Twin Research and Human Genetics
Volume 16 ▌ Number 1 ▌ pp. 252–267 ▌ © The Authors 2012 ▌ doi:10.1017/thg.2012.118

The Young Netherlands Twin Register (YNTR): Longitudinal Twin and Family Studies in Over 70,000 Children

Catharina E. M. van Beijsterveldt,[1] Maria Groen-Blokhuis,[1] Jouke Jan Hottenga,[1] Sanja Franić,[1] James J. Hudziak,[2] Diane Lamb,[1] Charlotte Huppertz,[1] Eveline de Zeeuw,[1] Michel Nivard,[1] Nienke Schutte,[1] Suzanne Swagerman,[1] Tina Glasner,[1] Michelle van Fulpen,[1] Cyrina Brouwer,[1] Therese Stroet,[1] Dustin Nowotny,[3,4] Erik A. Ehli,[3] Gareth E. Davies,[3,4] Paul Scheet,[5] Jacob F. Orlebeke,[1] Kees-Jan Kan,[1] Dirk Smit,[1] Conor V. Dolan,[1] Christel M. Middeldorp,[1] Eco J. C. de Geus,[1] Meike Bartels,[1] and Dorret I. Boomsma[1]

[1]Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands
[2]Department of Psychiatry and Medicine (Division of Human Genetics), Center for Children, Youth and Families, College of Medicine, University of Vermont, Burlington, VT, USA
[3]Avera Institute for Human Genetics, Sioux Falls, SD, USA
[4]Department of Psychiatry, University of South Dakota, Vermillion, SD, USA
[5]Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

The Netherlands Twin Register (NTR) began in 1987 with data collection in twins and their families, including families with newborn twins and triplets. Twenty-five years later, the NTR has collected at least one survey for 70,784 children, born after 1985. For the majority of twins, longitudinal data collection has been done by age-specific surveys. Shortly after giving birth, mothers receive a first survey with items on pregnancy and birth. At age 2, a survey on growth and achievement of milestones is sent. At ages 3, 7, 9/10, and 12 parents and teachers receive a series of surveys that are targeted at the development of emotional and behavior problems. From age 14 years onward, adolescent twins and their siblings report on their behavior problems, health, and lifestyle. When the twins are 18 years and older, parents are also invited to take part in survey studies. In sub-groups of different ages, in-depth phenotyping was done for IQ, electroencephalography , MRI, growth, hormones, neuropsychological assessments, and cardiovascular measures. DNA and biological samples have also been collected and large numbers of twin pairs and parents have been genotyped for zygosity by either micro-satellites or sets of short nucleotide polymorphisms and repeat polymorphisms in candidate genes. Subject recruitment and data collection is still ongoing and the longitudinal database is growing. Data collection by record linkage in the Netherlands is beginning and we expect these combined longitudinal data to provide increased insights into the genetic etiology of development of mental and physical health in children and adolescents.

■ **Keywords:** newborn twins, longitudinal data collection, development, cognition, psychopathology

The contribution of the Netherlands Twin Register (NTR) to this special issue has a festive touch: it coincides with the 25th anniversary of the NTR. In 1987, the NTR started by recruiting twins and their families, with the main goal to investigate individual differences in mental and physical health. In children, the focus was on understanding the factors contributing to normal and abnormal development of cognitive function, psychopathology, physical, and psychological well-being. These aims have not changed, but the scientific approaches have. Advances in behavioral and epidemiological genetic research have led to new types of research questions (Martin et al., 1997; van Dongen et al., 2012), which in turn require new types of data and data collection such as genotype data

RECEIVED 20 September 2012; ACCEPTED 12 October 2012. First published online 28 November 2012.

ADDRESS FOR CORRESPONDENCE: C. E. M. van Beijsterveldt, Department of Biological Psychology, VU University Amsterdam, Van der Boechorststraat 1, 1081 BT Amsterdam, The Netherlands. E-mail: t.van.beijsterveldt@vu.nl

or data from extended families. Advances in computer technology have led to changes in database management, and increased computer use and Internet availability in the Netherlands have led to the development of Internet surveys and increased sharing of information with participants through social media (Stroo et al., 2012). These developments have changed the NTR from a twin- to a twin family-based register (Boomsma et al., 2006a) with a dynamic database that handles the pedigree information and biological and social relations among participants (Boomsma et al., 2008a), and with data collection from participants as well as from teachers (de Zeeuw et al., 2012) and increasingly through record linkage with national databases (Lamb et al., 2012).

The NTR consists of two large sub-groups: families with young and adolescent twins (YNTR) and families with adult twins (ANTR). They are distinguished by recruitment and ways of data collection. Twins participating in the YNTR are registered at birth by their parents, and are recruited with the help of a commercial baby organization that visits parents of newborns and the 'Dutch association for parents of multiples' (NVOM; Bartels et al., 2007; Boomsma et al., 2002). The data collection for the YNTR and ANTR differs, simply because as long as twins are under age 14 their parents and their teachers are the main informants, whereas at later ages, the data are collected through self-reports. When participants in the YNTR reach the age of 18 years they are invited to take part in the ANTR. This article will give an overview of 25-year data collection of the YNTR. The data collection in the ANTR is described in detail in this issue (Willemsen et al., 2012).

YNTR Phenotyping: Longitudinal Survey Studies

The main phenotype assessment in the YNTR is through collection of surveys. Since 1987, newborn twins enter the register and surveys are collected that are conditional on the age of the children. We approach parents of twins and triplets and other multiples when the children are registered, which is usually a few weeks or months after birth, and at ages 2, 3, 5, 7, 9/10, and 12. At ages 7 through 12, the teachers of twins also provide information, and after age 12 we start to collect data from twins themselves. Because of its longitudinal character, our approach is to keep the content of the surveys fairly similar. On the other hand, new developments, insights, and trends require updates and extensions of items in the surveys. In the following paragraph, we describe the main content of the surveys of the YNTR and the most important innovations. In the past 25 years, we have collected data from 70,784 children (i.e., for all children there is at least one survey available). A survey could be filled in by a parent, a teacher, or by the children themselves. In Figure 1 an overview is given of the collected surveys for twins born between 1986 and 2011. The data collection for triplets is slightly different and is described in separate paragraph.

Parental Reports for Twins

1. After registration of newborn twins, which is before the age of 12 months for 89% of the twins, the first survey, sent to mothers, focuses on conception (spontaneous or otherwise), information from parents themselves (demographics, height, weight), pregnancy (e.g., duration, smoking and drinking behaviors of both parents during pregnancy), birth, and health of the children shortly after birth. For the majority of the twins, both parents were born in the Netherlands (90.7%). For the remaining families at least one parent was born in another Western county (3.9%), or in Turkey and Morocco (1.2%), and the other 4.2% were born in other non-Western countries. In 2005, all mothers registered with the NTR were mailed a two-page survey (referred as 'NTR maternal survey'; Hoekstra et al., 2008). In this short survey we collected information on familial twinning and the twin pregnancy. Nearly 70% returned the survey (18,292 YNTR mothers, including 1,537 YNTR mothers who had never participated before in survey studies). For birth cohorts 2005 and later, we combined the original first survey and the 'NTR maternal survey' into a renewed survey-1. An online version of this survey-1 is in preparation.
2. The survey at age 2 years (sent to mothers only) focuses on motor development, growth data as obtained from the Dutch National Health Services, breastfeeding, and handedness of twins and relatives. From birth cohort 2005 onwards, new items on well-being and temperament were added.
3. At age 3, a survey is sent to both parents. The survey includes the CBCL 1.5-5 (Achenbach & Rescorla, 2000), items on zygosity, health, child care, demographic characteristics, including religious background and parental education and occupation. From 2008 onwards, additional items on well-being, leisure activities, and sport activities of parents are included. To test if we could increase the response rate of the fathers, for three birth cohorts the father and the mother surveys were put into one booklet. This had no effect on the response rate of the fathers. If the mother returned the surveys, 75% of the fathers also completed a survey.
4. The survey at age 5 is sent to mothers and includes a two-page section for fathers of twins about behavior problems. The content of this survey remained unchanged throughout the years and includes items on child care, growth, zygosity, toilet training, asthma and eczema, fine motor coordination, language development, and problem behaviors as assessed by short version of the Devereux Child Behavior checklist (DCB; Spivack and Spotts, 1966; van Beijsterveldt et al., 2004).

