

Two Cohort and Three Independent Anonymous Twin Projects at the Keio Twin Research Center (KoTReC)

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The Keio Twin Research Center has conducted two longitudinal twin cohort projects and has collected three independent and anonymous twin data sets for studies of phenotypes related to psychological, socio-economic, and mental health factors. The Keio Twin Study has examined adolescent and adult cohorts, with a total of over 2,400 pairs of twins and their parents. DNA samples are available for approximately 600 of these twin pairs. The Tokyo Twin Cohort Project has followed a total of 1,600 twin pairs from infancy to early childhood. The large-scale cross-sectional twin study (CROSS) has collected data from over 4,000 twin pairs, from 3 to 26 years of age, and from two high school twin cohorts containing a total of 1,000 pairs of twins. These data sets of anonymous twin studies have mainly targeted academic performance, attitude, and social environment. The present article introduces the research designs and major findings of our center, such as genetic structures of cognitive abilities, personality traits, and academic performances, developmental effects of genes and environment on attitude, socio-cognitive ability and parenting, genes x environment interaction on attitude and conduct problem, and statistical methodological challenges and so on. We discuss the challenges in conducting twin research in Japan.

■ **Keywords:** twin cohort, longitudinal, infancy, childhood, adolescence, adulthood

The Keio Twin Research Center (KoTReC) was established in 2009 as an integrated organization of two twin cohort projects at Keio University; the Keio Twin Study (KTS) for adolescence and adulthood, and the Tokyo Twin Cohort Project (ToTCOP) for infancy and childhood. These two

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twin projects have been independently conducting various psychological, behavioral, neurophysiological, and molecular genetic studies for several years, and have involved a range of funding sources and research teams. The early work of the KTS and ToTCoP was reported by Shikishima et al. (2006) and Ando et al. (2006), respectively. The KoTReC has also collected three anonymous one-shot twin data sets, one of which uses a cross-sectional design.

The current article provides a brief outline of the current status and main findings at the KoTReC.

Purpose of Twin Studies

Twin studies typically have three main aims: to obtain relevant information *of* twins, *by* twins, and *for* twins. Studies *of* twins are designed to collect information about factors differentiating twins from singletons, such as the development of linguistic abilities and sibling relationships. Studies *by* twins are typically behavioral genetic studies in which genetically and environmentally systematic information of twins are utilized as a biometrical method. This second type of research is the focus of the KoTReC. Studies *for* twins are focused on providing evidence-based support for nursing and educating twins, mainly in infancy and childhood, by producing relevant information about the causes of parenting stress and environmental effects on infant growth and development.

The age ranges of the two cohorts in the KTS and ToTCoP are different, and the aims of the projects also differ. In the KTS, which includes participants between 15 and 40 years of age, almost all the research is conducted with a behavioral genetic focus (i.e., a *by*-twins study), including psychological, psychiatric, sociological, socio-economic, neurological, and molecular genetic characteristics. On the other hand, the ToTCoP, which examines twin participants from birth to 6 years of age, includes all three of the main aims of twin studies. For studies *of* twins, a set of singleton data that is comparable to twin data were obtained for several important variables.

Recruitment of Twin Participants

The strategy to recruit twins and their families in both the KTS and ToTCoP is to send letters to twin families identified by the Basic Resident Register (BRR; nation-wide census). The BRR is a quasi-complete (i.e., complete at a specific time in a specific area) residential record of each municipal area, which contains each resident's name, gender, residential address, and date of birth. This information gathering is authorized by each municipal area's regulations, and data are available at city halls. Twins or higher multiples can be identified as individuals who share the same date of birth and address. With this method, it is difficult to recruit newborn and adult twins because it takes several months for newborns to be registered on the BRR and because most adult twins live apart. Because these data are not ob-

tained electronically, but rather by printed documents with a substantial cost, well-trained staff are required to identify multiple births and transfer the information manually. This strategy has a number of methodological shortcomings (see Ando et al., 2006), but it is the only way to obtain 'population-based', rather than hospital-based or twin support group-based, twin data in Japan.

These research projects included three residential twin data collection periods from the BRR (1998–2002, 2003–2004, 2009), and cover the Tokyo metropolis and the neighboring prefectures (Kanagawa, Chiba, and Saitama). The 1998–2002 data contain approximately 10,000 pairs, which substantially overlap with the 2003–2004 data set, which contains 46,000 pairs. In addition, data from around 1,000 pairs of twins under 3 years of age were added in 2009. Currently, approximately 48,000 sets of multiple birth families are registered at our center.

Additional recruitment of twin child cohorts was conducted by voluntary participation through a poster campaign in public healthcare centers in the target areas and magazine advertisements in publications distributed nation-wide.

