblings per family), in 1997, and in 2000. In 2000 twins aged between 25 and 30 years were asked if they had a spouse/partner who was willing to fill out a questionnaire. In the offspring generation (twins and siblings) 10,437 individuals have taken part in the study at least once. Of this group, 40% has participated only once (these subjects were mainly recruited into the study in 1997 and 2000). Of the twins, 12% has participated on four and 5.3% at all five occasions.

### Research Projects: Survey Studies

In large groups (i.e., in all twins of the NTR that are registered with a current address) we collect longitudinal data by mailed surveys. In the young twin sample, these surveys focus on longitudinal studies of development and psychopathology.

**Table 2A**

Number of Twin Pairs for Whom Questionnaires are Returned by Parents and Teachers (11-02-2002).

COHORT	0	2	3	5	7	10	12 years
1986	155	109	82	101	89	103	103
1987	940	810	634	592	534	580	544
1988	993	815	673	635	601	586	521
1989	1022	795	800	710	720	636	460
1990	1188	945	945	861	802	745	42
1991	1235	988	900	867	836	669	
1992	1336	1072	973	872	906	95	
1993	1477	1161	1080	956	930		
1994	1533	1190	1000	928	569		
1995	1588	1132	1057	1017			
1996	1749	1261	1151	764			
1997	1664	1196	1156				
1998	1776	1476	555				
1999	1685	901	3				
2000	1411						
2001	851						

Note: Two questionnaires are sent to parents at ages 3, 5, 7, 10 and 12 years. The numbers in the table indicate that at least one of these is returned.

**Table 2B**

Questionnaires (TRF) Mailed to Teachers at Ages 7, 10 and 12 Years

TRF	Twin 1	Twin 2
Twins age 7	1225	1236
Twins age 10	1089	1095
Twins age 12	872	874

Note: Questionnaires are mailed out for each child (Twin 1 and Twin 2) separately

**Table 2C**

Conners' Mailed to Both Parents and Teachers at Ages 7, 10 and 12 Years

Conners' 4–18	Parent form		Teacher form	
	Mother	Father	Twin1	Twin2
Twins age 7	693	501	438	447
Twins age 10	417	448	338	328
Twins age 12	340	359	166	168

**Table 3**

Adolescent and Adult Twins and Their Family Members Who Participated in (Longitudinal) Survey Studies

	1991	1993	1995	1997	2000*
Fathers	1483	1778	1573	—	—
Mothers	1623	1919	1685	—	—
MZ Twins	1304	1576	1382	1371	2041
DZ Twins	2091	2309	2022	1760	2278
Twins, zygosity unknown	—	—	11	13	182
Siblings	—	—	1482	1435	1377
Spouses	—	—	—	—	701
Total	6501	7582	8155	4579	6579

Note: \*ongoing

**Table 4A**

Health Questions and Behavioral and Emotional Problems Rated by Parents of 3-, 5, 7, 10- and 12-year-old Twins; Behavioral Problems Are Also Assessed by Teachers (at Ages 7,10 and 12)

Health
Asthma, chronic bronchitis, bronchial asthma
Rhinitis / sinusitis
Eczema / dermatitis
Intestinal problems
Back problems / abnormalities
Epilepsy
Heart disease
Diabetes
Cancer
Liver problems / cirrhosis
Kidney problems
(Rheumatoid) arthritis
Deaf / hearing problems
Blind / visual handicap
Spasticity
Impaired movement / orthopedic problems
Metabolic disease
Other inborn or chronic diseases

Questionnaires are mailed to parents of twins at ages 0, 2, 3, 5, 7, 10 and 12 years. Table 4A and 4B give an overview of the health and behavior problems that are assessed in this way. In addition, we also ask parents about, for example, their own height and weight, place of birth, religious background, educational attainment and their socioeconomic status (SES). For birth cohorts 1986–1994, 19.4% of families were of low, 47.1% of middle and 33.5% of high SES.

In the young twins, data are collected at registration on birth weight and height (Van Baal et al., 1998) parental height/weight, pregnancy and birth complications, medication, smoking and alcohol use during pregnancy, and malformations.

When the twins are aged 2, growth data as measured by the Youth Health Services (Boomsma et al., 1992) are collected as well as information about breast feeding, motor development and behavior problems.

Research on twins from age 3 onwards is targeted at the development of psychopathology. At age 3 the CBCL2-3 (Child Behavior Checklist; Achenbach, 1992; Koot et al., 1997) is sent to both parents for the first time. This rating instrument is used to assess possible childhood psychopathology. Behavioral and emotional syndromes include overactive, oppositional and aggressive behavior, withdrawn/depressed and anxious behavior, sleep and somatic problems. In addition, the mother of the twins is asked about health problems (see Table 4A), and growth (height and weight), and both parents are asked about their religion, profession and education. Analyses of CBCL syndrome scales show high heritabilities for most behavioral problems in 3-year-old children (e.g., Van den Oord et al., 1997, 2000; Van der Valk et al., 1998, 2001).