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

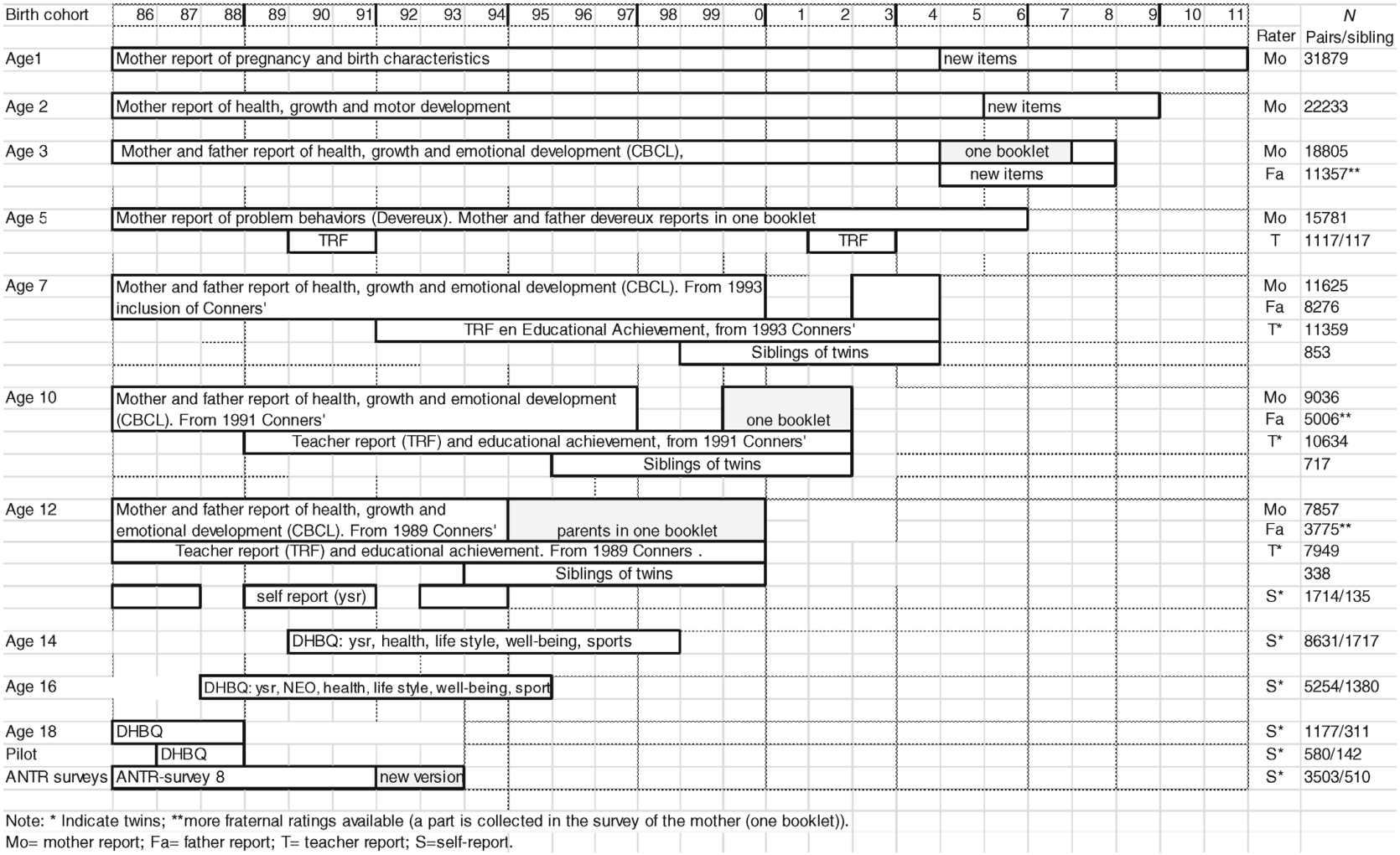

FIGURE 1

Overview of the YNTR data collection in twins born between 1986 and 2011.

TABLE 1
Overview of the Returned Surveys for Triplets (Born Between 1986 and 2011)

	N triplets*
Age 1	393
Age 2	39
Combination age 1/2	273
CBCL1.5-5	60 mother 35 father
CBCL6-12	217 mother 160 father
DHBQ self-report	295 triplets/32 siblings
Teacher report	256 triplets

Note: *Number indicates triplet families (except for teacher and DHBQ self-reports).

5. At ages, 7, 9/10, and 12 years, information on the twins is collected from both parents and targets the development of emotional and behavior problems, as assessed by the Child Behavior Checklist 6-18 (CBCL; Achenbach & Rescorla, 2001). From 2000 onwards, the Conners' Parent Rating Scale — Revised (CPRS-R) was also included (Conners et al., 1998; Conners, 2001). In addition, these surveys included items on zygosity, medical conditions, vaccinations, tonsillectomy (from 2005), demographic characteristics, growth, sport and leisure activity of both children and parents, and additional items on well-being (from 2008). Due to the transition to a new administration database (Boomsma et al., 2008a) and a shortage of staff, no surveys for ages 7 and 10 years were collected between 2008 and the last quarter of 2009.

Data Collection for Triplets

The NTR has registered 677 complete YNTR and 43 complete ANTR sets of triplets. Table 1 gives a summary of the data collection in triplets. The content of the triplet surveys is to a large extent similar to the content of the twin surveys. The survey at age 1 includes items on pregnancy, gestational age, smoking and alcohol consumption during pregnancy, and mode of delivery. Survey 2 includes information on breastfeeding, motor development, and growth. In 2008, a combination of survey 1 and 2 was sent to mothers of all triplets. The survey was sent to 535 mothers and was returned by 264 mothers. Since 2009, the combined survey 1 and 2 served as the first survey for newly registered triplets, and the number of returned surveys is now 273. The Child Behavior Checklist (Achenbach & Rescorla, 2000, 2001) was sent to parents of triplets aged 3 through 12 years, together with a short general questionnaire on parental and triplet characteristics (e.g., parental employment and religion, triplet school achievements and health).

Teacher Reports for Twins, Triplets, and Siblings of Twins and Triplets

To assess cognitive development and behavior and emotional problems of children in classroom settings, the NTR collects information from teachers for twins at ages 7, 9/10, and 12 from 1998 onwards. In addition, teacher data were collected in a few sub-groups of 5-year-olds (Polderman et al., 2006b). First, parents of twins are asked if they consent to approaching the teacher of their children. In this step, we collect the name and addresses of teachers and schools. Initially, collection of the parental consent was by mail, but for 2 years the consents have been gathered via online forms. If parents agree, one or more teachers, depending on whether children go to the same class/school, are approached to fill in a survey. The teacher surveys include the Teacher Report Form from the Achenbach System of Empirically Based Assessment (ASEBA) instruments (Achenbach & Rescorla, 2001), items on adaptive functioning, school achievement (de Zeeuw et al., 2012), and the Conners' Teacher Rating Scales Revised, short version CTSR-R (Conners, 2001). Recently, items were added on classroom size and on bullying and being bullied. From 2007 onward, teachers of any additional children in the family have been approached too (regardless of the age of the siblings, as long as they are in elementary school). After 14 years of data collection from teachers, the average response rate is around 65% but has decreased in recent years (Polderman et al., 2011). In 2012, we started with an Internet version of the teacher survey. When the survey is not completed, we send a paper-and-pencil version and a link to the Internet survey with a reminder letter. Also, to compare the willingness to respond to a paper-and-pencil survey and an Internet survey, we randomly split the group of teachers of a cohort of 9-year-old twins into two parts. Half of the teachers received a paper-and-pencil questionnaire and the other half were requested to fill in the same questionnaire online. The response rate seemed not to be overly affected by the survey approach. Initial results (before a reminder was sent) showed a response rate of 39.4% for the paper-and-pencil version and 47% for the online version. Starting in 2009, the teacher survey has also been collected for triplets (Lamb et al., 2011). After obtaining parental consent to approach the children's teachers (62% responded positively), we obtained a teacher questionnaire for 256 triplets.

Self-Report Surveys From Twins, Triplets, and Their Siblings

In 2005 we started with the data collection of self-report surveys in YNTR twins, triplets aged 14, 16, and 18 years, and in their siblings. All multiples are asked to complete the Dutch Health and Behavior Questionnaire (DHBQ), which focuses on emotional and behavior problems assessed by the Youth Self-Report (Achenbach, 1991), subjective well-being (Bartels & Boomsma, 2009a), lifestyle (Geels et al., 2011), exercise behavior (van der Aa, et al., 2010a), sedentary

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

TABLE 2

Overview of Longitudinal Data Collection in Parents of Twins, By Self-Reports (After Age 12), and of Teachers of Young Twins and Their Siblings

Parental report	Age 1	x	x	x	x	x	X	x
	Age 2	x	x	x	x	x	X	x
	Age 3	x	x	x	x	x	X	
	Age 5	x	x	x	x	x		
	Age 7	x	x	x	x			
	Age 10	x	x	x				
	Age 12	x	x					
	>12*	x						
		5,845	8,680	10,934	16,778	25,030	33,844	44,230
Self-report^	Age 14	x	x					
	Age 16	x	x	x				
	Age 18+	x		x				
		1,134	3,628	2,259				
Teacher report^	Age 7	x	x					
	Age 10	x	x	x				
	Age 12	x		x				
		1,721	4,778	3,733				

Note: >12* indicates that at least one self-report is returned (age >12 years); ^number indicates individuals (twins and siblings for self- and teacher report); age 18+ indicates returned survey could be YNTR-DHBQ or ANTR-Q8.

behavior (van der Aa et al., 2012), and family functioning (van der Aa et al., 2010b). Before inviting the twins and their siblings, parents are contacted to ask for permission to send their children a self-report survey. We also ask the parents to register non-twin siblings of the twins. Initially the parental consents were sent out at the same time as the surveys. From 2007 onwards, the parental consent form has been obtained separately. In addition, starting in 2007 the survey is sent only to 14- and 16-year-olds, and partly different elements of the DHBQ are used at ages 14 and 16. For instance, the 60-item short form of the Neuroticism Extraversion Openness Five Factor Inventory (NEO-FFI) was included for 16-year-olds (Costa & McCrae, 1992). At the same time, the DHBQ became available online. The invitation letter contained a hyperlink, which could be used to log in to the online survey. All participants who did not complete the online survey after 2 months were reminded by e-mail. The overall response rate is 47%, and is higher for the online than for the paper-and-pencil version. In 2009, twins and triplets from birth cohorts 1986 to 1991 and their siblings were invited to participate in the online ANTR survey-8 (see Willemsen et al., 2012). From 2010 onwards, 18-year-old twins and their siblings were invited to fill in a new version of the ANTR survey-8 and these cohorts are also invited for ANTR survey 9. Self-report data have additionally been collected in sub-samples of twins and siblings aged 11 and 12 who took part in studies of cognition, attention problems, or brain imaging (Bartels et al., 2002a; Polderman et al., 2006a; van Soelen et al., 2012). A total of 1,714 twins and 127 of their siblings who enrolled in these studies have returned a self-report survey. The collection of data for the adolescent triplets is the same as in twins. In total, 295 triplets and 32 siblings filled in the DHBQ survey.