Zygoty Diagnosis and DNA Data

In order to identify twins' zygoty, the KTS project mainly used a three-item questionnaire administered to twins themselves (Ooki et al., 1990), whereas the ToTCoP administered the questionnaire to parents (Ooki & Asaka, 2004). These questionnaires asked for judgments about the twins' physical similarities, and experiences of being mistaken for each other. The items in the ToTCoP questionnaire (and the KTS questionnaire) were as follows: 'Were your twin children (you and your co-twin) as alike as two peas in a pod?' 'Were your twin children (you and your co-twin) mixed up (as children)?' and 'If so, by whom were your twin children (you) mixed up?' This questionnaire has been found to have almost 95% accuracy by comparison with genetic markers (Ooki & Asaka, 2004).

DNA samples were collected from approximately 600 pairs of adult twins (KTS) by analyzing blood (approximately 240 pairs in 1998, partially replicated in 1999), buccal smear (approximately 200 pairs in 2005), nail or hair roots (approximately 100 pairs in 2010), and saliva (approximately 60 pairs in 2011; Table 1). These DNA data were also used to identify zygoty. Agreement rate between the DNA-based diagnoses and the questionnaire-based diagnoses was 93.0% (94.3% for monozygotic (MZ) and 87.5% for dizygotic (DZ)); a preliminary result was reported by Shikishima et al., 2007).

In the following sections, the research design and major findings of each of the sub-projects at the KoTReC are introduced.

TABLE 1

Data Collection History of the KTS (KTP)

			N	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Publications
Entry	Twin	Wave 1	315 pairs	—————→															
		Wave 2	45 pairs	—————→															
		Wave 3	354 pairs	—————→															
		Wave 4	312 pairs	—————→															
		Wave 5	672 pairs	—————→															
		Wave 6	548 pairs	—————→															
	Parent of	Wave 1 & 2	196 Mothers 173 Fathers					X											
		Wave 6	600 Mothers					X											
Survey	On campus			X	X	X	X	X	X	X	X	X	X			X	X	X	
	On mail						X	X		X	X	X				X	X	X	
	Online														X	X	X		
Biology	DNA ^a	N of pairs	BI 238	BI (70)							Bc 218	Bl 7				N & H/BI 101/11	S/BI 59/6		
	Hormone (teststerone)											X							
Cognition	Nx_full										X	X				X	X		Shikishima et al., 2009
	Nx_sub		X	X			X			X	X								Ando et al., 2001
	BAROCO_full									X	X					X	X		Shikishima et al., 2009
	BAROCO Short															X	X		Shikishima, et al., 2011a
	WAIS				X	X	X	X											
	ERP/EEG				X	X	X	X											
	Reaction time				X														
Inspection time				X															
Working memory		X																	Ando et al., 2001
3D mental rotation		X	X			X			X	X									Suzuki et al., 2011
Decision making	Economic game ^b										PG				PG	PG	D/U		
	Time preference														X		X		
	Allais paradox															X	X		
	Ellsberg paradox															X	X		
Personality	NEO-PI-R		X	X		X	X												Ono et al., 2000/ Yamagata et al., 2006/ Jang et al., 2001/ Jang et al., 2008 / McCrae et al., 2008/ Ekehammar et al., 2010
	NEO-FFI										X		X				X	X	
	TCI			X		X	X								X	X		X	Ando et al., 2004/ Rushton et al., 2009
	BIS/BAS							X				X						X	Takahashi et al., 2007
SPSRQ								X	X										
EC								X											Yamagata et al., 2005a

TABLE 1

Continued.

		N	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Publications
Mental health	HADS		X			X	X					X						Ono et al., 2002
	SUBI			X		X	X								X		X	
	AQ						X	X										
	STAI							X										
	SDS							X										
	QIDS-SR										X							
	QLS										X							
Attitude	Social attitude						X	X							X			Shikishima et al., 2006
	Voting behavior													X	X			
	RSES			X		X	X	X								X	X	
	General trust			X		X	X									X	X	
	Empathy			X		X	X								X			
Authoritarianism						X	X									X	Shikishima et al., 2008b Shikishima et al., 2011b Shikishima et al., 2008	
Gender	PSAI										X							Hiraishi et al., 2011
	Klein Grid										X							
	BSRI			X		X	X											
Eating	EAT					X	X											Kamakura et al., 2003
	EDI								X	X								
	TFEQ-R21									X								
Physical	Smoking/drinking			X		X	X										X	Hur et al., 2008 Hiraishi et al., 2011
	Height/weight			X		X	X		X	X							X	
	2D4D								X	X								
	Laterality									X							X	
Environment	SIDE						X	X										Shikishima et al., in press Shikishima & Ando, 2004
	School background															X		
	PBI			X		X	X								X		X	
Parental data	FACESIII						X	X										Shikishima & Ando, 2004
	PBI						X											
	RSES						X											
	SUBI						X											
	HADS						X											
	TCI																X	

Note: ^aBI = blood; S = saliva; Bc = buccal smear; N = nail; H = hair root.^bPG = public game; D = dictator game; U = ultimatum game.