At age 5 detailed questions are asked about asthma, allergies, eczema, handedness, growth, speech problems, stuttering, vision, birth weight and height in siblings of the twins and behavioral problems, based on a selection of items from the Devereux Child Behavior Rating Scale (Van Beijsterveldt et al., 2001).

At ages 7, 10 and 12 years items concerned with the development of childhood psychopathology are again rated by both parents (CBCL4-18; Achenbach, 1991; Connors et al., 1998a). The syndromes overlap with the CBCL2-3

**Table 4B**

Behavior Problems Assessed at Different Ages

CBCL (3 year)	Devereux (5 year)	CBCL (7,10,12 year)	TRF (7,10,12 year)
Aggressive	Aggressive	Aggressive	Aggressive
Overactive	Distraction	Attention problems	Attention problems
Oppositional	Inability to delay Physical coordination	Rule breaking	Rule breaking
Withdrawn	Timid with peer	Withdrawn	Withdrawn
Anxiety	Anxiety	Anxious/depressed	Anxious/depressed
Somatic problems		Somatic problems	Sleep problems
Sleep problems	Independence	Social problems Thought problems	Social problems Thought problems

Note: Child Behavior Checklists (CBCL) and Devereux rating list are sent to both fathers and mothers of twins; Teacher Rating Forms (TRF) to teachers.

to a large extent. Teacher data (TRF) are for the first time collected at age 7, and subsequently at ages 10 and 12. Teachers complete the TRF (Achenbach, 1991b) and the Conners' Teaching Rating Scale (Conners et al., 1998b). When the twins are 12 years of age, their parents and teachers are also asked about the child's CITO score (the score on an educational achievement test which is taken by most 12-year-old children in The Netherlands).

Most questionnaires to parents of young twins contain a series of items on parental beliefs about zygosity, twin resemblance for physical characteristics and confusion of twins by parents, family members and strangers. In combination with information on zygosity based on blood group and DNA polymorphisms, these data have been used to assign zygosity to same-sex twins (Rietveld et al., 2000).

In the families of adolescent and adult twins, longitudinal survey studies of lifestyle, personality and psychopathology have been conducted in 1991, 1993, 1995, 1997, and 2000. A new survey is scheduled for 2002. A more detailed list of data, which are collected in the older participants, is given in Table 5. For twins and siblings in the register who are older than 25 years of age the SES distribution is very similar to the distribution of the parents of young twins: 22,1% is of low, 43,8% of middle and 34,1% of high SES. Smoking behavior of twins (Boomsma et al., 1994; Koopmans et al., 1999) is comparable to that in the Dutch population. The religious background of the participating families of adolescent and adult twins also is comparable to the Dutch population (Boomsma et al., 1999).

### Research Projects: Other Studies

In addition to participating in the longitudinal questionnaire studies, smaller samples of twins are invited to take part in experimental and laboratory studies of brain development, cognition, neuropsychological functioning, cardiovascular risk and anxious depression.

For example, studies of cognition have been carried out in several age groups. Table 6 gives an overview of the heritability of IQ in individuals between 5 and 50 years of age (Boomsma, 1997; Boomsma & Van Baal, 1998; Posthuma et al., 2001; Rietveld et al., 2000; Rijdsdijk & Boomsma, 1997). There is a clear trend for heritability of IQ to increase between ages 5 and 16 years (from 25% to 62%). Between ages 18 and 50 years, the heritability is high (around 80%) and does not seem to change. For a large number of twins participating in the cognitive studies, there are also data on brain function (e.g., Posthuma et al., 2001; Van Beijsterveldt et al., 1998; Van Baal et al., 2001a, 2001b), brain structure (Posthuma et al., 2002) and other cognitive endophenotypes such as reaction times, inspection time and working memory.

Studies on cardiovascular risk factors have been carried out in adolescent twins and their parents, and in adult twins. The high heritabilities that were found for some cardiovascular variables (Snieder et al., 1997) have led to a genome search for quantitative trait loci (QTLs) influencing quantitative levels of blood pressure, lipids and lipoproteins (Beekman et al., 2001).

The search for QTLs influencing cardiovascular risk is carried out in an unselected sample of DZ twins. In contrast the data on anxiety and depression and on nicotine dependence collected over a 10-year period, have been used to select families with sibling pairs concordant or discordant for anxious depression (Boomsma et al., 2000) or for nicotine dependence and DNA samples have been collected in these families, or are currently being collected. These DAN samples are used to measure DNA markers for linkage and association analyses.