Longitudinal Survey Data

The structure of the age-specific data collection is presented in Table 2. Because not all cohorts have yet reached a specific age, the numbers for these cohorts in the final column are still growing for children aged 2–18. Up until 2012, 31,879 mothers of 63,758 twins returned the first survey. Of these twins, there are still 56,724 actively registered (89%). Participants can leave the register at any time and if possible, we ask them for their reason to do so. The following reasons were given: unwilling to participate any longer (77.8%), disabled/ill (0.8%), deceased (2.9%), temporarily inactive (0.4%), and moved to a new address without notifying the NTR (18.1%). We will try to trace this last group and, if found, they usually become active participants again.

The procedure for the collection of surveys is as follows: Parents who did not return surveys within 2–4 months receive a written reminder. If resources allow, persistent non-responders are contacted by phone. From 2010 onwards, we sent a reminder by e-mail if an e-mail address is known. In Figure 1 and in Table 2 it can be seen that with increasing ages the sample sizes decrease. The primary reason is of course that not all of the birth cohorts have yet reached a specific age (see also Figure 1) and due to attrition of participating families (Bartels et al., 2007). In Table 3, an overview is given of the response rate for each age. Here, rates are averaged over all years. In recent years the participation rate has decreased. It is also important to note that a substantial proportion of the families can miss participation for a particular survey, but that these families often participate again in later surveys. In Table 3 an overview is given of the number families with such 'partial' participation. Sometimes families do not want to participate at a specific age, and they agree to be approached at a later age, but the majority of these families have moved to a new address that was

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

TABLE 3

Overview of Response Rate and Response Pattern of Parental Surveys for Twins at Ages 1 to 18 for Birth Cohorts 1986–2011

	Response rate*	Total of returned surveys	Two of the two preceding surveys returned	One of the two preceding surveys returned	None of the two preceding surveys returned
Q1	–	31,879			
Q2	71%/76%	22,233		21,969^	
Q3**	63%/67%	19,110	16,835	2,089	186
Q5	57%/61%	15,781	12,856	2,276	649
Q6/7**	53%/55%	11,812	9,083	2,081	648
Q9/10**	47%/50%	9,157	6,127	2,241	789
Q12**	42%/46%	7,951	5,153	2,021	777
DHBQ	47%	11,873	7,161	2,712	2,000

Note: *The first number refer to the absolute response rate; the second is conditional on least one survey returned. ^For survey 2 (Q1), there is only one previous survey.**Indicates that at least one parental survey is returned.

retraced only after a survey had been closed. Low response rates may reflect bias and threaten the generalizability of the results. A number of studies have explicitly addressed potential bias. Using the 'NTR maternal survey' data collected by Hoekstra et al. (2010), we compared characteristics of twin mothers between responders and non-responders. Non-response was associated with offspring zygosity (more monozygotic (MZ) mothers participated), maternal smoking during pregnancy, being younger at twin birth, and having a lower educational level. No differences were found for the use of fertility treatments, familial twinning, or religion. Importantly, the pattern of non-response did not differ between MZ and dizygotic (DZ) mothers. For internalizing and externalizing problems, Bartels et al. (2007) compared the level of problem behaviors in 3-year-old twins between three groups: a group that participated at ages 3, 7, 10, and 12, a group that participated only at age 3, and a group with temporary non-participation. No significant mean differences in externalizing and internalizing behavior problems were found between the three groups. Around age 14, parental consent is obtained before approaching the 14- and 16-year-old twins and siblings. Around 40% of the parents return these forms. Comparisons of parental and twins' characteristics between responders and non-responders of the self-report revealed that responders were more likely to have returned multiple earlier surveys, and that non-response was associated with a lower socio-economic status, lower educational levels, higher prevalence of mothers and fathers who smoked during pregnancy, and more problem behavior in the children. However, the differences were small, significance reflecting mainly the large sample sizes. Non-response was associated with more externalizing behaviors at age 7 and 12 (with effect sizes of 0.17 and 0.24), but not at age 3 (Bartels et al., 2011). For internalizing problems, no differences were found between the two groups. It is important to note that the children in 13% of the families that did not respond to the parental consent completed an ANTR survey 8 after having reached age 18. Thus, non-response in adolescence does not signal permanent dropout.

YNTR Phenotyping: Standardized Tests Collected in School Settings

To assess cognitive performance, we started to collect information on the Dutch Cito-elementary test in 2000 (Bartels et al., 2002b). The Cito-test is a standardized test for educational achievement that is currently administered in the final grade (when children are 11 or 12 years old) of elementary school in February/March (Eindtoets Basisonderwijs, 2002; www.cito.nl). The Cito-test results weigh heavily in the primary school's advice, which is often binding, on the most appropriate level of secondary education. The Cito-test consists of items on language, mathematics, study skills, and world orientation. Together the scores on the four tests form a standardized final Cito-score. Initially, we obtained the Cito-scores from teachers. Because results are only available near the very end of the school year, we later asked the parents to report the scores on the Cito-test and currently we also ask the twins to report their own Cito-scores. The correlation between the Cito-scores as provided by the parents and from the self-reports of the twins is 0.975 ($N =$ 2,433), between teachers and twins the correlation is 0.932 ($N = 845$). Recently, we started a pilot project to obtain the full set of Cito-scores of all twins by linking our data to the national Cito database. In 2008, we started with a pilot to collect data of the Pupil Monitoring System (PMS), providing information on educational attainment, not just in the sixth grade but throughout elementary school. Schools are free to use the PMS, and are free in their choice of tests they want to use. About 95% of all schools in the Netherlands use the PMS. PMS tests are administered at fixed time points (i.e., beginning, halfway, and/or end of the school year) in each grade. Like the Cito-test, the PMS has scores on various academic skills, and teachers indicated reading comprehension, mathematics, word reading, and spelling as the most

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

informative of cognitive skills (Polderman et al., 2010). In 2008 and 2009 in respectively 92% and 95% of completed teacher surveys a PMS form was included (Polderman et al., 2011).

Record Linkage to National Registers and Databanks

Valuable information on twins and singletons can be obtained by linking the YNTR databases to national population-based registers. Examples of information available in national databases include socio-economic status, educational attainment, and information on chorionicity as well as tissue samples in the Pathologisch Anatomisch Landelijk Geautomatiseerd Archief [Pathological Anatomy National Automatic Archive] (PALGA)(the nationwide network and register of histopathology and cytopathology) database and biobank (Casparie et al., 2007). Linking these registers with YNTR data may form an enrichment of research questions in two directions. For the YNTR, linkage with external databases may provide information that we do not have or that we cannot collect with surveys for many reasons. For example, linkage with the pathology databanks will provide us information on the placenta and chorionicity of twins/triplets on a large scale. Recently, we conducted a pilot linkage study to PALGA for 334 mothers of triplets who gave permission to link data (Lamb et al., 2012). The linkage was successful for 70% of the mothers, and for 75% of these mothers (175 trios) the pathology records provided relevant information about triplet chorionicity. A part of the unsuccessful linking may be explained by the fact that PALGA only has full nation-wide coverage since 1992, whereas some of the triplets in the pilot study were born before that time (when there was only partial coverage in the nation-wide database). To obtain information of chorionicity for all YNTR twin pairs, a linkage of the PALGA database with YNTR twins is underway. The other way around, providing twin data to a national register may enrich medical databases with information on twin status. For example, we just started to link data of 600 twin pairs with a national database with blood samples obtained in the first week after birth. The aim of this study is to examine the heritability of the set point of thyroxine (Kempers et al., 2005). Permission for record linkage is obtained from mothers who returned the 'NTR maternal survey' that was sent to all mothers registered with the NTR in 2005 (Hoekstra et al., 2008). The mailing included a question asking for permission for record linkage. Starting with birth cohort 2005, the first survey to mothers now includes a question on permission for linkage. Around 90% of the YNTR mothers gave permission for linkage. Mothers who gave no permission for record linkage have a lower educational attainment, and were more often born in a non-Western country. Zygosity, age of mother, and religion did not differ between mothers who gave permission or who did not.

DNA Collection and Zygosity Typing

DNA collection from twins and their family members started in sub-groups that participated in interview studies (Brouwer et al., 2006; Derks et al., 2008), laboratory projects (described in Table 4), and increasingly in families characterized by a wealth of phenotype data. DNA is collected from whole blood (e.g., van Dijk et al., 1996) or, in the majority of YNTR families, by buccal swabs (Meulenbelt et al., 1995), which has proven to be successful for microsatellite and short nucleotide polymorphism (SNP) genotyping (Min et al., 2006). Presently, whole-genome SNP data from blood or buccal DNA are available for 3,813 YNTR family members (360 persons participated in both YNTR and ANTR). This group includes 2,665 twins, 278 siblings, and 870 parents of twins and these data have been used in the first genome-wide association studies (GWAS) projects (Paternoster et al., 2012).

Zygosity typing was initially based on micro-satellite markers or relatively small numbers of SNPs and is now based on a larger number of SNPs and some repeat polymorphisms in a set of candidate genes. Different sets of SNPs and variable number tandem repeats (VNTRs) in candidate genes have been used over time; Appendix provides an overview of the specific SNPs and VNTRs that were genotyped in each set (Tables A1a and A1b). Moreover, the appendix provides information on the rationale behind the selection of SNPs for the marker set currently in use and additional information on the genotyping process.