Abbreviation of instruments not introduced in the text.

SPSRQ (The Sensitivity to Punishment and Sensitivity to Reward Questionnaire; Torrubia et al., 2001); EC (Japanese version of Effortful Control Scale; Yamagata et al., 2005b); Klein Grid (Klein et al., 1985); RSES (Rosenberg Self-Esteem Scale; Rosenberg, 1965); PSAI (Pre-School Activities Inventory; Golombok & Rust, 1993); BSRI (Bem Sex Role Inventory; Bem, 1974); EAT (Eating Attitude Test; Garner et al., 1982); TFEQ-R21 (Three Factor Eating Questionnaire; Stunkard & Messick, 1985); SIDE (The Sibling Inventory of Differential Experience; Daniels & Plomin, 1985); PBI (Parental Bonding Instrument; Parker et al., 1979); FACESIII (Family Adaptability and Cohesion Scale; Olson, 1985).

The KTS

The KTS, originally named the Keio Twin Project (Shikishima et al., 2006), was established in 1998 to conduct behavioral genetic studies in adolescence and early adulthood. Twins entering the study in 1998 were aged between 15 and 30 years of age, and new participants within the same age range were added subsequently. Table 1 shows the major variables and survey administration year by year. As shown in the table, there were six entry time points in 1998, 1999, 2001, 2002, 2007, and 2011, totaling more than 2,000 twin pair data sets, some of which include their parents' data.

The variables investigated include cognition (general and specific cognitive abilities), decision-making tasks, personality traits (two-, five-, and seven-factor models), mental health, attitude and gender, eating, physical traits, and family and school environment.

Cognition and Decision Making

Cognition has been an important phenotype of interest in the history of behavioral genetics. At the KoTRC, the Kyodai Nx15- (Lynn et al., 1987; Osaka & Umamoto, 1973; Shikishima et al., 2009) is used as a full-scale intelligence test to measure individual difference of general cognitive ability in adolescence and adulthood. The Kyodai Nx15- is the most systematic group intelligence test available for this age range in Japan, and consists of 12 sub-scales covering verbal and spatial aspects of reasoning, memory, and processing speed. In situations where the full-scale version is too long to be administered (i.e., in an experimental session with many variables), a four-subscale version with two verbal and two spatial sub-tests is used.

Overall, our results indicate that the cognitive domain is a unitary feature of its genetic structure. Ando et al. (2001) reported that different aspects of working memory, storage, and executive functions of verbal and spatial modalities are mediated by a single latent genetic factor that also explains general cognitive ability, measured by the sub-scale version of the Kyodai Nx-15. Shikishima and colleagues developed a syllogistic reasoning test called BAROCO (Shikishima et al., 2009), named from a mnemonic word to memorize a syllogism form in classical logic, with 100 items, and reported that its genetic component completely overlapped with those of the Kyodai Nx-15. Based on these findings, a shortened five-item version, the BAROCO Short, was developed and validated (Shikishima et al., 2011a).

Researchers in our project recently began investigating the possibility of a 'general intelligence gene' by comparing epigenetic differences of discordant identical twin siblings (Yu et al., 2012).

Gender differences in spatial ability were independently investigated using a mental rotation task (Suzuki et al., 2011; Vandenberg & Kuse, 1978). A sex limitation analysis revealed that there were no gender-specific genetic factors

affecting this trait, but that the additive genetic influence was greater in males.

Endophenotypes of cognitive abilities, event-related potential (ERP) indices in a working memory task and electroencephalography under resting conditions with eyes open and closed were measured individually for approximately 150 pairs of twins, together with the full-scale Wechsler Adult Intelligence Scale and specific cognitive abilities, simple reaction time and inspection time, in an international collaborative study (Wright et al., 2001). These data, and data from another endophenotype (structural brain imaging examined using magnetic resonance imaging), will be analyzed and published in the near future.

We recently began to conduct behavioral genetic studies of 'decision-making' tasks, such as economic games (a public goods task, and the dictator and ultimatum games), time preferences, and Allais and Ellsberg paradoxes, which are commonly used tasks in behavioral economics. Collaborative studies with economists are also underway at the center.

