

## Article

# The Netherlands Twin Register: Longitudinal Research Based on Twin and Twin-Family Designs

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## Abstract

The Netherlands Twin Register (NTR) is a national register in which twins, multiples and their parents, siblings, spouses and other family members participate. Here we describe the NTR resources that were created from more than 30 years of data collections; the development and maintenance of the newly developed database systems, and the possibilities these resources create for future research. Since the early 1980s, the NTR has enrolled around 120,000 twins and a roughly equal number of their relatives. The majority of twin families have participated in survey studies, and subsamples took part in biomaterial collection (e.g., DNA) and dedicated projects, for example, for neuropsychological, biomarker and behavioral traits. The recruitment into the NTR is all inclusive without any restrictions on enrollment. These resources — the longitudinal phenotyping, the extended pedigree structures and the multigeneration genotyping — allow for future twin-family research that will contribute to gene discovery, causality modeling, and studies of genetic and cultural inheritance.

**Keywords:** Twins; multigeneration pedigree; zygosity; biobank; endophenotyping; longitudinal phenotyping

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For more than three decades, the Netherlands Twin Register (NTR) has invited twins and their family members to take part in research studies of development, health and behavior. The recruitment has mostly included two-generation extended families. The NTR has created a rich resource of data to assess genetic and nongenetic (cultural) transmission, and to contribute to gene-finding studies for complex human phenotypes. In this article we describe, by necessity in broad strokes, our recruitment procedures, the number of participants, the phenotyping through surveys and other data collection methods, and the collection of genotype and other omics data. We also summarize how we are constructing a data warehouse to archive the numerous datasets and make them optimally accessible for researchers, even when data were collected two or more decades ago.

This paper is incremental in a series of earlier papers on NTR resources (Bartels et al., 2007; Boomsma et al., 1992, 2002, 2006, 2008; Hoekstra et al., 2004; van Beijsterveldt et al., 2013; Willemsen et al., 2010, 2013), and aims to give an overview

from the first twin-family studies in the 1980s, via the formal establishment of the Young and Adult NTR and the NTR biobank to our latest projects. Participants who register with NTR are invited for research projects entailing surveys, interviews and other phenotyping studies, biomarker and omics research projects, and are also asked for permission for record linkage. Since the first twin-family studies were carried out for cardiovascular risk factors in the early 1980s on several hundreds of participants, data have been collected on tens of thousands of children and adults, and across a wide range of phenotypes.

## Recruitment

Starting around 1986, NTR systematically started to approach parents to register their newborn twins in the Young NTR (YNTR) with the help of a commercial 'birth felicitation' service. Additional recruitment of newborn twins and triplets and their parents is done with the support of the Dutch Society of Parents of Multiples (Nederlandse Vereniging van Ouders van Meerlingen: NVOM; <https://www.nvom.nl>). After parents return an informed consent form, the family is registered with the NTR and mothers receive a first survey with items on pre- and perinatal variables. When the twins are 2 years of age, a survey on growth, health, developmental and motor milestones is sent. At ages 3, 5, 7, 9/10 and 12 years, both parents receive additional surveys addressing a broader set of behavioral and health-related

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**Table 1.** Number of registered participants as of 2019 by role and age group

	0–11 years	12–17 years	18+	Total
Multiples	27,474	20,949	73,734	122,157
Siblings	628	2338	11,938	14,904
Parents	0	0	114,278	114,278
Spouses	0	0	2616	2616
Offspring	280	28	778	1086
Other	1	4	683	688
Total	28,383	23,319	204,027	255,729 <sup>a</sup>

<sup>a</sup>Due to unknown age, 56 participants are not listed in this table.

traits. At ages 7, 9/10 and 12 years, we also ask parents for permission to approach the twins' teachers. At that same moment, we ask if there are any additional siblings in the family who are in elementary school and whose teachers may be approached. From age 14 onward, adolescent twins and their siblings are invited to provide self-reports (after their parents give permission), largely substituting parental for self-report form this age onward. Once they become adults, the YNTR twins can opt to join the Adult NTR (ANTR).

Apart from the YNTR influx, ANTR twins have been recruited through city councils, starting in 1985 with smaller numbers of twin families in and around Amsterdam. In 1990–1993, a nationwide appeal to city councils for permission to approach adolescent and young adult twins and multiples and their parents was successful and created the basis for the ANTR, with the first surveys collected in 1991. Additional means of recruitment are the yearly NTR newsletter *Twinfo*, the NTR website and national events organized by, for example, the Dutch Twin Society. Surveys have been sent to ANTR participants about every 2–3 years since 1991. In total, 13 large-scale surveys have been collected. In contrast to the YNTR, where data collection is based on birth cohort and age, the ANTR data collection is independent of age and includes all adult participants.

As a result of the recruitment efforts described above, the NTR has registered about 52% of all Dutch twin-pairs born between 1987 and 2017, based on data from Statistics Netherlands (CBS; <https://www.cbs.nl/nl-nl/nieuws/2016/39/minder-tweelingen-geboren>). Most of these twin-pairs were recruited shortly after birth. The birth cohorts between 1970 and 1981 are also well represented (29%). For other birth cohorts, coverage is considerably lower due to lack of systematic recruitment.

### Number of Participants

Using the twin-pair as the probands, recruitment in both ANTR and YNTR initially included parents, and in later years also siblings, spouses and offspring of twins. This has resulted in a database with roughly equal proportions of participants who are and who are not twins. Over the years, a total of 280,569 participants were registered at the NTR, 231,088 of whom are still contactable. The total group of participants includes 255,785 members of twin families and 24,784 participants contributing as a teacher of a child registered at the NTR. Table 1 provides information about the age distribution in twins, parents, siblings and other groups of participants. Until now, the NTR has collected phenotypic data on 70% of all registered twin-family participants (i.e., excluding teachers). This includes self-report data, survey data supplied by parents or teachers, and data collected in dedicated projects such as cardiovascular and magnetic resonance imaging (MRI) studies.

**Table 2.** Number of MZ and DZ twins by sex and age group

	0–11 years	12–17 years	18+	Total
MZ male	2126	2402	8780	13,308
DZ male	1972	2946	9540	14,458
MZ female	2056	2544	11,974	16,574
DZ female	1892	2642	9794	14,328
DZ opposite sex	8908	6904	22,112	37,932
Total	16,954	17,438	62,200	96,600 <sup>a</sup>

<sup>a</sup>Numbers represent individuals, not pairs, and only include twins, not higher order multiples. Zygosity is not known for all registered twins; however, in the case of an opposite sex pair, no survey or DNA information is needed to determine zygosity. Therefore, this group is relatively large compared to the other zygosity groups.