Quality control was performed for each set separately. SNPs were tested for concordance in duplicate samples, Hardy–Weinberg equilibrium, Mendelian errors, call rate, and frequency differences relative to the HapMap CEU population. In some cases, SNPs passed quality control in one set but not in others. Samples were cleaned based on call rate, questionnaire-based versus genotypic relatedness, Mendelian errors, and sex errors. Fingerprint data based on this rich set of markers are currently available for 6,347 individuals (see Table 5), and include 4,030 twins, 418 siblings, and 1,899 parents.

Experimental Studies and In-Depth Phenotyping and Endophenotyping Studies

In addition to survey studies, twin families have been invited to participate in in-depth phenotyping and endophenotyping studies. Depending on the research question, selection of the families is based on the administration database, phenotypic database, or both. For example, studies have selected random groups of twins and siblings, or twins with a specific age or specific birth cohort (Polderman et al., 2006a) or with a specific phenotypic characteristic such as twin pairs having a older sibling (van Leeuwen et al., 2009; van Soelen et al., 2010), twin pairs concordant or discordant for anesthesia (Bartels et al., 2009b) or discordant for attention problems (Derks et al., 2008; Polderman et al., 2006a)

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

TABLE 4

Overview of Experimental and Laboratory Studies of the YNTR

		Measures							
Laboratory studies	Number	IQ	NPT	Brain	CVM	Growth	Hormone	Cohort	Age
Electroencephalogram (EEG) and Cognition I[1]	418	X		X	X	X	X	1986–1988	5,7,10, & 12
EEG and Cognition II[2*]	507 (twins/siblings)	X	X		X	X		1986–1988	18
Attention and IQ I[3]	474 (55 siblings)	X	X			X	X	1990–1991	5,12, & 17
Attention and IQ II[4***]	332	X	X			X	X	1990–1991	17
MRI and cognition[5]	327 (twins/siblings)	X	X	X	X	X	X	1995–1996	9 & 11
Magnetoencephalography[6]	74			X				1986–1989	18–21
Magnetic resonance imaging (MRI)-ADHD[7]	74			X				1986–1993	13–17
Computerized Neurocognitive Battery-Penn (CNP)[13]	93 (twins, siblings + parents)							1987–2001	16
Sport & Health (start 2012)	100 (500 planned to do)				X	X		1995–1996	17
MRI and cognition, age 16[5], (start 2012)		X	X	X	X	X	X	1995–1996	16
Interview									
ADHD clinical study[8]	1,006							1986–1993	10–13
Motor milestones[9]	470							2003–2004	0.5–1.5
Anesthesia project (2012)	950							1986–1997	14–25
Other									
DZ twinning[10]	604 mothers of DZ twins and 397 of MZ twins								
Chimerism[11]	1,104 twins/72 triplets							1987–1988	4
Follicle-stimulating hormone (FSH) in DZ mothers[12]	16								

Note: IQ refers to intelligence testing; NPT = neuropsychological tests; CVM = cardiovascular measures (including measures of autonomic functioning); growth (including anthropometry);*80% also participated in EEG and Cognition I;***80% participated in Attention and IQ I.
[1]Bartels et al., 2002a; [2]Hoekstra et al., 2007; [3]Polderman et al., 2006a, 2007; [4]Bartels et al., 2012; [5]van Soelen et al., 2012; [6]Van 't Ent et al., 2010; [7]Van 't Ent et al., 2007; [8]Derks et al., 2008; [9]Langendonk et al., 2007; [10]Meulemans et al., 1996; [11]van Dijk et al., 1996; [12]Lambalk et al., 1998; [13]Gur et al., 2001.

TABLE 5

Overview of Number of Individuals, SNPs, and VNTRs Genotyped in Subsets of the YNTR

Set	Number of SNPs genotyped	Number of VNTRs genotyped	Number of individuals genotyped
Set 1	38	5	4,592
Set 2	37	0	342
Set 3	36	7	982
Set 4	30	0	431 (ongoing)

Note: See Appendix, Tables A1a and A1b for an overview of specific SNPs and VNTRs genotyped in each subset.

or pairs with both twins having either a low or a high level of attention problems (van 't Ent et al., 2007). An overview of such projects is given in Table 4. The main variables assessed in the projects were brain measures (measured by electroencephalography (EEG), magnetoencephalography (MEG), and by magnetic resonance imaging (MRI)), neuropsychological testing (NPT), IQ measures, cardiovascular and autonomic measures, growth/anthropometry, and hormones. IQ testing was done with standardized tests, depending on age and study. In total, IQ scores are collected for 1,834 twins, 249 siblings, and 214 parents between the ages of 5 and 18. For 23% of this group, IQ was measured five times, for 63% between two to four times, and 14% had one measurement (Haworth et al., 2010). Brain measures were obtained in longitudinal studies that examined brain development in critical periods and include EEG (van Baal et al., 1996; van Beijsterveldt et al., 1996), MRI (van 't Ent et al., 2007; van Soelen et al., 2012), and MEG (van 't Ent et al., 2010). Extensive NPT was included in most of these studies. Anthropometry, cortisol and testosterone, and pubertal development were also measured in various studies (Estourgie-van Burk et al., 2010; van Soelen et al., 2011).

Telephone interviews were held to verify zygosity with the mothers of DZ twins (Meulemans et al., 1996), collect additional information on exposures to anesthesia (Groen-Blokhuis et al., 2012), to assess attainment of motor milestones (Langendonk et al., 2007), or to confirm clinical ADHD with the DSM-IV interviews for twin pairs with at least one twin with a high attention problem score (Derks et al., 2008). Recently, a study was started in 16-year-olds establishing the acute physiological and psychological response to (sub)-maximal exercise, as well as a pilot study of a computerized neuropsychological test battery (CNP) in twins, siblings, and their parents (Gur et al., 2001), as preparation for the third assessment (age 16) of MRI and cognition, that will include the CNP.

YNTR Findings

Summary of YNTR Results

The longitudinal genetically informative data collection and the in-depth phenotyping in young Dutch twins resulted in a large number of scientific papers on a range of topics. We showed that genetic factors not only contribute to variation in problem behaviors in childhood, but also to the

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

continuity of behavior and emotional problems during childhood (e.g., Boomsma et al., 2008b; Rietveld et al., 2004). In general, we found that shared environment accounted for less of the phenotypic variation and stability in behavior problems than genetic factors, especially when recognizing that part of this shared environment is actually represented by stability in rater bias (Bartels et al., 2007). By collecting longitudinal data on problem behavior as rated by teachers, stability will be further investigated in future studies. Using standard measures across YNTR and ANTR, such as the measurements of the ASEBA, it is feasible to assess problem behavior across the life span. Recently, we investigated attention problems in a quasi-longitudinal design spanning age 3 through 60 years, and found that the heritability of attention problems is higher than previously suggested, with early genetic and environmental factors having long-lasting effects (Kan et al., in press). A similar approach was used to analyze the genetic architecture of anxious-depressed symptom scores across the life span (Nivard et al., submitted). The question of a potential change in genetic architecture of traits across childhood and adolescence was also addressed for lifestyle traits. As an example, we point to our studies on leisure time exercise behavior (Huppertz et al., 2012; van der Aa et al., 2010a) that showed a very strong shift from shared environmental determination to genetic determination across the age span of 7–19. At age 7, both MZ and DZ correlations were around 0.90, suggesting a strong contribution of the environment of the twins to childhood exercise behavior. During adolescence, the MZ correlations decrease a bit to about 0.80, but DZ correlations drop especially in boys. At age 17–19, MZ correlations are still as high as 0.70, but DZ correlation by this time has decreased to 0.34 for females and 0.48 for males (Huppertz et al., 2012; van der Aa et al., 2010a), suggesting that late adolescent exercise behavior reflects heritable factors, which we hypothesize to include exercise ability and the acute psychological response to exercise (de Geus & de Moor, 2011).

The wealth of item-level data contained in the NTR offers possibilities for multivariate item-level analyses. Franić et al. (2012) applied multivariate genetic item analyses to the items of the *Internalizing* grouping of the Child Behavior Checklist 4-18 (CBCL 4-18), and showed that the indeterminacy of the phenotypic factor structure of internalizing problems in children as measured by the CBCL (in the sense of a three- and a four-factor model both providing a satisfactory account of the data) is a result of several different models pertaining to genetic and environmental influences, giving rise to the observed covariance structure. While additive genetic influences conform to a two-factor model, the individual-specific environmental influences form a four-dimensional structure. Interestingly, shared environmental influences form a unidimensional structure, therefore affecting *Internalizing* items across the board. This not only offers important insights for the test constructor, but may also aid diagnosis (e.g., by elucidating the etiology of comorbidity of anxiety and depression; genetically, these two syndromes appear to form a unidimensional structure, while unique environmental influences appear to drive their differentiation). In addition, the results of this type of analysis may assist studies aimed at identifying genetic variation relevant to phenotypic individual differences, by providing insights needed to optimally define the phenotype.

The large data collections have made it possible to investigate variation in individual differences in behaviors that are less common in the population, or where information about prevalence is lacking. For example, for gender identity disorder (when there is a disassociation between the biological sex and feelings of gender identity), information about the prevalence in children is scarce and derived from adult clinical data (Zucker & Bradley, 1995). On the basis of two items in the CBCL, we found that the prevalence of cross-gender behavior was 3.2% and 5.2% for 7-year-old boys and girls, respectively, and decreased to 2.4% and 3.3% for 10-year-old boys and girls, respectively. Surprisingly, the prevalence rate of cross-gender behavior of girls with a male co-twin was lower than of girls with a female co-twin. Genetics played an important role in the development of cross-gender behaviors (van Beijsterveldt et al., 2006). Another advantage of the large data collections is that we can select discordant MZ twins, even for traits that are highly heritable. Comparisons of discordant MZ twins offer an alternative to the traditional case–control study, since cases and controls are perfectly matched for age, sex, and genetic background. For example, Lehn et al. (2007) used MZ twins discordant for attention problems to investigate specific environmental influences on attention problems and found low birth weight and delayed motor development as risk factors for attention problems. Using the same twins, Ehli et al. (2012) recently found that copy number variations (CNVs) were associated with attention problems and found evidence for the presence of two de novo CNVs.