Personality and Mental Health

Personality traits have been another important research focus in behavioral genetics, and studies in our project have investigated the genetic structure of personality and related phenotypes. Ono and colleagues reported the results of a univariate genetic analysis of the five-factor model of personality using the NEO Personality Inventory Revised Test (NEO-PI-R, Costa & McCrae, 1992; Yoshimura et al., 1998). The results clearly replicated a very robust finding of this field that there are substantial genetic and non-shared environmental influences on personality traits (Ono et al., 2000). Yamagata conducted an international comparative study of the five-factor model by conducting genetic factor analysis based upon 30 sub-scales of the NEO-PI-R, revealing that the genetic structure is strikingly congruent among Japan, Germany, and Canada (Yamagata et al., 2006). Using the same data set, Jang reported genetic comorbidity between Neuroticism and Agreeableness, and their molecular bases (Jang et al., 2001), and proposed a two-higher-order-genetic-factor structure of the Big Five factors (Jang et al., 2006). Furthermore, McCrae, who originally developed the NEO-PI-R, reported that these higher-order genetic factors contained artifacts as well as substance effects (McCrae et al., 2008). Conversely, Rushton proposed a single general personality factor model and reported its genetic validity using our NEO-PI-R and the Temperament and Character Inventory (TCI) data (Rushton et al., 2009).

The TCI was developed by Cloninger, based upon his theory of personality development (Cloninger et al., 1993), which proposes that four temperamental traits (Novelty Seeking, Harm Avoidance, Reward Dependence, and Persistence) are driven by genetic neurotransmission-related factors, whereas three character traits (Self-Directedness, Cooperativeness, and Self-Transcendence) are determined

by post-natal experience. A study in our project attempted to verify this theory, revealing that Novelty Seeking, Harm Avoidance, and Reward Dependence are genetically independent, as Cloninger et al.'s (1993) theory predicts, but persistence and the three character traits exhibited genetic overlap with the three temperamental traits (Ando et al., 2002). In addition, we found that one facet of Novelty Seeking (Exploratory Excitement) is strongly genetically correlated with Harm Avoidance, so should be rearranged by changing combination of facets to make scales genetically consistent (Ando et al., 2004). Yamagata and colleagues (2005) applied the same methodology to examine the genetic structure of Effortful Control (Rothbart et al., 2000) and confirmed its genetic coherence, supporting the validity of the theory.

Ono and colleagues investigated the genetic and environmental overlap between temperamental TCI traits and depressive symptoms measured by the Hospital Anxiety Depression Scale (Kitamura, 1993; Zigmond & Snaith, 1983), suggesting that there are no independent 'depression-specific genes', but that depressive symptoms are dependent on genetic factors involved in normal temperamental dimensions under specific unique environments (Ono et al., 2002). The twin studies at KoTReC are not hospital-based studies, and no medically diagnosed participants have been identified. However, the data from several scales related to mental health and psychiatry, including the Subjective Well-Being Inventory (SUBI; Sell & Nagpal, 1992), the Autism-Spectrum Questionnaire (AQ; Baron-Cohen et al., 2006), the State and Trait Anxiety Inventory (STAI; Spielberger et al., 1970), the Zung Self-Rating Depression Scale (SDS), the Quick Inventory of Depressive Symptomatology (QID-SR), and the Quality of Life Scale (QLS; Rush et al., 2003), are available for our normal twin samples. In addition, a univariate genetic analysis of Eating Disorder Inventory (EDI) data in this sample revealed substantial shared environmental influences on four of five sub-scales of the EDI (Kamakura et al., 2003).

Because the KTS is designed in a longitudinal fashion as shown in Table 1, several cognitive and personality phenotypes were measured at different time points for the same individuals. Developmental changes and the stability of the Behavioral Inhibition System (BIS) and Behavioral Activation System (BAS; Carver & White, 1994) — two measures of temperament based on Gray's reinforcement sensitivity theory — have been investigated (Takahashi et al., 2007). The results indicated that genetic influences contribute only to continuity, whereas environmental influences contribute to both continuity and change in the two traits, and that the degree of genetic influences does not differ across time.

Attitudes

Results similar to those reported by Takahashi et al.'s (2007) BIS/BAS longitudinal study were reported for the self-

esteem scale (Kamakura et al., 2007). Developmental stability was affected by genetic and non-shared environmental factors, whereas developmental changes were affected by non-shared environmental factors. However, the degree of genetic influence increased during adolescence and young adulthood.

Self-esteem is a personality trait, and can be considered as a type of attitude. Our twin studies have involved a number of measures of attitudes other than self-esteem, such as general trust, voting behavior, empathy, and authoritarianism (Table 1). Shikishima reported a series of behavioral genetic studies on attitude variables traditionally thought to be transmitted through the family environment. The results revealed a substantial genetic influence on authoritarianism (Shikishima et al., 2008) and trust (Shikishima et al., 2006), with no significant effect of shared environmental factors. However, significant environment \times environment interactions were found, indicating that shared family environmental factors significantly affected empathy for individuals exhibiting high or very low parental warmth (Shikishima et al., 2011b). A study using direction of causation (DOC) analysis (Heath et al., 1993), an application of behavioral genetic methodology, revealed that the level of general trust can be predicted by personality factors (Extraversion and Agreeableness; Hiraishi et al., 2008a), indicating that humans adaptively control the activation of domain-specific mental mechanisms in accord with domain-general genetic traits like personality.