### Zygosity

Most NTR surveys contain a standard series of items for zygosity assessment, addressing several aspects of physical resemblance and the degree to which the twins are confused by parents, other relatives and strangers. In YNTR and ANTR data, a series of discriminant analyses was performed to assess the accuracy of zygosity classification based on survey items, using information from blood group and DNA polymorphisms as the index of true zygosity. In the YNTR, the accuracy of classification at age 3 years and older was 97.2%, based on a set of 10 questionnaire items. In the ANTR, where a set of eight items is used, the correspondence was 95.9%. For more details on the zygosity classification, see the Appendix. Table 2 shows the numbers of monozygotic (MZ) and dizygotic (DZ) twins within the NTR, stratified by age and sex.

### YNTR and ANTR: Longitudinal Survey Collection

Tables 3 and 4 summarize the numbers of twins and family members who have participated in YNTR and ANTR survey research as of April 2019. Separate columns indicate for how many participants the phenotypic data from the surveys were enriched by DNA collection and genomewide single nucleotide polymorphism (SNP) genotyping.

Longitudinal data collection by surveys began by paper and pencil and has now been replaced by online survey collection. The YNTR surveys focus on growth and physical development, wellbeing, health status and health behaviors, and behavioral and emotional problems as rated by parents and at ages 7, 9/10 and 12 years by teachers. Over time, surveys have been kept as similar as possible, relying on standardized inventories such as the Achenbach System of Empirically Based Assessment (ASEBA; Achenbach et al., 2017) which allows for multi-informant assessment at ages 1½ through 90+ years (i.e., parents, teacher and self-assessment), and the Conners' Teacher Rating Scales-Revised for teachers (Conners et al., 1998b), the Conners' Parent Rating Scale-Revised (Conners et al., 1998a) for parents of young twins and the Conners' Adult ADHD Rating Scales for adults (Conners et al., 1999). New instruments have been added in recent years, e.g. the Child Behavior Questionnaire (CBQ; Rothbart et al., 2001), for assessment of behavioral control at age 5 by parents, and the Social Skills Rating System (SSRS; Gresham & Elliott, 1990) for assessment of social competence at ages 7, 9 and 12 by teachers and wellbeing (Cantril Ladder; Cantril, 1965). Other topics addressed in the YNTR surveys include the use

**Table 3.** Numbers of available surveys per type of rater for young multiples and siblings (YNTR)

Survey	Mother (N twins)	Father (N twins)	Teacher (N twins/triplets/sibs)	Self-report (N twins/triplets/sibs)	Total N with genomewide SNP data (children)	Total N with genomewide SNP data (mother/father)	Birth cohort
Age 1	67,400				7392	2527/1871	1986–2014
Age 2	47,816	890 <sup>a</sup>			6449	2105/1596	1986–2013
Age 3	39,400	26,672			6382 <sup>b</sup>	2066/1586	1986–2009
Age 5	35,988 <sup>c</sup>		1115/0/176		6275	2002/1554	1986–2010
Age 6/7	25,776	18,426	15,124/184/1463		5562 <sup>b</sup> /6418 <sup>d</sup>	1615/1234	1986–2007
Age 9/10	23,170	15,962	14,196/148/1356		5715 <sup>b</sup> /6240 <sup>d</sup>	1712/1357	1986–2008
Age 12	20,584	13,752	11,122/168/595	1714/4/126	4818 <sup>b</sup> /5169 <sup>d</sup>	1303/987	1986–2006
Age 14–18 (pilot)				581/0/141	431		1987–1988
Age 14				9761/200/1922	2691		1990–1999
Age 16				6966/132/1625	2748		1988–1996
Age 18				1778/32/313	798		1986–1988

YNTR = Young Netherlands Twin Register.

<sup>a</sup>Data collection in fathers started in 2016.<sup>b</sup>Mother or father report.<sup>c</sup>Survey includes a father report.<sup>d</sup>Mother or father or teacher report.**Table 4.** Numbers of completed surveys for adult participants (multiples, siblings, parents, spouses and offspring)

ANTR							Total N	Total N with genomewide SNP data
Survey (start year)	Multiples	Siblings	Parents	Spouses	Offspring	Total N		
Survey 1 (1991)	3374	4	3013	0	0	6391	2610	
Survey 2 (1993)	4235	8	3662	0	0	7905	3736	
Survey 3 (1995)	3429	1481	3234	1	0	8145	3938	
Survey 4 (1997)	3233	1506	4	0	0	4743	2749	
Survey 5 (2000)	4619	1470	28	675	0	6792	3771	
Survey 6 (2002)	4538	1452	2851	1442	1	10,285	5862	
Survey 7 (2004)	6760	1663	4075	969	335	13,804	7689	
Survey 0.1 (2008) <sup>a</sup>	1216	52	156	157	120	1711	235	
Survey 8 (2009)	10,176	2142	6928	810	288	20,368	9587	
Survey 9 (2011)	5870	1269	4011	596	191	11,943	6639	
Survey 10 (2013)	9419	1854	7205	924	270	19,696	8629	
Survey 11 (2015)	8320	1639	5729	666	249	16,619	8310	
Survey 0.2 (2015) <sup>a</sup>	886	105	627	30	38	1690	442	
Survey 12 (2015)	10,604	1761	8692	503	216	21,787	9439	
YNTR/ANTR						Total N	Total N with genomewide SNP data	
Mothers of twins survey						20,140	3135	

ANTR = Adult Netherlands Twin Register.

<sup>a</sup>The surveys numbered 0.x run over longer periods and are sent to new ANTR participants; survey 0.2 is currently ongoing.

of assisted reproduction techniques in the first survey, motor milestones at age 2 years, and academic performance and sharing the same school and/or classroom at ages 7 through 12 years.

ANTR surveys have focused on demographics, health and health behaviors, personality and wellbeing, as well as a broad range of behavioral and emotional problems as assessed by

ASEBA, Beck Depression Inventory, Personality Assessment Inventory — Borderline (PAI-BOR) scale, NEO Five-Factor Inventory and other instruments (Achenbach et al., 2017; Bouman et al., 1985; Costa & McCrae, 1989; Morey, 2003). ANTR survey studies all address the general theme of individual differences of mental and physical health but often have a specific

**Table 5.** Number of children with longitudinal parent and teacher reports (YNTR)

Number of reports per child	1	2	3	4	5	6	7
Mother report	14,398	11,354	8996	9160	8244	8674	9448
Father report	16,000	8510	7590	4978			
Teacher report	19,324	9017	2746				

YNTR = Young Netherlands Twin Register.