The increasing number of genotyping data in combination with longitudinal survey data can be used for testing of gene-environment interactions. In a large twin study on asthma liability and tests for gene-environment interactions, we found that being a boy, born in the 1990s, premature birth, longer incubator time, and child care outside the home all increased the risk for asthma (van Beijsterveldt & Boomsma, 2008). Although our findings revealed almost no evidence for gene-environment interaction, the risk factors that were identified could be useful in studies with measured genotypes. An important risk factor could be birth cohort, and since we recruit children from 1986, the YNTR asthma data could be very informative.

Collecting biological material for DNA may be more difficult in children than in adults. Recently, we found that DNA from buccal samples seems to be a reliable alternative for blood sampling. Utilizing Affymetrix SNP 6.0 microarrays from 372 DNA samples of children, we found

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

high concordance in genotype and CNVs across blood and buccal samples (Scheet et al., 2012). Genotype concordance was very high ($R^2 > 99\%$) between co-twins from 43 MZ pairs, suggesting that DNA from buccal samples perform as well as DNA from blood samples on the current generation of microarray technologies.

Representativeness and Participation of Twins

A recurring question for twin researchers concerns the representativeness of twins. We compared heritability estimates for growth at birth and in early childhood (Mook-Kanamori et al., 2012) between Dutch twins and singleton offspring and found comparable heritability estimates. At birth the heritability estimates for body length were 26% for singletons and 27% for twins, and for weight heritability estimates were 26% in singletons and 29% in twins. At age 3, the heritability estimates for height were 63% and 72% for respectively singletons and twins, while the heritability for weight for twins was 71% and higher than for singletons (42%). Regarding physical and psychological development, comparisons of twins and singletons revealed no large differences. Compared to singletons, twins have a growth delay during early childhood (van Dommelen et al., 2008), but catch up their growth during childhood (Estourgie-van Burk et al., 2006). At age 18 twins attained the same height and weight as their peers in the general population (Estourgie-van Burk et al., 2010). Compared to their siblings, twins were as tall as their siblings, but were leaner. Motor development of twins, as measured by achievement of motor milestones, showed no deviations compared to a normative database of singletons (Brouwer et al., 2006). In childhood, the developmental trajectories of externalizing problems are similar in twins and singletons (Robbers et al., 2010). The course of development trajectories of internalizing problems are broadly the same, but after age 9, twins showed less internalizing problems than singletons. Small differences in the cognitive abilities have been reported between twins and singletons at age 6, but these differences were less than one IQ-point and disappeared at later grades (Webbink et al., 2008). In a within-family design, we found that during primary school twins had lower ratings on arithmetic, reading, and language than their non-twin siblings. However, the differences observed between twins and their non-twin siblings in educational achievement seemed to be dependent on the birth order within the family. Twins who were first in birth order within the family had the same, or even higher, ratings as their non-twin sibling, while twins with a sibling who was first in birth order within the family had lower ratings than their non-twin siblings (de Zeeuw et al., 2012).

To keep study samples representative, it is important to retain families in the register and in the studies. Not all families contact the register when they move to a new address and not all families are motivated to fill out all surveys. One way to motivate families is by informing the participants about ongoing research studies and results. This is done in various ways. We have a Web site (http://www.tweelingenregister.org/) and a yearly newsletter TWINFO that is mailed to all families and also published on the Web site (http://www.tweelingenregister.org/twinfo/). Recently, the NTR also started to use social media. This includes Facebook (https://www.facebook.com/NederlandsTweelingenRegister), Twitter (https://twitter.com/NTR_VU), and Hyves (http://ntr-vu.hyves.nl). With social media, information can be shared on a more informal level, updates on study results can be given on a regular basis, and contacts with the participants can be established quickly (Stroo et al., 2012). In addition, the NTR has started building a personal portal for participants. The purpose is to provide customized insight into their own results (e.g., personality profile), in addition to the general information.

Concluding Remarks

Critics of twin studies often argue that the utility of twin samples is limited to the determination of genetic and environmental influences on specific phenotypes. As already presented, the NTR provides more knowledge than important behavioral genetic findings. The NTR has already provided findings using molecular genetic, neuroimaging, endophenotypic, and family study methodologies to provide hints on the links between the genome, brain, and behavior. A little understood, but equally important gain from the NTR has been the non-genetic exploration of the validity, stability, sensitivity, and specificity of new nosologic studies. Through the use of the NTR, advances have been made in understanding the quantitative nature of obsessive-compulsive behavior (Hudziak et al., 2004; van Grootheest et al., 2007), pediatric bipolar disorder (Boomsma et al., 2006b), oppositional defiant disorder (Derks et al., 2007), and aggression (Ligthart et al., 2005). Although it is true that behavioral genetic analyses have yielded insights into the genetic architecture of each of these traits, additional approaches such as latent class, latent trait, and item level analyses have helped advance criteria-based understanding of each of these common disorders. In every case, other groups have picked up on these 'diagnostic' conceptualizations and advanced them in other, non-twin, databases. The NTR, specifically because of its longitudinal developmental infrastructure, provides an unparalleled opportunity to study the resilience of children and adolescence. As time goes forward, investigators will not only be able to investigate the genetic and environmental contributions to the development of adolescent and young adult medical and psychiatric illness, but also shed light on why some children recover from early psychopathology to lead lives well. The NTR and its capacity to continue to collect both phenotypic and biological data will provide an excellent resource to better understand the biological relations between the environment, the (epi)genome, and medical outcomes.

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

Acknowledgments

We thank all twin families who took part in the NTR studies. We also thank all funding agencies that made this work possible in the last 25 years.

The study was supported by Spinozapremie (NWO/SPI 56-464-14192); European Research Council Genetics of Mental Illness (ERC-230374); the Twin-Family Database for Behavior Genetics and Genomics Studies (NWO 480-04-004); a collaborative study of the genetics of DZ twinning (NIH R01 HD042157-01A1); Longitudinal Survey and Panel Funding Application 2008 (NWO-481-08-011); NARSAD; Brain & Behavior Research Foundation-18633; The US National Institute of Mental Health as part of the American Recovery and Reinvestment Act of 2009: Genomics of Developmental Trajectories in Twins (1RC2MH089995-01) and Sport & Exercise: Determinants of Adolescent Exercise Behavior (NIH-1R01DK092127-01); part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the US National Institutes of Health, the (NIMH, MH081802); Genetic and Family Influences on Adolescent Psychopathology and Wellness (NWO 463-06-001); A Twin-Sib Study of Adolescent Wellness (NWO-VENI 451- 04-034*);* The Interplay of Genetic and Environmental Factors in the Development of Anxious Depression in Children and Adults (NWO-VENI grant 916-76-125); Genetic Influences on Stability and Change in Psychopathology From Childhood to Young Adulthood (ZonMW 912-10-020).

References

Achenbach, T. M. (1991). *Manual for the Youth Self-Report and 1991 profiles*. Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M., & Rescorla, L. A. (2000). *Manual for the ASEBA Preschool Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth & Families.

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth & Families.

Bartels, M., Althoff, R. R., & Boomsma, D. I. (2009b). Anesthesia and cognitive performance in children: No evidence for a causal relationship. *Twin Research and Human Genetics*, *12*, 246–253.

Bartels, M., & Boomsma, D. I. (2009a). Born to be happy? The etiology of subjective well-being. *Behavior Genetics*, *39*, 605–615.

Bartels, M., Rietveld, M. J., van Baal, G. C., & Boomsma, D. I. (2002a). Genetic and environmental influences on the development of intelligence. *Behavior Genetics*, *32*, 237–249.

Bartels, M., Rietveld, M. J., van Baal, G.C., & Boomsma, D. I. (2002b). Heritability of educational achievement in 12-year-olds and the overlap with cognitive ability. *Twin Research and Human Genetics*, *5*, 544–553.

Bartels, M., van Beijsterveldt, C. E. M., Derks, E. M., Stroet, T. M., Polderman, T. J. C., Hudziak, J. J., & Boomsma, D. I. (2007). Young Netherlands Twin Register (Y-NTR): A longitudinal multiple informant study of problem behavior. *Twin Research and Human Genetics*, *10*, 3–11.

Bartels, M., van der Aa, N., van Beijsterveldt, C. E. M., Middeldorp, C. M., & Boomsma, D. I. (2011). Adolescent self-report of emotional and behavioral problems: Interactions of genetic factors with sex and age. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *20*, 35–52.

Bartels, M., van Weegen, F. I., van Beijsterveldt, C. E. M., Carlier, M. L., Polderman, T. J. C., Hoekstra, R. A., & Boomsma, D. I. (2012). The five factor model of personality and intelligence: A twin study on the relationship between the two constructs. *Personality and Individual Differences*, *53*, 368–373.

Boomsma, D. I., de Geus, E. J. C., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., . . . Willemsen, G. (2006a). Netherlands Twin Register: From twins to twin families. *Twin Research and Human Genetics*, *9*, 849–857.

