

The Quebec Newborn Twin Study Into Adolescence: 15 Years Later

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The Quebec Newborn Twin Study (QNTS) is an ongoing prospective longitudinal follow-up of a birth cohort of twins born between 1995 and 1998 in the greater Montreal area, Québec, Canada. The goal of QNTS is to document individual differences in the cognitive, behavioral, and social-emotional aspects of developmental health across childhood, their early bio-social determinants, as well as their putative role in later social-emotional adjustment, school and health outcomes. A total of 662 families of twins were initially assessed when the twins were aged 6 months. These twins and their family were then followed regularly. QNTS has 14 waves of data collected or planned, including 5 in preschool. Over the past 15 years, a broad range of physiological, cognitive, behavioral, school, and health phenotypes were documented longitudinally through multi-informant and multi-method measurements. QNTS also entails extended and detailed multi-level assessments of proximal (e.g., parenting behaviors, peer relationships) and distal (e.g., family income) features of the child's environment. This detailed longitudinal information makes QNTS uniquely suited for the study of the role of the early years and gene-environment transactions in development.

■ **Keywords:** longitudinal twin study, gene-environment transactions, infancy, preschool, social-emotional development, peer relationships, language, numeracy, school readiness and achievement

As its name entails, the Quebec Newborn Twin Study (QNTS) is an ongoing prospective longitudinal follow-up of a birth cohort of twins born between 1995 and 1998 in the greater Montreal area. The goal of QNTS is to document individual differences in the cognitive, behavioral, social-emotional aspects of developmental health across childhood, their early bio-social determinants, as well as their putative role in later social-emotional adjustment, school and health outcomes. A population-based sample of more than 660 families of twins were initially enrolled in QNTS, and then assessed longitudinally at regular intervals. The QNTS is part of a larger research initiative that also involves the Québec Longitudinal Study of Child Development (QLSCD), a longitudinal follow-up of a representative sample of more than 2,000 children born as singletons in 1998 in the province of Québec, Canada. The QNTS and the QLSCD are companion studies sharing many features

(measures and time of assessments), including an extensive and detailed set of evaluations in the early years, and thus providing together a unique window into the role of early childhood in development.

Recruitment and Initial Assessment

The recruitment phase of the QNTS was initiated in 1995 with the Québec Newborn Twin Registry, which identified

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all twin births occurring in the Province of Québec between April 1995 and December 1998. Names, addresses, and phone numbers of all the mothers of newborn twins were collected from the computerized birth records of the Québec Bureau of Statistics. Recruitment and maintenance of families in the registry was achieved through a series of periodic mail and telephone contacts, newsletters, and follow-ups. Starting 1 June, 1996, all parents in the registry who lived in seven administrative social health districts comprising and surrounding the Greater Montreal Area were asked to enroll with their twins in the QNTS. Parents were contacted by letter and by phone, and laboratory appointments were scheduled for when the twins were aged between 59 and 61 weeks from conception, or 5 months corrected for gestational age. A total of 989 families were contacted, of which 662 actually participated in the first wave (i.e., at age 6.3 months on average, $SD = 0.73$) between June 1996 and November 1998. A sub-group of these families of twins (with their mothers) was seen in the lab (322 families) where extensive psycho-physiological, hormonal, and observational-behavioral measures were taken. These laboratory assessments were followed within 2 weeks by a home visit to obtain exhaustive social, demographic, health, and further behavioral data on the twins and their families, mainly through interviews of both parents, self-report questionnaires filled out by both parents, and direct observation of the infant, home, and neighborhood by the interviewer. Interviews were done in French or English, depending on the language of the respondents.

Longitudinal Follow-Up

As shown in Table 1, these twin children and their parents were then followed up longitudinally and assessed at 20 months (families seen in the lab at 5 months were again seen in the lab and at home; families seen only at home at 5 months were seen only at home), 32 months (home interview and questionnaires to parents only), 50 months (home interview and questionnaires to parents only), and then again the summer before school entry at 63 months (lab visit in the summer for a battery of cognitive measures), in Kindergarten (school-based assessments: behavioral observations; cognitive assessments; peer and teacher assessments), Grade 1 (similar to kindergarten), Grade 3 (only teacher ratings), Grade 4 (self-, peer, and teacher assessments), and Grade 6. The twins and their families are now being followed into secondary school, with assessments in Secondary 1, Secondary 2 (ongoing), Secondary 3 (ongoing), and Secondary 5 (planned for 2013–2015).