**Table 6.** Number of participants with longitudinal self-reports (YNTR and ANTR)

N self-reports YNTR	1	2	3	4	5	6	7	8	9	10	11	12
Multiples	8103	3978	2138	1315	1216	966	420	146	23	7	6	3
Siblings	1633	796	405	283	218	159	61	15	4	3	0	0
Parents	6153	2115	1417	1088	855	185	28	4	0	1	0	0
Total	15,889	6889	3960	2686	2289	1310	509	165	27	11	6	3
N self-reports ANTR	1	2	3	4	5	6	7	8	9	10	11	12
Multiples	3520	1692	1553	1307	1081	1100	705	626	422	387	290	153
Siblings	957	647	426	310	243	221	186	162	163	78	1	1
Parents	2442	1258	969	556	458	379	356	294	393	255	2	0
Spouses	825	519	314	210	171	165	149	30	0	0	0	0
Offspring	184	125	92	80	72	53	0	0	0	0	0	0
Other	13	6	9	3	3	0	0	0	0	0	0	0
Total	7941	4247	3363	2466	2028	1918	1396	1112	978	720	293	154

YNTR = Young Netherlands Twin Register; ANTR = Adult Netherlands Twin Register.

Note: The top part of the table, ('YNTR') includes self-reports from YNTR twins and siblings at ages 14, 16 and 18 years, as well as self-reports by these participants after they enrolled in the ANTR as adults. In the bottom part of the table, ('ANTR') are the participants who registered as adolescents between 1991 and 1995, and adults.

focus, for example, survey 9 was focused on pain, survey 11 mainly included a large food preference assessment and survey 12 was developed for online phenotyping of major depressive disorder (BIONIC; Bot *et al.*, 2017). At the same time, we try to maintain continuity for topics such as substance use, sports and exercise behavior, personality and wellbeing, in order to facilitate longitudinal comparisons. Tables 5 and 6 show the longitudinal survey participation in YNTR and ANTR: the number of participations of any single individual across studies. Please note that these tables are snapshots of the current numbers, as data collection and data entry are ongoing processes. A distinction is made between self-reports (YNTR and ANTR) and parent and teacher reports (YNTR only).

Triplets are a unique group of multiples within the NTR. The NTR has registered a total of 871 complete sets of triplets. ANTR triplets complete the regular surveys sent to all NTR participants. In the YNTR, dedicated triplet surveys are used, which follow the line of the twin data collection.

### Nonsurvey-Based Phenotyping

In addition to participating in the longitudinal surveys, subgroups of twins and their family members are invited to take part in dedicated studies of, for example, autonomic nervous system functioning and cardiovascular risk, brain structure and function, neurocognitive test performance and IQ, or (an)aerobic fitness and daily physical activity. A second approach to phenotyping has been to conduct large-scale telephone interviews. These

included monthly interviews on motor development and psychopathology with mothers about their young twins (Langendonk *et al.*, 2007) and interviews to assess clinical diagnoses of ADHD (Derks *et al.*, 2008). In ANTR participants, a series of Composite International Diagnostic Interviews (Wittchen, 1994) were conducted to obtain DSM-IV diagnoses of mental disorders, including major depressive disorder (Middeldorp *et al.*, 2005).

A subset of these studies is presented in Table 7. The table indicates the type of data collected per study, categorized into broad domains and a primary reference for each study. The same study domains are listed in Table 8, where the overlap with other data collections is shown. Selection for participation in nonsurvey studies may be random, but can also be based on survey data, demographic information, or the availability of, for example, omics data. Examples are studies that included twins from specific age or birth cohorts (Bartels *et al.*, 2002b; Hoekstra *et al.*, 2007; Polderman, Posthuma *et al.*, 2006; Rietveld *et al.*, 2003; van Baal *et al.*, 1998), twins with an older sibling (van Leeuwen *et al.*, 2009; van Soelen *et al.*, 2010) or twin-pairs concordant or discordant for a specific phenotype, such as anaesthesia (Bartels *et al.*, 2009), attention problems (Derks *et al.*, 2008; Polderman, Posthuma *et al.*, 2006; van't Ent *et al.*, 2007), depression (de Geus *et al.*, 2007), obsessive-compulsive symptoms (den Braber *et al.*, 2008) and body mass index (Doornweerd *et al.*, 2016).

The academic achievement tests administered by Dutch schools are another important resource for phenotype information. Data are obtained from a standardized test administered in Grade 6, and the Pupil Monitoring System (PMS; Grades 1–6). In addition,

**Table 7.** Overview of nonsurvey projects

Project	Main reference	Study population (age)	N	Primary research domain					Specifics
				CV	EEG	IMG	NC/IQ	PA	
Stress and CV 1	Boomsma et al. (1996)	Adolescent twins (14–21) + parents	642	x					Lab: rest and stress measures, incl. BP
Stress and CV 2	Snieder et al. (1997)	Adult twins	427	x					Lab: rest and stress measures, incl. BP
Diabetes study	Simonis-Bik et al. (2008)	Adult twins and sibs	189	x					Diabetes, $\beta$ -cell function, glucose tolerance, BP
NTR-VUAMS1	de Geus et al. (2005)	Adult twins and sibs	458	x					24 h ambulatory assessment incl. BP
NTR-VUAMS2	Kupper et al. (2004)	Adult twins and sibs	428	x					24 h ambulatory assessment incl. BP
NTR-VUAMS3	Neijts et al. (2015)	Adult twins and sibs	593	x					24 h ambulatory assessment incl. BP, short stress test
EEG & Cognition	Van Baal et al. (1998)	Young twins (5/7)	418		x		x		EEG, ERP, IQ, HR
Cognition <sup>a</sup>	Rietveld et al. (2003)	Young twins (10)	394				x		IQ
Cognition <sup>a</sup>	Bartels et al. (2002a)	Young twins (12)	364				x		IQ, hormones
Cognition and hormones <sup>b</sup>	Hoekstra et al. (2007)	Adolescent twins and sibs (18)	508	x			x		IQ, cardiovascular measures, growth, hormones
WebCNP	Swagerman et al. (2016)	Twins, sibs, parents (10–86)	1110	x			x		Computerized Neuropsychological Testing System
Attention and IQ	Groot et al. (2004)	Young twins (5)	474				x		IQ, ANT, behavior surveys
Attention and IQ	Polderman, Gosso et al. (2006)	Young twins (12) and sibs	409	x			x		IQ, ANT, hormones, behavior surveys
Attention and IQ	Huguet et al. (2017)	Adolescent twins (17/18)	332	x			x		Raven advanced, NEO
EEG adolescents	van Beijsterveldt et al. (1996)	Adolescent twins (16/18)	426		x		x		EEG, ERP, IQ, HR, NCV
EEG adults	Posthuma et al. (2005)	Adult twins and sibs (25–55)	785		x		x		EEG, cognitive tasks, IQ, HR, BP
Brainscale1	van Leeuwen et al. (2008)	Young twins (9) and sibs	330			x	x		MRI, IQ, neurocognitive tests, hormones
Brainscale2	van Soelen et al. (2012)	Young twins (12) and sibs	261			x	x		MRI, IQ, neurocognitive tests, hormones, behavior surveys
Brainscale3	Teeuw et al. (2019)	Adolescent twins (17) and sibs	246	x		x	x		MRI, IQ, neurocognitive tests, hormones, behavior surveys
MRI	Baaré et al. (2001)	Adult twins and siblings	160			x			MRI
MRI OCD	den Braber et al. (2010)	Adult twins (conc./disc. OCD)	209			x			MRI, The Padua Inventory Abbreviated
MRI DOS	den Braber et al. (2013)	Adult opposite sex twins	40			x			MRI
MRI MDD	de Geus et al. 2007	Adult twins (conc./disc. MDD)	65			x			MRI
MRI BMI	Doornweerd et al. (2018)	Adult twins (disc. BMI)	92			x			MRI, interview, anthropometric data, BP
MRI/PET	Konijnenberg et al. (2018)	Elderly MZ twin-pairs (60+)	205			x			MRI, PET, neuropsychological tests, physical examination, carotid artery ultrasound, BP
MRI ADHD	van't Ent et al. (2009)	Young MZ twins (conc./disc. ADHD)	74			x			MRI, Stroop task, flanker task