Boomsma, D. I., Rebollo, I., Derks, E. M., van Beijsterveldt, C. E. M., Althoff, R. R., Rettew, D. C., & Hudziak, J. J. (2006b). Longitudinal stability of the CBCL-juvenile bipolar disorder phenotype: A study in Dutch twins. *Biological Psychiatry*, *60*, 912–920.

Boomsma, D. I., van Beijsterveldt, C. E. M., Bartels, M., & Hudziak, J. J. (2008b). Genetic and environmental influences on anxious/depression: A longitudinal study in 3- to 12-year-old children. *Developmental Psychopathology and Wellness: Genetic and Environmental Influences*, 161–189.

Boomsma, D. I., Vink, J. M., van Beijsterveldt, C. E. M., de Geus, E. J. C., Beem, A. L., Mulder, E. J., . . . van Baal, G. C. (2002). Netherlands Twin Register: A focus on longitudinal research. *Twin Research*, *5*, 401–406.

Boomsma, D. I., Willemsen, G., Vink, J. M., Bartels, M., Groot, P., Hottenga, J. J., . . . van der Kleij, F. (2008a). Design and implementation of a twin-family database for behavior genetics and genomics studies. *Twin Research and Human Genetics*, *11*, 342–348.

Brouwer, S. I., van Beijsterveldt, C. E. M., Bartels, M., Hudziak, J. J., & Boomsma, D. I. (2006). Influences on achieving motor milestones: A twin-singleton study. *Twin Research and Human Genetics*, *9*, 424–430.

Casparie, M., Tiebosch, A. T., Burger, G., Blauwgeers, H., van de Pol, A., van Krieken, J. H., & Meijer, G. A. (2007). Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cellular Oncology*, *29*, 19–24.

Conners, C. K. (2001). *Conners' Rating Scales — revised*. New York and Toronto: Multi-Health Systems.

Conners, C. K., Sitarenios, G., Parker, J. D., & Epstein, J. N. (1998). The revised Conners' Parent Rating Scale (CPRS-R): Factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology*, *26*, 257–268.

Costa, P. T., & McCrae, R.R. (1992). *Revised NEO personality inventory (NEO PI-R) and the NEO five factor inventory (NEO-FFI) professional manual*. Odessa, FL: Psychological Assessment Resources.

de Geus, E. J. C., & de Moor, M. H. M. (2011). Genes, exercise, and psychological factors. In C. Bouchard & E. P. Hoffman (Eds.), *Genetic and molecular aspects of sport performance* (pp. 294–305). Chichester, UK: Blackwell Publishing.

de Zeeuw, E. L., van Beijsterveldt, C. E. M., de Geus, E. J. C., & Boomsma, D. I. (2012). Twin specific risk factors in primary school achievements. *Twin Research and Human Genetics*, *15*, 107–115.

Derks, E. M., Dolan, C. V., Hudziak, J. J., Neale, M. C., & Boomsma, D. I. (2007). Assessment and etiology of attention deficit hyperactivity disorder and oppositional defiant disorder in boys and girls. *Behavior Genetics*, *37*, 559–566.

Derks, E. M., Hudziak, J. J., Dolan, C. V., van Beijsterveldt, C. E. M., Verhulst, F. C., & Boomsma, D. I. (2008). Genetic and environmental influences on the relation between attention problems and attention deficit hyperactivity disorder. *Behavior Genetics*, *38*, 11–23.

Ehli, E. A., Abdellaoui, A., Hu, Y., Hottenga, J. J., Kattenberg, M., van Beijsterveldt, C. E. M., ... Davies, G. E. (2012). De novo and inherited CNVs in MZ twin pairs selected for discordance and concordance on Attention Problems. *European Journal of Human Genetics, EJHG Open*.

Eindtoets Basisonderwijs (2002). Arnhem: Citogroep.

Estourgie-van Burk, G. F., Bartels, M., Boomsma, D. I., & Delemarre-van de Waal, H. A. (2010). Body size of twins compared with siblings and the general population: From birth to late adolescence. *Journal of Pediatrics*, *156*, 586–591.

Estourgie-van Burk, G. F., Bartels, M., van Beijsterveldt, C. E. M., Delemarre-van de Waal, H. A., & Boomsma, D. I. (2006). Body size in five-year-old twins: Heritability and comparison to singleton standards. *Twin Research and Human Genetics*, *9*, 646–655.

Franić, S., Dolan, C. V., Borsboom, D., Hudziak, J. J., Van Beijsterveldt, C. E. M., & Boomsma, D. I. (in press). Can genetics help psychometrics? Improving dimensionality assessment through genetic factor modeling. *Psychological Methods*.

Geels, L., Bartels, M., van Beijsterveldt, C. E. M., Willemsen, G., van der Aa, N., Boomsma, D. I., & Vink, J. M. (2011). Trends in adolescent alcohol use: Effects of age, sex and cohort on prevalence and heritability. *Addiction*, *107*, 518–527.

Gur, R. C., Ragland, J. D., Moberg, P. J., Turner, T. H., Bilker, W. B., Kohler, C., ... Gur, R. E. (2001). Computerized Neurocognitive Scanning: I. Methodology and validation in healthy people. *Neuropsychopharmacology*, *25*, 766–761.

Haworth, C. M., Wright, M. J., Luciano, M., Martin, N. G., de Geus, E. J. C., van Beijsterveldt, C. E. M., ... Plomin, R. (2010). The heritability of general cognitive ability increases linearly from childhood to young adulthood. *Molecular Psychiatry*, *15*, 1112–1120.

Hoekstra, C., Willemsen, G., van Beijsterveldt, C. E. M., Lambalk, C. B., Montgomery, G. W., & Boomsma, D. I. (2010). Body composition, smoking, and spontaneous dizygotic twinning. *Fertility & Sterility*, *93*, 885–893.

Hoekstra, C., Willemsen, G., van Beijsterveldt, C. E. M., Montgomery, G. W., & Boomsma, D. I. (2008). Familial twinning and fertility in Dutch mothers of twins. *American Journal of Medical Genetics*, *146A*, 3147–3156.

Hoekstra, R. A., Bartels, M., & Boomsma, D. I. (2007). Longitudinal genetic study of verbal and nonverbal IQ from early childhood to young adulthood. *Learning and Individual Differences*, *17*, 97–114.

Hudziak, J. J., van Beijsterveldt, C. E. M., Althoff, R. R., Stanger, C., Rettew, D. C., Nelson, E. C., ... Boomsma, D. I. (2004). Genetic and environmental contributions to the Child Behavior Checklist Obsessive-Compulsive Scale: A cross-cultural twin study. *Archives of General Psychiatry*, *61*, 608–616.

Huppertz, C., Bartels, M., van Beijsterveldt, C. E. M., Boomsma, D. I., Hudziak, J. J., & de Geus, E. J. C. (2012). The impact of shared environmental factors on exercise behavior from age 7 to 12. *Medicine & Science in Sports & Exercise*, *44*, 2025–2032.

Kan, K. J., Dolan, C. V., Nivard, M. C., Middeldorp, C. M., van Beijsterveldt, C. E. M., Willemsen, G., & Boomsma, D. I. (in press). Genetic and environmental stability in attention problems across the lifespan: Evidence from the Netherlands Twin Register. *Journal of the American Academy of Child and Adolescent Psychiatry*. doi:10.1016/j.jaac.2012.10.009

Kempers, M. J., van Trotsenburg, A. S., van Tijn, D. A., Bakker, E., Wiedijk, B. M., Endert, E., ... Vulsma, T. (2005). Disturbance of the fetal thyroid hormone state has long-term consequences for treatment of thyroidal and central congenital hypothyroidism. *Journal of Clinical Endocrinology and Metabolism*, *90*, 4094–4100.

Lamb, D. J., Middeldorp, C. M., van Beijsterveldt, C. E. M., Vink, J. M., Haak, M. C., & Boomsma, D. I. (2011). Birth weight in a large series of triplets. *BMC Pediatrics*, *11*, 24.

Lamb, D. J., Vink, J. M., Middeldorp, C. M., van Beijsterveldt, C. E. M., Haak, M. C., Overbeek, L. I., & Boomsma, D. I. (2012). Effects of chorionicity and zygosity on triplet birth weight. *Twin Research and Human Genetics*, *15*, 149–157.

Lambalk, C. B., Boomsma, D. I., De Boer, L., De Koning, C. H., Schoute, E., Popp-Snijders, C., & Schoemaker, J. (1998). Increased levels and pulsatility of follicle-stimulating hormone in mothers of hereditary dizygotic twins. *Journal of Clinical Endocrinology and Metabolism*, *83*, 481–486.

Langendonk, J. M., van Beijsterveldt, C. E. M., Brouwer, S. I., Stroet, T., Hudziak, J. J., & Boomsma, D. I. (2007). Assessment of motor milestones in twins. *Twin Research and Human Genetics*, *10*, 835–839.

Lehn, H., Derks, E. M., Hudziak, J. J., Heutink, P., van Beijsterveldt, C. E. M., & Boomsma, D. I. (2007). Attention problems and attention-deficit/hyperactivity disorder in discordant and concordant monozygotic twins: Evidence of environmental mediators. *Journal of the American Academy of Child and Adolescent Psychiatry*, *46*, 83–91.

Ligthart, L., Bartels, M., Hoekstra, R. A., Hudziak, J. J., & Boomsma, D. I. (2005). Genetic contributions to subtypes of aggression. *Twin Research and Human Genetics*, *8*, 483–491.

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

Martin, N. G., Boomsma, D. I., & Machin, G. (1997). A twin-pronged attack on complex traits. *Nature Genetics*, *17*, 387–392.