Other Variables

As shown in Table 1, a large number of variables have been investigated in previous studies, some of which have been published. These variables include eating disorder symptoms (EDI; Kamakura et al., 2003), gender role personality factors (Sasaki et al., 2009), testosterone (Uchida et al., 2006), the relationship between second to fourth finger ratio (2D4D) and sexual orientation (Hiraishi et al., 2012), and parenting (Shikishima et al., in press).

In 2002, 2010, and 2011, parents of the twin participants in our studies provided information about several additional variables. Since 2009, the Web interface of our project (<http://www.futago-labo.net/> in Japanese only) has been available to supplement some experimental and questionnaire data.

The ToTCoP

ToTCoP was established to conduct a longitudinal cohort twin study starting from 2003 (Ando et al., 2006) and continues to conduct studies *of*, *by*, and *for* twins from infancy. This project consists of four data sources: (1) questionnaires (Table 2), (2) cognitive and social investigations in the home (Table 3), (3) cognitive, linguistic, and social investigations in university-based laboratories (Table 4), and (4) brain

TABLE 2
Timeline of Investigation Tools in Questionnaire-Based Research

	Month	Entry	9 months	12 months	15 months	18 months	24–30 months	36 months	42 months	48 months	Preschool	1st grade	
Children's characteristics	Body sizes	1,639 x	142 x	256 x	376 x	458 x	249 x	585 x	675 x	475 x	392	303	
	Stressful life events		x	x	x	x	x	x	x	x			
	Zygoty				x		x	x					
	Laterality		x	x	x		x	x					
	Motor development			DenverII			DenverII						
	Temperament			IBQ-R	R-ITQ		ECBQ			CBQ		BIS/BAS	BIS/BAS
	Developmental disorder-related symptoms/social behavior	Excerpt from M-CHAT	M-CHAT		Yale Screener	M-CHAT	Yale Screener					ADHD school refusal behavior (original)	ADHD school refusal behavior (original)
	Sleeping behavior	x			BISQ		BISQMEQ		BISQ	MEQ	MEQ	MEQ	MEQ
Problem behavior							SDQ		SDQ	SDQ	SDQ	ODBI KINDL	
Nutrition	x	x	CFQ			x			x				
Parenting behavior/ environment	Attachment		MAI		MAI								
	Cultural/ educational environment					x			x		x (original)	x (original)	
	Parental behavior		x		x				x		x (original)	x (original)	
	Home environment		EES			EES					EES (6 items)	EES (6 items)	
	Twin situation		x		x		x			x	SIB (18 items)		
Parenting stress	Depressive symptom			SDS		SDS		SDS		SDS			
	Parental stress		PSI		PSI						PSI	PSI	
	Social support		x	x	x		x	x		x	x(1 item)	x(1 item)	
	Marital status										RAM Short	RAM Short	

Note: ADHD-RS-IV = ADHD Rating Scale — IV, (DuPaul et al., 1998); BISQ = Brief Infant Sleep Questionnaire, (Sadeh, 2004); BIS/BAS = Behavioral Inhibition and Activation Systems Scales (Carver & White, 1994); CBQ = Children's Behavior Questionnaire (Ahadi et al., 1993); CFQ = Child Feeding Questionnaire (Birch, 2001), Denver II (Frankenburg et al., 1992); ECBQ = Early Childhood Behavior Questionnaire (Putman et al., 2002); EES = Evaluation of Environmental Stimulation (Anne, 1997); MAI = Maternal Attachment Inventory (Müller, 1994); IBQ-R = Infant Behavior Questionnaire-Revised (Gartstein & Rothbart, 2003; Nakagawa & Sukigawa, 2005); MEQ = Morningness-Eveningness Questionnaire (Horne & Östberg, 1976); M-CHAT = Modified Checklist for Autism in Toddlers (Baron-Cohen et al., 1992; Robins et al., 2001); ODBI = Oppositional Defiant Behavior Inventory (Harada et al., 2004); PTCI = Preschool Temperament & Character Inventory (Constantino et al., 2002); KINDL = Questionnaire for Measuring Health-Related Quality of Life in Children and Adolescents (Bullinger et al., 1994); RAM = Relationship Attribution Measure (Fincham & Bradbury, 1992); SDQ = Strength and Difficulty Questionnaire (Goodman, 1999); SDS = Self-rated Depression Scale (Zung, 1965); SIB = The Sibling Inventory of Behavior (Volling & Blandon, 2005); Short Marital-Adjustment Scale (Locke & Wallace, 1959).