(Continued)

Table 7. (Continued)

Project	Main reference	Study population (age)	N	Primary research domain					Specifics
				CV	EEG	IMG	NC/IQ	PA	
MEG	van't Ent et al. (2010)	Young adult twins	74			x			MEG-recorded somatosensory-evoked fields
Fitness	Schutte et al. (2016)	Adolescent twins and sibs	481					x	Lab experiment, surveys and interview
Physical activity	Schutte et al. (2019)	Young adult twins	774					x	7 days triaxial accelerometer (hip)
CIDI	Middeldorp et al. (2007)	Twins, sibs and parents	1438					x	CIDI
ADHD clinical study	Derks et al. (2008)	Young twins (mother report)	1116					x	Diagnostic Interview Schedule for Children
Motor milestones	Langendonk et al. (2007)	Young twins (0.5–2)	470					x	Telephone interview motor milestones

CV = cardiovascular/(ambulatory) autonomic nervous system; EEG = EEG/ERP; IMG = imaging; NC/IQ = neurocognitive testing/IQ; PA = fitness/physical exercise; INT = interview; BP = blood pressure; HR = heart rate; ANT = Amsterdam Neuropsychological Tasks; NCV = nerve conduction velocity; conc. = concordant; disc. = discordant; OCD = obsessive-compulsive disorder; MDD = major depressive disorder; BMI = body mass index; CIDI = composite international diagnostic interview; NTR-VUAMS = NTR Vrije Universiteit Ambulatory Monitoring System; ERP = Event related potential; WebCNP = The (web-based) University of Pennsylvania Computerized Neuropsychological Test Battery; MRI = magnetic resonance imaging; DOS = dizygotic opposite sex twins; PET = Positron emission tomography; ADHD = Attention deficit hyperactivity disorder; MEG = Magnetoencephalography.

<sup>a</sup>EEG & Cognition sample.

<sup>b</sup>EEG & Cognition sample plus additional participants.

the regular surveys to parents and teachers during the elementary school years include items on children's school grades (van Bergen et al., 2018). NTR started to collect information on standardized educational achievement tests in 2000 (Bartels et al., 2002b). The test is administered nationwide over a period of 3 days in the last grade of elementary school at ~12 year (Eindtoets Basisonderwijs 2002; www.cito.nl). Initially, these scores were obtained from teachers. Because results become available near the very end of the school year, we later asked parents and twins to report these scores. The correlation between scores provided by parents and twins is 0.96 ( $N = 3314$ ) and between teachers and twins the correlation is 0.93 ( $N = 922$ ).

In 2008, the NTR started to collect PMS data, providing information on academic achievement throughout elementary school. Since 2014/2015, all elementary schools are required to use a PMS. The PMS consists of grade appropriate tests, independent of teaching methods, which are administered at fixed time points (i.e., beginning, halfway and/or end of the school year) in each grade. The PMS has tests on all important educational domains, for example, reading, mathematics and spelling (de Zeeuw et al., 2016). Currently, around one-third of the teachers who fill out a survey also send us a student report with the results from the PMS tests of the current and previous grades.

## Twinning

A phenotype of special interest for which nearly every NTR participant is informative is the trait 'being a twin' or 'being a parent of twins'. Among the earliest NTR projects was a study on the inheritance of twinning. In the early 1990s, telephone interviews were held with the mothers of DZ twins (Meulemans et al., 1996) to collect information on twinning in their extended pedigree for segregation analyses, and to assess zygosity in the proband twins and any additional twins in the pedigree. These pedigree data formed the basis for several blood collection projects in 'twinning pedigrees', initially with the purpose of linkage studies in sister-pairs (Painter et al., 2010)

and later for genomewide association studies (Mbarek et al., 2016). A large survey study collected information on risk factors for twinning in both mothers from the YNTR/ANTR and the ANTR (Hoekstra et al., 2008, 2010). Table 9 describes the characteristics of the pedigrees within NTR, including special groups of participants relevant to twinning research, such as families with more than one twin-pair.

## Record Linkage/Cross-Referencing the NTR Database to Other National Databases

Starting with ANTR survey 6, participants were asked for permission for record linkage with other databases in the Netherlands. Across surveys, on average 81% of participants agreed with record linkage, 14% did not agree and 5% did not answer the question. Subjects who did not agree or did not answer the question are excluded from record linkage.

Projects that have made use of record linkage include a large study of chorionicity (van Beijsterveldt et al., 2016). Information on chorionicity through record linkage as well as access to tissue samples was available from in the Pathological Anatomy National Automatic Archive (PALGA), a nationwide network and register of histopathology and cytopathology database and biobank. Other projects that made use of the PALGA database include studies on triplets and a study of cervix smear abnormalities (Lamb et al., 2012; van Beijsterveldt et al., 2016; Vink et al., 2011). Valuable information can also be obtained by linking the NTR databases to national population-based registers such as the cancer registration in the Netherlands. A facility has been created in collaboration with Statistics Netherlands (<https://www.cbs.nl/en-gb>), to combine data from multiple databases for analysis. This 'ODISSEI Data Facility (ODF)' ([www.odissei-data.nl](http://www.odissei-data.nl)) enables, for example, the analysis of NTR genotype information with phenotype information from Statistics Netherlands.