Meulemans, W. J., Lewis, C. M., Boomsma, D. I., Derom, C. A., van den Berghe, H., Orlebeke, J. F., . . . Derom, R. M. (1996). Genetic modelling of dizygotic twinning in pedigrees of spontaneous dizygotic twins. *American Journal of Medical Genetics*, *61*, 258–263.

Meulenbelt, I., Droog, S., Trommelen, G. J., Boomsma, D. I., & Slagboom, P. E. (1995). High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *American Journal of Human Genetics*, *57*, 1252–1254.

Min, J. L., Lakenberg, N., Bakker-Verweij, M., Suchiman, E., Boomsma, D. I., Slagboom, P. E., & Meulenbelt, I. (2006). High microsatellite and SNP genotyping success rates established in a large number of genomic DNA samples extracted from mouth swabs and genotypes. *Twin Research and Human Genetics*, *9*, 501–506.

Mook-Kanamori, D. O., van Beijsterveldt, C. E. M., Steegers, E. A., Aulchenko, Y. S., Raat, H., Hofman, A., . . . Jaddoe, V. W. (2012). Heritability estimates of body size in fetal life and early childhood. *PLoS One*, *7*, e39901.

Nivard, M. G., Dolan, C. V., Kendler, K. S., Willemsen, G., van Beijsterveldt, C. E. M., Lindauer, R. J. L., . . . Boomsma, D. I. (submitted). Stability and change in symptoms of anxiety and depression as a function of genotype and environment: A longitudinal twin study from age 3 to 65 years. *Journal.*

Paternoster, L., Standl, M., Chen, C. M., Ramasamy, A., Bønnelykke, K., Duijts, L., . . . Early Genetics & Lifecourse Epidemiology (EAGLE) Consortium. (2012). Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nature Genetics*, *44*, 187–192.

Polderman, T. J. C., Boomsma, D. I., Bartels, M., Verhulst, F. C., & Huizink, A. C. (2010). A systematic review of prospective studies on attention problems and academic achievement. *Acta Psychiatrica Scandinavica*, *122*, 271–284.

Polderman, T. J. C., Gosso, M. F., Posthuma, D., van Beijsterveldt, C. E. M., Heutink, P., Verhulst, F. C., & Boomsma, D. I. (2006a). A longitudinal twin study on IQ, executive functioning, and attention problems during childhood and early adolescence. *Acta Neurologica Belgica*, *106*, 191–207.

Polderman, T. J. C., Huizink, A. C., Verhulst, F. C., van Beijsterveldt, C. E. M., Boomsma, D. I., & Bartels, M. (2011). A genetic study on attention problems and academic skills: Results of a longitudinal study in twins. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *20*, 22–34.

Polderman, T. J. C., Posthuma, D., De Sonneville, L. M., Stins, J. F., Verhulst, F. C., & Boomsma, D. I. (2007). Genetic analyses of the stability of executive functioning during childhood. *Biological Psychology*, *76*, 11–20.

Polderman, T. J. C., Posthuma, D., De Sonneville, L. M., Verhulst, F. C., & Boomsma, D. I. (2006b). Genetic analyses of teacher ratings of problem behavior in 5-year-old twins. *Twin Research and Human Genetics*, *9*, 122–130.

Rietveld, M. J., Hudziak, J. J., Bartels, M., van Beijsterveldt, C. E. M., & Boomsma, D. I. (2004). Heritability of attention problems in children: Longitudinal results from a study of twins, age 3 to 12. *Journal of Child Psychology and Psychiatry*, *45*, 577–588.

Robbers, S. C., Bartels, M., van Oort, F. V., van Beijsterveldt, C. E. M., van der Ende, J., Verhulst, F. C., . . . Huizink, A. C. (2010). A twin-singleton comparison of developmental trajectories of externalizing and internalizing problems in 6- to 12-year-old children. *Twin Research and Human Genetics*, *13*, 79–87.

Scheet, P., Ehli, E. A., Xiao, X., van Beijsterveldt, C. E. M., Abdellaoui, A., Althoff, R. R., . . . Boomsma, D. I. (2012). Twins, tissue and time: A comparison of genomic structures. *Twin Research and Human Genetics*, *28*, 1–9

Spivack, G., & Spotts, J. (1966). *The Devereux Child Behavior (DCB) Rating Scale.* Devon, UK: The Devereux Foundation.

Stroo, N., de Moor, M. H. M., Lighart, C., Brouwer, C., Willemsen, G., Boomsma, D. I., & Vink, J. M. (2012, April). *Social media experiences of the Netherlands Twin Register.* Paper presented to the International Congress on Twin Studies 2012 (Florence, Italy).

van 't Ent, D., Lehn, H., Derks, E. M., Hudziak, J. J., van Strien, N. M., Veltman, D. J., . . . Boomsma, D. I. (2007). A structural MRI study in monozygotic twins concordant or discordant for attention/hyperactivity problems: Evidence for genetic and environmental heterogeneity in the developing brain. *Neuroimage*, *35*, 1004–1020.

van 't Ent, D., van Soelen, I. L., Stam, K. J., de Geus, E. J. C., & Boomsma, D. I. (2010). Genetic influence demonstrated for MEG-recorded somatosensory evoked responses. *Psychophysiology*, *47*, 1040–1046.

van Baal, G. C., de Geus, E. J. C., & Boomsma, D. I. (1996). Genetic architecture of EEG power spectra in early life. *Electroencephalography and Clinical Neurophysiology*, *98*, 502–514.

van Beijsterveldt, C. E. M., Hudziak, J. J., & Boomsma, D. I. (2006). Genetic and environmental influences on cross-gender behavior and relation to behavior problems: A study of Dutch twins at ages 7 and 10 years. *Archives of Sexual Behavior*, *35*, 647–658.

van Beijsterveldt, C. E. M., & Boomsma, D. I. (2008). An exploration of gene-environment interaction and asthma in a large sample of 5-year-old Dutch twins. *Twin Research and Human Genetics*, *11*, 143–149.

van Beijsterveldt, C. E. M., Molenaar, P. C., de Geus, E. J. C., & Boomsma, D. I. (1996). Heritability of human brain functioning as assessed by electroencephalography. *American Journal of Human Genetics*, *58*, 562–573.

van Beijsterveldt, C. E. M., Verhulst, F. C., Molenaar, P. C., & Boomsma, D. I. (2004). The genetic basis of problem behavior in 5-year-old Dutch twin pairs. *Behavior Genetics*, *34*, 229–242.

van der Aa, N., Bartels, M., te Velde, S. J., Boomsma, D. I., de Geus, E. J. C., & Brug, J. (2012). Genetic and environmental influences on individual differences in sedentary behavior during adolescence: A twin-family study. *Archives of Pediatrics & Adolescent Medicine*, *166*, 509–514.

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

van der Aa, N., Boomsma, D. I., Rebollo-Mesa, I., Hudziak, J. J., & Bartels, M. (2010b). Moderation of genetic factors by parental divorce in adolescents' evaluations of family functioning and subjective well-being. *Twin Research and Human Genetics*, *13*, 143–162.

van der Aa, N., de Geus, E. J. C., van Beijsterveldt, C. E. M., Boomsma, D. I., & Bartels, M. (2010a). Genetic influences on individual differences in exercise behavior during adolescence. *International Journal of Pediatrics*. [Epub]. doi:10.1155/2010/138345

van Dijk, B. A., Boomsma, D. I., & de Man, A. J. (1996). Blood group chimerism in human multiple births is not rare. *American Journal of Medical Genetics*, *61*, 264–268.

van Dommelen, P., de Gunst, M., van der Vaart, A., van Buuren, S., & Boomsma, D. I. (2008). Growth references for height, weight and body mass index of twins aged 0–2.5 years. *Acta Paediatrica*, *97*, 1099–1104.

van Dongen, J., Slagboom, P. E., Draisma, H. H., Martin, N. G., & Boomsma, D. I. (2012). The continuing value of twin studies in the omics era. *Nature Reviews Genetics*, *13*, 640–653.

van Grootheest, D. S., Cath, D. C., Beekman, A. T., & Boomsma, D. I. (2007). Genetic and environmental influences on obsessive-compulsive symptoms in adults: A population-based twin-family study. *Psychological Medicine*, *37*, 1635–1644.

van Leeuwen, M., van den Berg, S. M., Hoekstra, R. A., & Boomsma, D. I. (2009). The genetic and environmental structure of verbal and visuospatial memory in young adults and children. *Neuropsychology*, *23*, 792–802.

van Soelen, I. L. C., Brouwer, R. M., Peper, J. S., van Beijsterveldt, C. E. M., van Leeuwen, M., de Vries, L. S., . . . Boomsma, D. I. (2010). Effects of gestational age and birth weight on brain volumes in healthy 9 year-old children. *Journal of Pediatrics*, *156*, 896–901.

van Soelen, I. L. C., Brouwer, R. M., Peper, J. S., van Leeuwen, M., Koenis, M. M., van Beijsterveldt, C. E. M., . . . Boomsma, D. I. (2012). Brain SCALE: Brain structure and cognition: An adolescent longitudinal twin study into the genetic etiology of individual differences. *Twin Research and Human Genetics*, *15*, 453–467.

van Soelen, I. L. C., Brouwer, R. M., van Baal, G. C., Schnack, H. G., Peper, J. S., Chen, L., . . . Hulshoff Pol, H. E. (2011). Heritability of volumetric brain changes and height in children entering puberty. *Human Brain Mapping. 59*, 3871–3880.

Webbink, D., Posthuma, D., Boomsma, D. I., de Geus, E. J. C., & Visscher, P. M. (2008). Do twins have lower cognitive ability than singletons? *Intelligence*, *36*, 539–547.