TABLE 3
Timeline of Home Assessment

Age N (pairs)		12 months 127	18 months 236	24 months 277	36 months 279	48 months 188
	Cognitive ability	Bayley II	Bayley II	Bayley II	K-ABC	K-ABC
	Vocabulary	CDI	CDI	CDI		
	Socio-cognitive ability	ESCS	ESCS			
				ToM EF	ToM EF	ToM EF
Observation	Parent-child relationship	x	x	x	x	x
	Twin sibling relationship				x	x
Questionnaire	Parenting stress	PSI	PSI	PSI	PSI	PSI
	Parenting behavior	x	x	x	x	x
	Depressive symptom			SDS		
	Marital relation				Marital love	Marital love
	Mom's personality				NEO-FFI	
	Problem behavior					SDQ

Note: Bayley = Bayley Scales of Infant Development (Bayley, 1993); ESCS = Early Social Communication Scales (Mundy et al., 2003); K-ABC = Kaufman Assessment Battery for Children (Kaufman & Kaufman, 1983); CDI = MacArthur Communicative Developmental Inventories (Fenson et al., 1993); PSI = Parenting Stress Inventory (Abidin et al., 1995); Marital love (Locke & Wallace, 1959).

TABLE 4
Timeline of Laboratory Assessment

Age N (pairs)		42 months 245	60 months 175
	Cognitive ability	K-ABC	K-ABC
	Reading	x	x
	Socio-cognitive ability	EF ToM	
Observation	Parent-child relationship	x	x
	Twin sibling relationship	x	x
	Social communication (peer)	x	x
Questionnaire	Parenting Stress	PSI	PSI
	Parenting Behavior	x	x
			PFQ PDI
	Marital relation	Marital love	Marital love
	Mom's personality	NEO-FFI	
	Problem behavior	SDQ	SDQ
	Temperament		CBQ-VSF
	Twin sibling relationship		SIB
		MISR	MISR

Note: PFQ = Parental Feelings Questionnaire (Deater-Deckard, 1996; Deater-Deckard, 2000); PDI = Parental Discipline Interview, (Deater-Deckard, 2000); SIB = Sibling Inventory of Behavior (Volling & Blandon, 2005); MISR = Maternal Interview of Sibling Relationships (Stolker et al., 1989).

activity and motor skill experiments in university-based laboratories.

Questionnaire-Based Research

Table 2 shows the timeline of the questionnaire investigation tools used for each specific time point from infancy to childhood when twins enter elementary school. The variables in these questionnaires are related to children's characteristics and parents' characteristics, and both types of questions are given to both mothers and fathers until participants are 36 months old. The versions for fathers are partially shortened

or different from the versions for mothers, which contain additional items regarding parenting stress. When participants are aged 42 months or older, the questionnaires are administered only to twins' mothers, because asking twins' fathers to answer questionnaires tended to lower the total response rate, and the reliability of fathers' evaluations of twins' behavior was low.

As Table 2 indicates, the number of participating twin families (over 1,600) was relatively large at the first session, constituting approximately 55% of the total twin births in the target area. Although we observed a high degree of data attrition, we retained substantial numbers of twin pairs that could be investigated longitudinally. For example, Fujisawa and colleagues investigated the relationship between head circumference growth from birth to 10 months of age, and socio-cognitive ability at 19 months. Although no significant phenotypic correlation was found between them, significant genetic and shared environmental correlations in opposite directions (i.e., genetically negative and environmentally positive) were reported (Fujisawa et al., 2012a). In addition, Yamagata examined the longitudinal association between authoritative parenting and children's peer problems at 42 and 48 months using a longitudinal MZ twin difference design. They reported that when genetic and family environmental covariates were controlled, authoritative parenting and children's peer problems concurrently influenced each other, peer problems increased authoritative parenting, and authoritative parenting decreased peer problems, canceling each other out (Yamagata et al., in press).

For preschool and first grade elementary school children, additional twin families were recruited. The main research target of these two age groups is social adaptation to changes in educational environmental conditions from preschool to elementary school. To tap these