## Harmonization and Data Repository

Given the large amount of data collected over the last decades, and the diversity in research topics and data collections that have

**Table 8.** Sample sizes biological and nonsurvey data, and overlap with surveys

	Total N	YNTR survey	ANTR survey
<i>Survey data</i>			
YNTR survey <sup>a</sup>	147,360	147,360	24,072
ANTR survey	52,039	24,072	52,039
<i>Biological samples</i>			
DNA (blood or buccal)	29,138	15,984	19,397
Genomewide SNPs	26,146	14,511	17,704
DNA methylation whole blood 450K	3025	355	2942
DNA methylation buccal 450K	22	22	21
DNA methylation buccal 850K	1526	1526	58
DNA sequencing whole blood	349	9	330
RNA expression U219 array	3370	284	3231
RNA sequencing	1606	256	1561
RNA sequencing LPS-stimulated samples	390	13	380
Metabolomics in serum or plasma	6485	621	6311
Metabolomics in urine	1564	1564	156
Microbiome	568	187	561
Hair sample	222	7	222
<i>Biomarkers<sup>b</sup></i>			
Cholesterol, HDL, LDL, triglycerides	10,860	1328	9729
Immune parameters	10,115	1318	9240
Cortisol (in saliva)	2980	1303	2535
<i>Non-survey projects<sup>c</sup></i>			
Cardiovascular/ambulatory ANS	3589	1132	3141
EEG	1520	437	1319
Imaging (MRI, MEG, PET)	893	220	830
Neurocognitive/IQ	3726	2081	2955
Physical activity	1255	831	1139
Interview	3013	1597	1994
<i>Other</i>			
Blood pressure	3548	263	3238

YNTR = Young Netherlands Twin Register; ANTR = Adult Netherlands Twin Register; LPS = Lipopolysaccharide; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ANS = autonomic nervous system; EEG = electroencephalography; MRI = magnetic resonance imaging; MEG = Magnetoencephalography; PET = Positron emission tomography; IQ = Intelligence Quotient.

<sup>a</sup>Count includes children whose parents and teachers reported about them as well as parents reporting about themselves and each other.

<sup>b</sup>Limited selection; not all measured biomarkers are listed.

<sup>c</sup>See also Table 7.

evolved over the years, the need arose for the NTR to harmonize the data collected across multiple projects and to bring all these resources together. This should ensure that the large amount of data collected since the beginning of the NTR becomes accessible under Findable, Accessible, Interoperable, Reusable (FAIR) data stewardship principles and can be optimally used in research on behavior and health. For this purpose, we are building a new data repository for the phenotypic data, as well as an online data showcase, in collaboration with IT company The Hyve (<https://thehyve.nl/>).

## NTR Data Showcase

The NTR Data Showcase was designed to facilitate data requests from NTR and affiliated researchers, by allowing them to see what data are available in the NTR. The NTR Data Showcase does not contain the actual research data, only metadata. Users can search items by keyword, project or research domain. The research domains are based on the domain structure as used by Maelstrom Research (<https://www.maelstrom-research.org>), supplemented with NTR-specific subdomains (e.g., twin-specific variables such as zygosity) where necessary. Researchers can view properties of the available variables, including the item name, keywords, a brief description of the measure, the original phrasing of survey questions in Dutch and English, the data type (string/numeric/categorical), labels and subject counts. Once a set of variables is identified, the items can be collected in a 'shopping cart' and exported to a JSON (JavaScript Object Notation) file, to become part of a data request.

## NTR Data Repository

The phenotypic research data are stored in a TranSMART data warehouse (<https://transmartfoundation.org/>), which can be accessed by the NTR data managers using the Glowing Bear application (<https://glowingbear.app/>). All data for a participant, which may have been collected in many separate research projects, are stored under the same numeric person ID. TranSMART also implements the use of 'concepts': if the same variable was collected in many different projects (e.g., a certain personality questionnaire that was included in many different projects), and was measured and coded in the same way across all projects, these variables can be linked to a single concept, after which all of them can be extracted at once with a simple query. Once a researcher has applied for a dataset and the request has been approved by the NTR data access committee, a JSON file exported from the NTR Data Showcase can be uploaded in the Glowing Bear application by a data manager to extract the requested dataset. In addition to importing these JSON files, which contain the variable selection, cohort selections can be specified by the data managers, based on, for example, participation in a particular research project, person characteristics (e.g., age or sex), availability of genotype data or family relationships (e.g., 'all individuals who are a mother of twins'). The queries used to extract a specific dataset can be stored and reused in case an update of the dataset is necessary.

Contact information of all participants is stored in the Person Administration of the Netherlands Twin Register (PANTR) database (Boomsma et al., 2008). This person-oriented database stores contact details and family relationships between persons (Boomsma et al., 2018) in a secure environment that is not connected to the internet. In the PANTR database, participants are stored under an administrative ID which is different from the IDs used for research data (e.g., phenotype and omics data), so that personal and research data remain separated.

## NTR Biomarker and Omics Projects

The first two large-scale NTR studies ( $N = 936$ ) on cardiovascular risk factors in twin families included assessment of biomarkers such as lipid levels, plant sterols, Lp(a) glucose and other biomarkers (Kempen et al., 1991; Snieder et al., 1997). Of note, 20 years later nearly half of the participants in the early 90s projects ( $N = 510$ ) also took part in the NTR biobank studies as described below, creating possibilities for longitudinal biomarker studies.

**Table 9.** The NTR Pedigree as of April 2019

NTR Pedigree information	Individuals	Complete pairs <sup>a</sup>
Total <i>N</i>	255,785	
Multiples	123,157 <sup>b</sup>	60,406
Twins — total	119,040	59,520
Twins — MZ	29,882	14,941
Twins — DZ-same sex	28,786	14,393
Twins — DZ-opposite sex	37,932	18,966
Twins — unknown zygosity	22,440	11,220
Triplets	2613	871
Quadruplets	52	13
Quintuplets	10	2
<i>Parental generation</i>		
Parents	115,702 (113,923 multiple parents)	
Multiples who are parents of multiples	1662 individuals (730 families <sup>c</sup> )	
<i>Families where twins/sibs have offspring</i>		
Families with sib pairs who both have offspring <sup>d</sup>	469	
Families with twin/multiple pairs who both have offspring <sup>d</sup>	136 <sup>e</sup>	
Families with sib pairs who both have twin/multiple offspring	378	
Families with twin-pairs who both have twin/multiple offspring	12 <sup>f</sup>	
<i>Family size</i>		
Number of extended families	59,694 (933 <i>n</i> = 1 families)	
Typical family size ( <i>n</i> )	4 (43,374 families)	
Largest family ( <i>n</i> )	39 (1 family)	
Typical number of twin-pairs/sets of multiples in a family ( <i>n</i> )	1 (56,468 families)	
Families with more than one multiple	2549 families	
Largest number of twin-pairs/sets of multiples in a family ( <i>n</i> )	7 (1 family)	
Typical number of generations ( <i>n</i> )	2 (57,167 families)	
Largest number of generations ( <i>n</i> )	4 (145 families)	

NTR = Netherlands Twin Register.