Willemsen, G., Vink, J. M., Abdellaoui, A., den Braber, A., van Beek, J. H. D. A., Draisma, H. H. M., . . . Boomsma, D. I. (2012). The Adult Netherlands Twin Register: 25 years of survey and biological data collection. *Twin Research and Human Genetics*, *13*.

Zucker, K. J., & Bradley, S. J. (1995). *Gender identity disorder and psychosexual problems in children and adolescents*. New York. Guilford Press.

Appendix A: Current Approach to Zygosity Determination

The single nucleotide polymorphisms (SNPs) selected for zygosity testing were balanced on their information as candidate markers to a diverse group of phenotypes (psychopathology, body mass index, etc.) as well as having a relatively high minor allele frequency (MAF). We selected 25 SNPs in separate genes but also included an additional 5 markers in the same or closely spaced genes due to our special interest as candidate genes (e.g. FTO, FADS2, and Apo lipoprotein E/J). The mean MAF (HapMap CEU) for the 25 SNPs with no putative linkage disequilibrium (LD) is approximately 0.30. We determined that for these SNPs, the probability of making a false positive call (calling a dizygotic relationship as monozygotic relationship) in a single sibling pair would be 3.2×10^{-9}.

SNP genotyping was performed using the TaqMan Genotyping Assay according to the instructions of the manufacturer (Life Technologies; Foster City, CA, USA). Each assay contains a mix of unlabeled polymerase chain reaction (PCR) primers and TaqMan dye-labeled probes, VIC and FAM, for allelic discrimination of the SNP alleles. The TaqMan SNP assays are designed to work with TaqMan Genotyping Master Mix, which contains a proprietary combination of DNA polymerase, dNTPs, and optimized buffer components. For each reaction, 1 μL of genomic DNA (10 ng/μL) was mixed with 2.5 μL of 2′ TaqMan Genotyping Master Mix, 0.125 μL of TaqMan Assay (40′), and 1.375 μL of AccuGene (Lonza; Basel, Switzerland) water to bring the final reaction volume to 5 μL. The resulting DNA/PCR mix was loaded onto a 384-well optical reaction plate (Life Technologies). The plate was placed into either an ABI Viia7 or ABI 7900HT Fast Real-Time PCR System and the PCR cycling conditions were as follows: 95 °C for 10 minutes followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. Genotype calling was performed using Viia7 and 7900HT SDS (Sequence Detection Systems) software versions 1.2.1 and 2.3, respectively. Quality control consisted of genotyping select samples in duplicate, along with confirming positive and negative controls. Table A1a describes each of the polymorphisms used in the zygosity determination (set 4) along with a description of the gene, db SNP ID#, polymorphism, minor allele, MAF, and the Life Technologies Assay ID Number. Table A2 lists the sequences of the primer and probes used for the custom designed assays.

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

TABLE A1a

Zygosity Determination Markers in Candidate Genes for all Subsets Genotyped in the YNTR, SNPs

Gene	dbSNP ID#	Genotyped in Set 1 (1 = yes, 0 = no)	Genotyped in Set 2 (1 = yes, 0 = no)	Genotyped in Set 3 (1 = yes, 0 = no)	Genotyped in Set 4 (current) (1 = yes, 0 = no)	Polymorphism	Minor allele	MAF (HapMap CEU)	Life Technologies Assay ID#
BDNF	rs6265	1	0	0	1	C>T	T	0.18	C__11592758_10
COMT	rs4680	1	1	1	1	A>G	G	0.48	C__25746809_50
DRD2	rs1800497	1	1	1	1	A>G	A	0.23	C___7486676_10
DBH	rs1611115	1	1	1	0	C>T	T	0.18	C___2535786_10
DBH	rs2519152	1	1	1	1	A>G	G	NA	AHHR67X - custom
SNAP-25	rs3746544	1	1	1	0	T>G	G	NA	C__27494002_10
SNAP-25	rs1051312	1	0	0	1	C>T	C	NA	AHCSEEK - custom
NET	rs998424	1	1	1	0	C>T	T	NA	C___3020067_10
NET	rs3785157	1	1	1	1	C>T	T	0.39	C__27481947_10
CLOCK	rs1801260	1	0	0	1	A>G	G	0.28	C___8746719_20
ApoE	rs7412	1	1	1	0	T>C	T	NA	C____904973_10
ApoE	rs429358	1	1	1	1	C>T	C	NA	C___3084793_20
NPY	rs16139	1	1	1	1	C>T	C	0.07	C__11164473_20
ADRA2A	rs1800544	1	1	1	1	C>G	G	NA	C___7611979_10
ADRA2A	rs1800545	1	1	1	0	A>G	A	NA	C___7611978_10
ADRA2A	rs553668	1	1	1	0	A>G	A	0.14	C____996424_20
AADAT	rs13145318	1	1	1	1	T>C	C	0.38	AH6Q6VX - custom
PCLO	rs2715148	1	1	1	1	A>C	C	0.48	C__16277334_10
PCLO	rs2522833	1	0	0	0	A>C	C	0.43	C___2553139_10
HTR1B	rs6296	1	1	1	1	C>G	G	0.34	C___2523534_20
HTR2A	rs6314	1	1	1	1	A>G	A	0.08	C__11696920_20
TPH2	rs1843809	1	1	1	0	G>T	G	0.13	C__11479729_10
TPH2	rs1386497	1	0	0	1	A>C	C	0.12	C___8872295_10
GRIN2A	rs8049651	1	1	1	1	C>T	T	0.25	C___2663544_10
DRD1	rs265981	1	0	0	1	A>G	A	0.39	C___1011775_20
FADS2	rs174575	1	1	1	1	C>G	G	0.28	C___2575522_20
FADS2	rs1535	1	1	1	1	A>G	G	0.37	C__2575539_1_
ApoE	rs2075650	1	0	0	1	A>G	G	0.14	C___3084828_20
CLU/ApoJ	rs11136000	1	1	1	1	C>T	T	0.30	C__11227737_10
PICALM	rs3851179	1	1	1	1	C>T	T	0.42	C___8748810_10
CR1	rs6656401	1	1	1	1	A>G	A	0.23	C__30033241_10
DAT	rs2652511	1	1	1	0	A>G	C	NA	C__16273213_10
DAT	rs40184	1	1	1	1	C>T	T	0.45	C___2960969_10
DRD4	rs1800955	1	0	0	1	C>T	C	NA	C___7470700_30
DRD4	rs3758653	1	1	1	0	C>T	C	0.21	C__27512407_10
SRC1	rs11125744	1	0	0	1	C>G	G	0.10	C__25972631_10
FTO	rs9939609	0	1	1	1	A>T	A	0.45	C__30090620_10
FTO	rs1121980	0	0	0	1	A>G	A	0.48	C___2031261_10
FTO	rs6499640	0	0	0	1	A>G	G	0.35	C__29387696_10
FTO	rs8050136	0	0	0	1	A>C	A	0.45	C___2031259_10
ADRB2	rs1042714	0	1	1	0	C>G	G	0.47	C__2084765_20
CHRNA5	rs16969968	0	1	1	0	A>G	A	0.43	C__26000428_20
FADS1	rs174550	0	1	1	0	C>T	C	0.37	C__2575539_10
GRM8	rs2237781	0	1	1	0	A>G	A	0.07	C__16170802_20
GRIK2	rs6570989	0	1	1	0	A>G	A	0.15	C__29435771_10
CD53	rs6679497	0	1	1	0	C>G	G	0.44	C__31638818_10
GABRG3	rs8036270	0	1	0	0	A>G	G	0.41	C___9408527_20

TABLE A1b

Zygosity Determination Markers in Candidate Genes for All Subsets Genotyped in the YNTR, VNTRs

Gene	Polymorphism	Genotyped in Set 1 (1 = yes, 0 = no)	Genotyped in Set 2 (1 = yes, 0 = no)	Genotyped in Set 3 (1 = yes, 0 = no)	Genotyped in Set 4 (current) (1 = yes, 0 = no)	Observed alleles
SERT	5-HTTLPR + rs25531 in promoter	1	0	1	0	S, Lg, La, XL
DRD4	48bp VNTR in Exon III	1	0	1	0	2,3,4,5,6,7,8,9,10,11
DAT1	40bp VNTR in 3′ UTR	1	0	1	0	400,440,480,520
DRD5	di-nucleotide repeat 18.5 kb upstream of txn start site	1	0	1	0	130,132,134,136,138, 140,142,144,146,148, 150,152,154,156,158, 166
MAOA	30bp VNTR in promoter	1	0	1	0	2,3,3.5,4,5
DRD4	120bp repeat in promoter	0	0	1	0	120,240,360.5,480
SERT	17bp repeat in Intron II	0	0	1	0	9,10,12

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press

TABLE A2

Primer and Probe Sequences for Custom Designed Assays

Assay ID	Forward primer sequence	Reverse primer sequence	Reporter 1 Sequence — VIC	Reporter 2 Sequence - FAM
AHHR67X – custom	GCGAAGCTGTGAGGAGTGA	CCCTTGCGTCTGCCTCAT	AGGGACAGGACCTCGAG	AGGGACAGGACCCCGAG
AHCSEEK – custom	CATTTGGTGGCTCTAACTCCTTGA	GCAAATGCCACCGAGGAGA	AAAATGAAAAATGAAACTCAAGAC	AAATGAAAAATGAAACTCAGGAC
AH6Q6VX – custom	GATGCCTCCTCTTGTGTCCAT	TGCTCAAGCTGAAGGAGAAAGAG	TGTTAGCAGCGTCCCCG	TTAGCAGCATCCCCG

https://doi.org/10.1017/thg.2012.118 Published online by Cambridge University Press