A final molecular genetics project does not use data from DZ twins and their siblings, but looks at pairs of sisters who both had DZ twins in an attempt to find the genes that influence familial DZ twinning (Montgomery et al., 2000).

The availability of large numbers of twins and family members who have phenotyped longitudinally, is not only valuable for genetic epidemiological studies, but is also increasingly valuable for molecular genetic studies.

**Table 5**

Measures in Adolescent and Adult Twins, Parents, Siblings and Spouses

	91	93	95	97	00
<b>Personality / Psychopathology Inventories</b>					
Beck Depression Inventory	—	x	—	x	—
Neuroticism, Extraversion					
Somatic Anxiety, Lie (ABV)	x	x	—	x	x
Trait Anxiety (Spielberger)	x	x	—	x	x
Trait Anger (Spielberger)	x	x	—	—	—
Disinhibition, Boredom					
Susceptibility, Experience-,					
Thrill & Adventure Seeking	x	x	—	x	x
Type-A (JAS)	x	—	—	—	—
Cognitive Failures	x	—	—	—	—
Phobia	—	—	—	x	x
Burn-out	—	—	—	—	x
Traumatic Life-Events	—	—	—	—	x
Anxious/Depressed,					
Withdrawn, Rule breaking,					
Aggression, Somatic-, Social-,					
Thought-, Attention					
Problems (YASR)	x	—	x	x	x
<b>Demographics / Health / Lifestyle</b>					
Demographics	x	x	x	x	x
Zygosity (twins)	x	x	x	x	x
Religion	x	x	x	x	x
Health / BMI	x	x	x	x	x
Smoking / Alcohol / Drugs	x	x	x	x	x
Coffee / Tea	x	x	—	—	x
Nutrition	x	—	—	—	—
Education	x	x	x	x	x
Profession	x	x	x	x	x
Sports participation / level	x	x	x	x	x
Leisure Activities	x	x	x	—	x
Talents	x	—	—	—	—

**Table 6**

Twin Correlations and Heritabilities for Total IQ in Dutch Twins (and Sibs) Aged 5 to 50 Years: Correlations and Number of Twin Pairs for Each Age by Zygosity Group, Followed by Maximum-likelihood Estimates for Heritability ( $h^2$ ) and the Percentage of Variance Explained by Common Family Environment ( $c^2$ ).

Age	MZF	DZF	MZM	DZM	DOS	MZ	DZ	$h^2$	$c^2$	Test
5 yr	.78	.73	.77	.53	.64	.77	.62	.26	.50	RAKIT
<i>N</i>	46	37	42	43	39	88	119			
7 yr	.74	.59	.61	.40	.53	.68	.50	.40	.29	RAKIT
<i>N</i>	41	34	37	41	38	78	113			
10 yr	.83	.47	.75	.66	.47	.82	.50	.54	.26	RAKIT
<i>N</i>	43	37	38	41	37	81	115			
12 yr	.85	.66	.85	.58	.31	.85	.54	.60	.25	WISC
<i>N</i>	43	37	36	39	35	79	111			
16 yr	.50	.35	.77	.24	.42	.66	.39	.62	—	RAVEN
<i>N</i>	46	36	37	31	44	83	111			
18 yr	.84	.44	.86	.19	.24	.85	.30	.82	—	WAIS
<i>N</i>	46	36	37	31	44	83	111			
26 yr	.87	.36	.88	.64	.45	.88	.45	.88	—	WAIS-3R
<i>N</i>	29	97	25	76	110	54	283			
50 yr	.85	.52	.85	.27	.42	.85	.42	.85	—	WAIS-3R
<i>N</i>	26	85	22	62	95	48	242			

Note: MZF = monozygotic females, DZF = dizygotic females, MZM = monozygotic males, DZM = dizygotic males, DOS = dizygotic opposite sex, MZ/DZ = all mono- and dizygotic pairs.

Siblings included as DZ twins at 26 and 50 years; for adult twins 26 ( $SD$  4.2) and 50 ( $SD$  7.5) are average ages (as opposed to the other age groups in which all Ss are of the same age).

The 5-, 7-, 10- and 12-year-olds participate in a longitudinal study; the 16- and 18-year-old twins also come from a longitudinal project; some of these twins are included in the 26 year group. Significance of  $h^2$  is .03 at age 5; of  $c^2$  it is .06 at age 7, .09 at age 10 and .08 at 12 years.

## Footnote

- 1 The web site address for the Netherlands Twin Register is <http://www.psy.vu.nl/ntr>

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