<sup>a</sup>A pair indicates a complete set of multiples, that is, two twins, three triplets, four quadruplets or five quintuplets.

<sup>b</sup>Number includes 1442 multiples who are registered without a co-twin. These are often individuals who registered because they are a parent or spouse of a twin. They are not included in counts of twins, triplets, and so on.

<sup>c</sup>Families where both parent and offspring are multiples, and the parent's co-twin is also registered.

<sup>d</sup>This includes multiple offspring as well as singleton offspring.

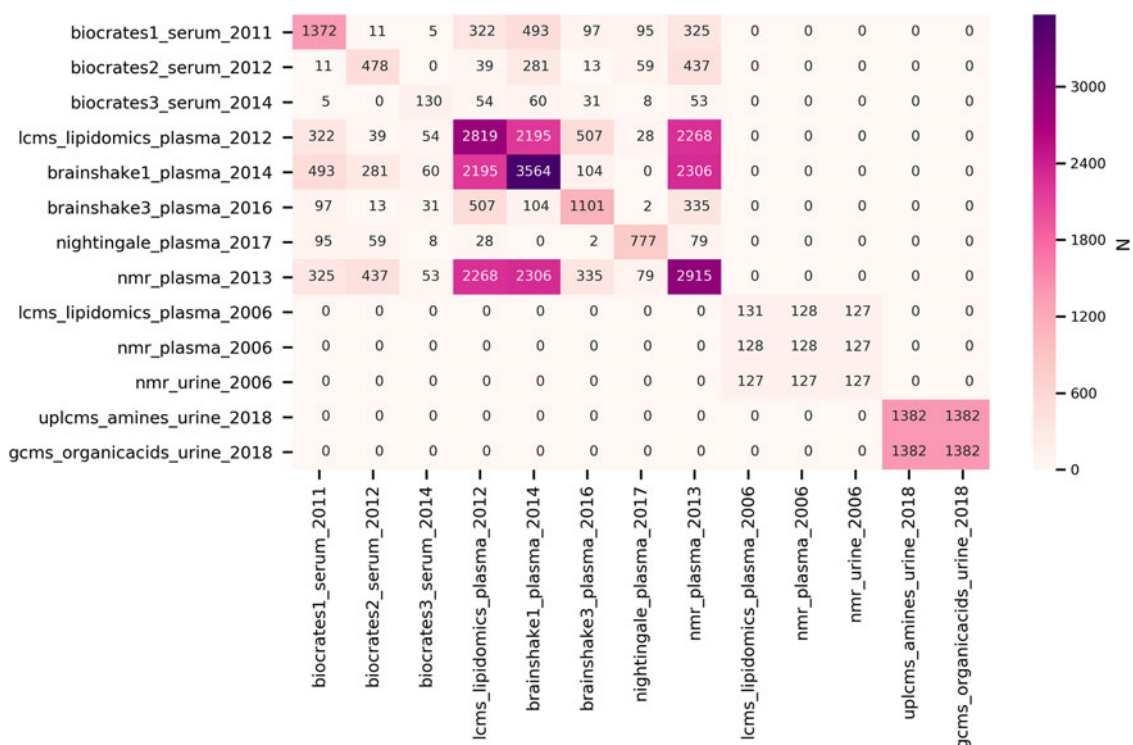
<sup>e</sup>Number includes 21 families in which one or more siblings and both members of a twin-pair all have offspring.

<sup>f</sup>Number includes two families in which one or more siblings and both members of a twin-pair all have twin/multiple offspring.

In 2004, the NTR started a large biological sample collection in nearly 10,000 participants to create a resource for future omics and biomarker studies as well as the creation of immortalized cell lines: the NTR biobank (Willemsen *et al.*, 2010). We first carried out a large pilot project (Hoekstra *et al.*, 2004) in which we established the feasibility of, for example, drawing blood from fertile women on day 2–4 of the menstrual cycle, or in their pill-free week. Between 2004 and 2008, a group of adult participants from NTR research projects was invited for the NTR biobank study, of which 69% participated. A comparison of nonparticipants with participants showed that nonparticipants were less often female (52% vs. 64%), were slightly younger (average birth year 1963 vs. 1960 in men, 1964 vs. 1962 in the women) and had less often received higher education (27% vs. 39% in men, 22% vs. 33% in women). During a morning home visit, eight tubes of fasting blood

and a morning urine sample were collected along with phenotypic information on health, medication use, body composition and smoking. A second project between 2008 and 2010 added collection of stool samples in mainly MZ twin-pairs (Sirota *et al.*, 2015). In both projects, extensive cell counts were realized in fresh blood samples (Lin *et al.*, 2017). These data on cell counts serve as important covariates in transcriptomics (e.g., Jansen *et al.*, 2014) and epigenomics studies (van Dongen *et al.*, 2016). Biomarkers assessed for all NTR biobank participants include lipids, glucose, insulin, HbA1c, liver enzymes (van Beek *et al.*, 2015), C-reactive protein, fibrinogen, interleukin (IL)-6, TNF- $\alpha$  and soluble IL-6 receptor (van Dongen *et al.*, 2015). Other biomarkers collected in (large) subsets of samples include cotinine in blood (Bot *et al.*, 2013), telomere length (Broer *et al.*, 2013) and microbiome datasets (Finnicum *et al.*, 2018).





**Fig. 1.** Subject overlap between the NTR metabolomics datasets. The datasets are denoted by metabolomics platform, sample material/tissue and year of measurement. Note that the values on the diagonal represent the sample size of the individual datasets. All data assessed before 2018 are from adults, all data assessed in 2018 are from 9-year-olds.