TABLE 5
The Variables List of the CROSS Study

		Early childhood	Middle childhood	Late childhood		Adolescence		Adult
		3–5 years	7–9 years	10–12 years		13–18 years		19–26 years
		3,291	3,196	3,396		5,279		5,095
Mailed		859	857	740		960		697
Returned (entry)		26.10%	26.80%	21.80%		18.90%		13.70%
Response rate				Child	Parent	Child	Parent	Child
Informant		Parent	Parent	Child	Parent	Child	Parent	Child
Family and parents	Family structure	X	X	X	X	X	X	X
	Age, sex, zygosity, and sib order	X	X	X	X	X	X	X
	Twinship							X
	Sharing toys, room, clothes, etc.	X	X	X	X	X	X	
	Assisted reproductive technology	X	X		X		X	
	Maternal smoking/drinking in pregnancy				X		X	
	Sib interaction	X	X					
	Child-rearing attitude				X		X	X
	Join twin-mother organization?	X	X					
Same class in nursery school?	X	X		X		X		
Academic-related variables	Income							X
	Name of schools	X	X	X	X	X	X	X
	Academic achievement		X	X	X	X	X	
	Time spent for academic learning		X	X	X	X	X	X
Academic-related variables	Academic motivation					X		
	Learning strategy					X		
	Contingent cognition of effortful results					X		
	Attribution of failure					X		
	Cognition of competition					X		
	Helplessness					X		
In classroom and out of classroom	Intrinsic/extrinsic motivation			X		X		
	Academic autonomy			X		X		
	Goal structure			X		X		
	Self-efficacy			X		X		
	Class mate			X		X		
	Teacher's explanation length			X		X		
	Skill for integrated learning			X		X		
	Class size			X		X		
	Classroom teacher			X		X		
	After-school activities			X		X		
	Academic rank of high school					X		X
	What is society/learning			X		X		X
	Club activity							X
	Family cultural environment		X	X		X		X
Learning out of school	X	X	X		X		X	
Mental health	Depression							X
	QOL							X
	Loneliness				X			X
	Adaptation to school			X		X		
	Suicide					X		X
	CFS			X				
	Problematic behavior	X	X	X		X		
	Gender orientation/identification	X	X	X	X	X	X	X
Personality and social attitude	Personality		X	X		X		X
	Language development	X						
	Correctivism					X		X
	Authoritarianism					X	X	X
	Value					X		
	Hobby							X
	Job supervisor							X
Body and physics	Height/weight	X	X	X		X		X
	Head/chest circumference	X						X
	Eye sight			X		X		X
	Sleeping	X	X	X		X		X
	Blood pressure/ fever					X		X
	Mense					X		
	Allergy	X	X		X			X
	Health			X		X		X
	Liability					X		
	Decade teeth			X				X
	Athletic ability			X		X		

TABLE 5
Continued.

		Early childhood	Middle childhood	Late childhood	Adolescence	Adult
		3–5 years	7–9 years	10–12 years	13–18 years	19–26 years
		3,291	3,196	3,396	5,279	5,095
		859	857	740	960	697
		26.10%	26.80%	21.80%	18.90%	13.70%
Mailed Returned (entry) Response rate Informant		Parent	Parent	Child Parent	Child Parent	Child
	Exercises	X	X			X
	Smoking/drinking					X
	Birth height/weight	X	X	X	X	
	Nutrition					X
	Eating/walking speed	X	X	X	X	X
	Ideal weight				X	
	Nutrition	X	X	X	X	X
Home environment	Home hygienic status	X	X	X	X	X
	Housing condition	X	X	X	X	X
	N of books	X	X	X	X	X
	Reading	X				
	School commuting			X	X	
	Coming home time					X
	Family cohesion				X	X
	Child rearing attitude	X	X	X	X	X
	Parenting		X		X	
	Parental intervention			X	X	
	Media exposure	X	X	X	X	X
	Cell phone			X	X	X
TV game	X					
Parent	Parental job	X	X	X	X	X
	Parental educational history	X	X	X	X	X
	Parental income	X	X	X	X	X
	Sleeping time	X	X			
	Religiousness				X	
	Life-long education			X	X	
	Life events			X	X	X
	Parental personality	X	X	X	X	
	Rearing burden	X				
	Social support	X				

time-specific features of environmental change, we conducted preliminary but relatively large-scale studies with approximately 1,000 non-twin individuals to develop appropriate items.

Performance-Based Research at Home and in the University Lab

Two independent performance-based studies (with some overlapping twin pairs) are currently underway, as shown in Table 3 (assessment and observation at home) and Table 4 (assessment in the university laboratory). Both studies involve individual cognitive ability tests (Bayley II for younger and Kaufman Assessment Battery for Children (K-ABC) for older twin children), theory of mind and executive function tasks, questionnaires, and observation of dyadic and triadic interactions between twin siblings and among twin siblings and parents.

One of the main purposes of our studies is to investigate the development of pre-reading skills and the relationship with cognitive abilities during early childhood. The Japanese *kana* writing system is different from alphabetic systems such as English. Our experiments are designed to

be comparable with English language experiments, such as Byrne et al.'s (2002) study. We developed a Japanese version of a test battery to measure pre-reading skills such as phonological awareness, non-word repetition, receptive vocabulary, and visual perceptual skills (Kakihana et al., 2009). Preliminary results revealed a significant influence of shared environmental factors on kana pre-reading skills, and no significant effect of genetic influence (Fujisawa et al., 2012b). However, we found that genetic factors had significant and stable effects on cognitive abilities (Fujisawa & Ando, 2010, 2011).