Longitudinal Measures of Child Phenotypes

Over these years, participants were assessed longitudinally on a broad range of characteristics through multi-informant (mothers, fathers, caregivers, teachers, age-peers,

TABLE 1
Longitudinal Follow-Up of the QNTS

Wave	Mean age (months)	SD	N of pairs
6 months	6.3	.73	662
20 months	19.6	.82	566
32 months	31.9	.99	524
50 months	50.2	1.9	442
63 months	63.5	3.3	446
Kindergarten	72.5	3.3	402
Grade 1	84.9	3.2	476
Grade 3	109.2	3.5	393
Grade 4	120	3.4	444
Grade 6	145.1	3.4	406
Sec 1	156.7	3.7	410
Sec 2	169.2	3.6	404
Sec 3	180.5	3.2	TBD

Note: SD = standard deviation; N of Pairs = number of twin pairs; Sec = secondary; TBD = to be determined.

and multi-method measurements (behavior ratings, semi-structured interviews, interactive interviews, peer assessments, direct observation). Whenever possible, a 'blind' procedure was used through which assessments were taken and rated by research personnel who were not exposed to both members of a twin pair, reducing potential biases related to the perceived zygosity of subjects. At each assessment time, computerized face-to-face interviews of the Person Most Knowledgeable about the child (PMK, usually the mother in the early years) were conducted. Regular assessments also included questionnaires completed by the mother, the father, and the interviewer, and direct and multi-faceted assessments of cognitive development, with a strong emphasis on school readiness at 63 months and in Kindergarten (e.g., literacy, vocabulary, numeracy, general IQ), and school achievement at each time point of grade school. Social behaviors were assessed yearly from 20 months on, through PMK, spouse and interviewer ratings, teacher ratings (in primary school), and self-ratings (starting at the end of primary school, and then in secondary school); a variant of the Child Social Behavior Questionnaire was used, which centers on behavior problems such as hyperactivity, impulsivity, inattention, aggression, and social withdrawal. In secondary school, the self-ratings also covered substance use, gambling, peer relationships, and psychological adjustment. Behavior observations were conducted in a controlled setting when the twins were aged 6 months and 20 months, and in Kindergarten. Cortisol samples were collected at 6 months, 20 months, 63 months, Kindergarten, and are now being collected in Secondary 2.

Longitudinal Measures of the Child's Environment

The QNTS also entails extended and detailed multi-level assessments of proximal (e.g., parenting behaviors) and distal (e.g., family income) features of the child's environment across development. Parenting behaviors were assessed yearly, starting at 5 months, through mother and

father self-reports of parental warmth, self-efficacy and impact, overprotection, and hostile-reactive behavior. Peer relationships and experiences (e.g., peer rejection and victimization) were assessed in Kindergarten, in Grade 1, and in Grade 4 through peer assessments and classroom sociometric interviews, a unique feature for a population-based study of this scale (see below for details). Other putative measures of the child environment collected regularly are about nutrition (see below), the mental health status of parents, marital satisfaction and status, as well as a variety of socio-economic and demographic characteristics (e.g., family income).

Additional Data Collection

In addition to the 14 regular data collections, QNTS was supplemented with unique ad hoc data collections aimed at:

1. *Pre-natal and perinatal health.* Medical records are available for 613 families of twins (based on the number of Apgar at 1 minute, available), and were supplemented with mother report self-report when the twins were 6 months old, allowing for the assessment of specific pre-natal and perinatal risk factors, including maternal smoking (about 26% of the mothers smoked during pregnancy), drinking or substance abuse during pregnancy (32% had alcohol at least once, 9% more than once, 3% more than once per week), newborn's birth weight (47% of the twins were born low birth weight (LBW), that is, less than 2.5 kg, 5% as very low birth weight (VLBW), that is, less than 1.5 kg.), APGAR score (i.e., an indication of child health at birth, muscle tone, reflex, breathing) at 1 minute and 5 minutes, hospital length of stay, type of birth (C-section), gestational diabetes, pre-eclampsia and hypertension during pregnancy, gestational age at delivery, and hypoxia.
2. *Genotyping.* Blood and saliva samples were collected in 375 families (the twin and their parents) when the twins were approximately 100 months of age, allowing for the genotyping of parents and twins of these families.
3. *Peer assessment and sociometry.* In Kindergarten, and then in Grade 1 and Grade 4, the participating twins' schools and classrooms were identified. With the authorized consents of the school boards, the school directors, the teachers, and the parents, the classmates of each twin were asked to participate in a peer nomination task, as well as to fill out a short questionnaire about their behavior. Using booklets of photographs, the participating children were asked to nominate three classmates for each of a series of behavioral and social descriptors. This information could then be used to assess: (1) the degree to which the twins were liked/disliked by their peers, (2) victimized by their peers, (3) the twin's behavior (e.g., aggressive, hyperactive, inattentive, impulsive, withdrawn, shy, or leadership) as perceived by their peers, as well as identify the classmates who were their friends. In Kindergarten, there were 569 participating classrooms in 325 schools, with an average of 14 'nominating' children per class (30% of the twins were in the same class). In Grade 1, the numbers were 636 classrooms in 362 schools, with an average of 14 'nominating' children per class (23% of the twins were in the same class). Finally, in Grade 4, the peer assessments were done in 581 classrooms in 348 schools, with an average of 19 'nominating' children per class (28% of the twins were in the same class). This rich information is a unique feature of QNTS and provides a unique window into the quality of the twins' peer experiences, as well as of their social behavior as perceived by their classmates.
4. *Nutrition.* In addition to nutrition and body weight data regularly collected over the years (e.g., breastfeeding, eating behaviors), a detailed nutrition survey was done with the twins when they were approximately 9 years of age. Nutrition data were collected with a 24-hour recall, repeated two times on separate days (including one weekend day) by trained nutritionists using wooden volume models to estimate portion size (Hill & Davies, 2001). From this database, energy, carbohydrates, fat, protein, vitamins and minerals, and food group consumption will be estimated.
5. *Neuropsychological assessments.* As part of a project aimed at the documenting the neuropsychological underpinnings of early inattention, impulsiveness, and restlessness, a sub-sample of 150 pairs of QNTS were seen in the lab when they were aged about 100 months between April 2004 and April 2006. These QNTS twins were probed for the presence of symptoms of inattention, impulsiveness, and hyperactivity, and assessed through a battery of different neuropsychological tests, including a mixed go/no-go and stop task (measuring both restrain and cancellation in a single paradigm), continuous performance task (oddball), and a visuo-spatial working memory task. Their brain electrical activity was also recorded using a large array of electrodes (electroencephalography/event-related potential (EEG/ERP)) during these tests, and brain anatomical magnetic resonance imaging data was collected.
6. *Mathematical abilities and number sense.* In addition to measures of early numeracy (number knowledge), and mathematical achievement and motivation, regularly collected in QNTS, a Web-based test battery developed as part of the Twins Early Development Study (Robert Plomin, Director), adapted and validated for international administration by the International Laboratory for Interdisciplinary Investigations into Individual Differences in Learning (in-Lab, Goldsmith College, University of London, Dr. Yulia Kovas, Director), is now being administered to the QNTS twins at the end of

Secondary 3, when the twins are aged 15. This battery of eight tasks covers a wide range of abilities (e.g., estimation of numbers and numerical magnitude, visuo-spatial working memory, speed of processing, problem verification) that underlie number sense and mathematical skills. It is now being used in different cohorts internationally, including in Russia, the United Kingdom (London), the United States (Ohio), and Kyrgyzia. The administration of this battery to the twins of QNTS will be completed by the summer of 2013.