DNA collection constituted an important element of the biobanking project, continuing the effort in many earlier NTR studies that also collected DNA samples from twin families, for instance, as part of linkage studies into depression and anxiety (Boomsma et al., 2000), asthma (Wu et al., 2010) and twinning pedigrees (Painter et al., 2010), or as part of the nonsurvey projects listed in Table 7. DNA collection continues for participants who provided phenotype information, for example, as part of a large transatlantic collaboration with the Avera Institute of Human Genetics (Ehli et al., 2017). DNA has been collected from whole blood for the majority of the ANTR subjects and by buccal swabs in the majority of YNTR families. Our buccal swab procedures yield high-quality DNA amenable to multiple omics arrays (Meulenbelt et al., 1995; Min et al., 2006). For twins, we aim to always collect buccal DNA, often in addition to DNA from whole blood.

In the DNA and RNA resources in the NTR biobank, substantial progress was made in assessing epigenetic, transcriptomics and metabolomics data. Epigenetic and RNA expression data have been measured in MZ and DZ twin-pairs (van Dongen et al., 2016; Wright et al., 2014). Analyses of these data, making use of the classical twin design, showed significant heritability for both the transcriptome and the epigenome. Figure 1 summarizes the metabolomics data that have been measured in blood for adults and in urine for 9-year-old twins. In adults, for over 5100 individuals, metabolomics data have been realized from 4 metabolomics platforms: the Nightingale Health 1H-NMR platform (previously referred to as Brainshake), a UPLC-MS Lipidomics platform, the LUMC 1H-NMR platform and the Biocrates Absolute-IDQTM p150 platform (Draisma et al., 2013; Hagenbeek et al., 2019). In children, metabolomics data were assessed across three platforms for amines, organic acids and steroid hormones. The NTR

biobank, combined with the extensive phenotypic information available within the NTR, provides a valuable resource for the study of genetic influences on individual differences in mental and physical health (see Table 8).

### Communication with Participants

We observe that families that do not participate at one of more waves of data collection often participate again in subsequent waves ('drop-in'). Reasons for this include true changes in motivation and availability, but also participants moving to new addresses, which may be traced only after data collection for the current wave is closed. To bolster the motivation to stay involved, we have a website (<http://www.tweelingenregister.org/>) and a yearly newsletter, *Twinfo*, that is mailed to all families and also published on the website (<http://www.tweelingenregister.org/twinfo/>). Two social media outlets are Facebook (<https://www.facebook.com/NederlandsTweelingenRegister/>) and Twitter ([https://twitter.com/NTR\\_VU](https://twitter.com/NTR_VU); <https://twitter.com/NTRscience>). In addition, within a national collaboration of cohorts/biobanks (<https://www.bbmri.nl/>), the NTR has pioneered building a portal for participants. This 'My NTR' portal (<https://www.mijnntr.nl/>) provides selected personalized feedback on data provided by the participants (e.g., personality profile), in addition to general information on the outcomes of the research participants have contributed to (Bovenberg et al., 2016).

### Discussion

We conclude this paper on the NTR by considering some exciting developments which we believe illustrate the continued value of twin-family data across multiple domains of research.

### ***Polygenic Risk Scores to Detect the Effects of Nurture***

Two papers published last year (Bates et al., 2018; Kong et al., 2018) detailed an innovative method to measure intergenerational transmission in families where whole-genome genotyping is available in both parents and in their offspring. Parents transmit only one copy of each chromosome (~50% of genome) to each offspring, but shape an environment for their offspring that is influenced by their own genome. The aggregate effect of an individual's genome on a trait can be summarized in a polygenic risk score (PRS). The PRS for the transmitted and nontransmitted parental genotypes can be separately associated with outcome traits. The nontransmitted genotypic effects on offspring's outcome traits can be interpreted as a direct measure of parenting behaviors and environments, unconfounded by the genomes transmitted to the offspring. This is also referred to as 'genetic nurturing'. Large genomewide association studies are required to estimate the transmitted and nontransmitted PRS, but these are increasingly becoming available. The second requirement is that offspring and parents are all genotyped (however, note that the phenotype information is only needed in the offspring). The NTR has nearly always aimed to collect DNA samples in extended twin families, that is, the index twins, their parents and siblings. In these NTR families, it is possible to construct PRS based on the transmitted and nontransmitted parental genomes for all traits with published summary statistics. These have now been calculated for 5902 offspring within NTR. We recently employed this design to seek evidence of genetic nurturing in adults' educational attainment and children's academic achievement and ADHD (de Zeeuw et al., 2019).

### ***Phenotypic Reference Panels***

When phenotyping for a trait, or a series of traits, has been carried out with different instruments, data integration methods for phenotype harmonization across instruments require or can greatly benefit from datasets where the different instruments have been administered to the same subjects (van Den Berg et al., 2014). In YNTR, we collected such a reference panel for phenotype data to harmonize multiple measures of aggression and other behavioral problems in schoolchildren as part of the ACTION project (Aggression in Children: Unraveling gene-environment interplay to inform Treatment and interventION strategies Consortium; <http://www.action-euproject.eu/>; Bartels et al., 2018; Luningham et al., 2017). Throughout 2016, the Child Behavior Check List (CBCL; Achenbach et al., 2017), the complete Strengths and Difficulties Questionnaire (Goodman, 1997) plus a selection of A-TAC items (Autism-Tics, ADHD, and other Comorbidities inventory; Larson et al., 2010) were completed by both parents of twins born between September 2005 and October 2008. Hendriks et al. (2019) analyzed aggression data across these different instruments. Although agreement with respect to phenotypic diagnoses and correlations between continuous scores were moderate, genetic correlations indicated that the underlying construct of childhood aggression was consistent across measures.

### ***Children-of-Twins Design/Numbers for Exceptional Families***

Infant twins who were enrolled by their parents and for whom we have data since infancy are now becoming parents themselves. The NTR has started collecting multigenerational data in this unique group. Other interesting groups include multigenerational families where both parent and offspring are twins, as well as families where two siblings are both parents of twins, that is, the twin offspring are cousins. These data enable application of

the Children-of-Twins (CoT) design (D'Onofrio et al., 2003), to disentangle the genetic and environmental transmission from parents to their offspring and give more insight into the causal role of the home environment on the development of children. The CoT design is based on the fact that offspring of MZ twins are as genetically related to the co-twin of their parent (uncle/aunt) as they are to their own parent, but only share a home environment with their own parents. For more details on the composition of the pedigrees within NTR, see Table 9.