As mentioned above, studies of twins typically have another important aim. As such, we compared twin siblings with non-twin siblings to investigate the relationship between sibling relationships and social adjustment among children. We found that the effects of sibling relationships on pro-social behaviors and conduct problems were stronger for twin siblings than for non-twin siblings, and positive relationships between siblings increased peer problems only among MZ twins; this is the opposite effect compared with that reported among DZ twins and non-twin siblings (Nozaki et al., in press).

TABLE 6
Items of Two Anonymous High School Twin Studies

Category		2009	2010
Entry		Anonymous Junior/senior high 570 families (1,062 twins, 553 mothers, & 459 fathers)	Anonymous Senior high 424 families (751 twins, 402 mothers, & 318 fathers)
Physical	Height/weight	X	
Academic	App/av motivation	X	
	Sense of belonging to school	X	X
Cognition	BAROCO Short	X	X
Social attitude	Social attitude	X	
	Party identification	X	
	Attitude to political issues	X	
	RSES	X	X
	Authoritarianism	X	X
Gender	Gender identity	X	
Environment	Class atmosphere	X	X
Environment	Parent party identification	X	
Environment	FACESIII	X	
Parental data	FACESIII	X	
Parental data	BAROCO Short	X	X

Brain Activity and Motor Skills

The stimulation of brain function by social stimuli such as mothers' vocalizations in infancy and early childhood twins was investigated using ERPs and near infrared spectroscopy at 6, 9, 18, and 36 months, and data from a total of 161 pairs of twins are currently being analyzed. Development of laterality, especially handedness, has also been investigated. The results of these studies indicate a non-additive genetic influence on handedness, suggesting that spatial constraint is a crucial factor for the expression of genetic effects on handedness in infants (Suzuki et al., 2009).

Three Independent Anonymous Twin Studies

Longitudinal studies place a heavy burden on participants, sometimes resulting in severe data attrition. To obtain large samples to verify specific research questions, the KoTReC conducted three independent 'anonymous' twin studies (i.e., twins who received questionnaire mails do not have to inform their names to the KoTReC, which lets them know that they are not followed longitudinally and reduces their burdens to collaborate in our research), a large-scale cross-sectional twin study (CROSS) and two high school twin studies.

The CROSS was conducted in 2007 with over 4,000 pairs of twins and their parents, with an age range of 3 to 26 years old. There were five age categories: early childhood from 3 to 5 years old, middle childhood from 6 to 9 years, late childhood from 10 to 12 years, adolescence from 12 to 18 years, and adulthood from 19 to 26 years.

The design and sample size of this study is shown in Table 5. As shown in the table, the item questions in the CROSS were not based upon standardized, well-organized, or internationally used psychological scales like those in our cohort

studies. Rather, the CROSS used independent measures focusing on specific questions, even though some were related and can be grouped in categories such as academic performance and parental stress. For example, Strengths and Difficulties Questionnaire (SDQ; Goodman, 1999) data were used to examine genetic and environmental influences on the relationship between negative parenting and conduct problems of children in terms of attention deficit hyperactivity disorder status (Fujisawa et al., 2012c).

Two high school twin studies (Table 6) were conducted to investigate the genetic and environmental relationships between educational attainment, cognitive ability, and family social environment. Murayama and colleagues (2011) applied academic motivation data to verify the performance-approach and performance-avoidance achievement goal theories (Murayama et al., 2011).

Ozaki (2008) challenged methodological limitations using paired comparison analysis applied to biometric modeling (Ozaki, 2008), non-normal structural equation modeling with higher order moments applied to DOC (Ozaki & Ando, 2009), and estimation of four parameters (additive genetic, non-additive genetic, shared, and non-shared environmental factors) at the same time (Ozaki et al., 2011).

Future Perspectives

The KoTReC has collected the largest active twin sample in Japan, with a total of approximately 9,000 twin pairs from infancy to young adulthood. Some of these data (approximately 2,000 pairs) are longitudinal, and data collection is ongoing. This is the largest Japanese twin research database ever developed. However, many aspects of the database are incomplete. We have not yet established a complete DNA sample from all twin participants in our project because of budget limitations, which have also led to difficulties in long-term planning and administration of well-organized

research. Moreover, there is no systematic system for education about the theories and methods of behavioral genetics in the official curriculums of Japanese universities.

Recruiting twins into research programs presents a further difficulty. We do not have free access to official electronic databases of Japanese residents for scientific use, and conducting manual searches of the BRR is expensive. Compared with many Western countries, Japanese citizens tend to be less willing to participate in scientific research, particularly in psychology and social sciences. The overall average participation rate in our field is around 20% (Ogiwara, 2009; Shinogi, 2010), so data attrition is a serious problem.

Twin research is transitioning from traditional, quantitative-only methodology to the new integrated methodology of neurogenomics research. Recently, researchers from other fields such as economics, sociology, and even philosophy have become involved in twin studies in Japan. We believe that this promising trend will lead to a 'paradigm shift' in the human sciences in Japan.

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