### ***Mendelian Randomization Combined With the Twin Design***

One of the greatest challenges in the social, behavioral and medical sciences is to determine the causality underlying associations between a (putative) risk factor and a behavioral or disease outcome. Simple regression analysis cannot properly address this question. The correlation between two variables can reflect reverse causation or unobserved factors that influence both (confounding). Mendelian randomization (MR) is a method based on instrumental variable analysis in econometrics that can be used to estimate the causal effect of a risk factor on a behavioral or health outcome, provided that genetic variants are known that influence the risk factor (Davey Smith & Hemani, 2014; Palmer et al., 2011; Pierce et al., 2010). However, many genetic variants derived from genomewide association (GWA) meta-analyses have small effects, and so, when used as instrumental variables, render MR liable to weak instrument bias. PRS have the advantage of larger effects, but may be characterized by horizontal pleiotropy, which violates a central assumption of MR (Pickrell et al., 2016). We recently developed the *MR-DoC twin model*, which allows one to test causal hypotheses and to obtain unbiased estimates of the causal effect given strong but pleiotropic instruments (like PRS) by controlling for genetic and environmental influences common to the outcome and exposure (Minica et al., 2018). This method greatly increases the value of the large twin-family cohorts like the NTR and many others in this special issue.

In conclusion, we believe that twin studies and twin-family studies will continue to blossom into the next decades, and with the new data repository and data showcase under development, the NTR should be well prepared for the future.

Detailed background information on the NTR may also be found on the website <http://www.tweelingenregister.org/>. This website originated as an information resource for participants and also includes the nearly complete list of publications.

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## Appendix. Zygosity

### Methods

To determine the accuracy of zygosity determination in children aged 0–16 years, the agreement between zygosity based on a blood group or DNA tests and zygosity based on discriminant analysis of survey items on resemblance was investigated. In earlier research, prediction accuracy of zygosity was around 93% in children

**Table A1.** Zygosity prediction accuracy of YNTR questionnaire items compared to DNA

YNTR questionnaire	Correctly classified (%)
Overall (multiple questionnaires)	96.8
Age 1	78.9
Age 2	93.8
Age 3 mother	94.9
Age 3 father	94.1
Age 5 mother	97.5
Age 7 mother	95.9
Age 7 father	96.5
Age 10 mother	96.1
Age 10 father	96.3
Age 12 mother	96.6
Age 12 father	95.9
Age 14 self	91.8
Age 16 self	94.9

(Rietveld *et al.*, 2000). Since then, an additional item was added to the NTR questionnaires and substantially more DNA data have become available. We reevaluated zygosity assignments, based on surveys obtained at ages 3, 5, 7, 10, 12, 14 and 16 years. These contain 10 zygosity items about resemblance between the twins (6 items about physical similarities and 4 items about confusion by parents and others). At ages 3–12 years, mothers and fathers and at ages 14–16 years the twins themselves filled out the questions. In adults aged 18–99 years, multiple surveys contain eight zygosity items (five items about resemblance and three items about confusion by parents and others). Here, the questions were answered by the twins.

Because knowledge of the result of a zygosity test may affect responses, we only included data from same-sex twins whose survey information had been completed before they, or their parents, received the results of the DNA tests. For the children, this resulted in a sample of 5776 twins and for adults in a sample of 3512 twins.

For children, the data were randomly divided into a training set (60%) and a testing set (40%). In the training set, linear discriminant analysis was applied to survey data from each informant (mother, father and self) and ages 3–16 years. Linear discriminant analysis determines the axes that maximize the separation of different classes, in our case MZ and DZ. This analysis generated a linear function of the weighted sum of the items, in which the weights were optimized to distinguish between MZ and DZ twin-pairs. The outcomes then were applied to the data from the testing set ( $N = 2260$ ). If multiple surveys were available (either multiple ages, multiple informants or both), the most frequently predicted zygosity was chosen as the assigned zygosity. If an equal number of MZ and DZ outcomes was observed, the mean probability of being MZ determined by the discriminant analyses was used (MZ: probability  $> .5$  and DZ: probability  $< .5$ ). To determine the accuracy of our zygosity classification by the questionnaire items, we determined the proportion in which the true zygosity and the assigned zygosity corresponded. If there was no childhood survey after age 2 years, the item about resemblance from the questionnaire collected at age 2 years was used (MZ: 'yes, but barely different' or 'yes, but well distinguishable' and DZ: 'no, not a lot' or 'no, not at all'). If there also was no information at age 2 years, the item from the questionnaire at age 1 year was used (MZ: 'MZ twins' and DZ: 'DZ twins').

**Table A2.** Ranks for the predictive value of the zygosity items in YNTR and ANTR surveys

Item	Age 3 mother-rated	Age 3 father-rated	Age 12 mother-rated	Age 12 father-rated	Age 16 self-rated	Adults self-rated
Facial appearance	7	3	6	5	5	7
Hair color	3	8	5	4	4	5
Face color	4	6	4	6	7	6
Eye color	5	4	7	7	2	4
Hair structure	2	2	3	3	3	-
Spitting image	10	9	9	9	1	1
Confusion by mom/dad	8	5	10	8	6	8
Confusion by other family members	1	1	2	2	8	3
Confusion by strangers	6	7	1	1	9	2
Confusion on photos	9	10	8	10	-	-

YNTR = Young Netherlands Twin Register; ANTR = Adult Netherlands Twin Register.  
Note: 1 = most predictive item; 10 = least predictive item.

For adults a similar scheme was used: 60% of the data were randomly assigned to be in the training set and 40% to be in the testing set. The first available survey with zygosity items was analyzed. The outcomes of the linear discriminant analysis of the training set were used to predict zygosity in the testing set ( $N = 1362$ ).

## Results

In children, results indicate that the entire procedure of zygosity prediction by the 10-item zygosity questionnaires correctly classified zygosity in 96.8%. Prediction accuracy for all YNTR questionnaires separately can be found in Table A1. For 95.8% of this sample, data on the 10-item zygosity list included in the questionnaires at ages 3–16 years were available. If only these participants were considered, the accuracy of zygosity classification was 97.2%. In the remaining sample, only surveys at earlier ages, when twins are 1 and 2 years, were available. These were completed by mothers and included one question at age 1 year ('According to you, the twins are', with answer options 'DZ twins' and 'MZ twins') and one question at age 2 years ('Do the children resemble each other' — with answer options 'yes, they are barely different', 'yes, but well distinguishable', 'no, not a lot' and 'no, not at all'). If only the questionnaire at age 2 years is used, zygosity prediction is accurate in 93.8% of the cases. When only the questionnaire at age 1 year is used, prediction accuracy of zygosity drops to 78.9%. Participants with data at age 1 or 2 years only are underrepresented in the sample, ( $N = 96$ ).

In adults, zygosity prediction based on survey items was accurate in 95.9%. In Table A2, the results of the linear discriminant analysis are displayed. In adults, the item that distinguishes best between MZ and DZ twins was 'Were you each other's spitting image as children?', whereas the item 'Did mother and father mix you up when you were young' does not distinguish well. In parent-reports on children, confusion by other family members than parents was a well-distinguishing item.