Zygosity Assessment

Zygosity was ascertained through the analysis of genetic markers for same-sex twin pairs, supplemented by diagnoses based on physical similarity when genetic diagnoses were inconclusive or could not be obtained. Eight or nine highly polymorphic genetic markers were used for genotyping. Any difference between the twins of a pair resulted in a dizygotic (DZ) diagnosis. The twins were diagnosed monozygotic (MZ) when concordant for every genetic marker. The diagnosis of zygosity based on genetic marker analysis can be considered certain. The genetic marker analysis could not be made for twins who drop out of the study early and for twins for whom parents refused consent. In some cases, the genetic material was insufficient for genotyping. In these cases, the pairs were diagnosed mainly on the basis of a shortened version of Goldsmith's Zygosity Questionnaire for Young Twins (Goldsmith, 1991) at 5 and 18 months. When compared with the diagnoses obtained by genetic marker analysis in a sub-sample of 237 same-sex pairs, the diagnoses based on physical similarity were accurate in 92% of the cases at 5 months and in 94% of cases at 19 months. Supplemented with chorionicity data, the accuracy rate climbed to 96% (Forget-Dubois et al., 2003). The final zygosity diagnosis for all same-sex pairs was constructed this way: (1) If a diagnosis by genetic marker analysis was available, it is considered the final diagnosis; (2) In the absence of a diagnosis based on genetic marker analysis, the final zygosity diagnosis is based on physical similarity at 5 and 18 months, supplemented whenever possible by data on blood group and chorionicity. The diagnoses based only on physical similarity were re-evaluated in April 2007 with physical similarity questionnaires (adapted from Spitz et al., 1996) answered by the parents during a brief telephone interview. A final diagnosis (September 2007) indicated that QNTS consisted in 254 pairs of MZ twins (125 boy pairs and 129 girl pairs), 210 pairs of same-sex DZ twins (105 boy pairs and 105 girl pairs), and 203 pairs of opposite-sex DZ twins.

Scientific Contribution

The QNTS is uniquely suited to document the genetic and environmental etiology of a variety of phenotypes early in development as well as longitudinally. Here are a few exam-

ples of QNTS-based contributions. We have documented variations in the genetic and environmental contributions to different forms and functions of aggressive behaviors, such as reactive versus proactive versus social aggression at school entry (Brendgen et al., 2005, 2006), as well as to physical aggression, language, and their association at 18 months of age (Dionne et al., 2003). We also showed that the stability, but not the episodic manifestations of disregard for rules, a central component of early opposition, has a significant genetic component (Petitclerc et al., 2011). In contrast, cognitive school readiness before school entry was found more strongly accounted for by environmental factors shared and uniquely experienced by children of the same family than by genetic predisposition. Cognitive school readiness was highly predictive of school achievement in the early grades, and this predictive association was also substantially accounted for by shared and unique environmental factors (Lemelin et al., 2007; see also Vitaro et al., 2012). We have provided evidence for the central role of early literacy training (i.e., parent reading to the child) in accounting for the link between socio-economic status and school readiness (Forget-Dubois et al., 2009), as well as shown how gestational diabetes may specifically hinder early language development (Dionne et al., 2008). This ongoing line of research clearly indicates that school achievement and readiness are mainly environmentally mediated and have their roots in early development.

Thanks to the detailed longitudinal information about the twins' development and their (measured) environment, the QNTS is uniquely suited for the investigation of gene-environment transactions in development, such as gene-environment correlations and gene-environment interaction processes. Many scientific contributions of QNTS are along those lines. For example, a series of studies on the genetic and environmental determinants of early (i.e., 5 months and 18 months) vulnerability to stress regulation has provided evidence for a complex pattern of time-dependent gene-by-environment interaction in the cortisol response (Ouellet-Morin et al., 2008, 2009). These patterns indicate that the role of genes in the stress response varies as a function of family adversity, but in a developmentally coherent way over time; significant heritability was found only in the presence of family adversity at 6 months, but a reverse pattern was revealed at 18 months (i.e., heritability in the absence of adversity, and no heritability in the presence of adversity). This pattern suggests an evolving process of gene \times environment interaction in the stress response, and is quite consistent with a programming effect of the environment (possibly through parenting) superseding genetic influence at 18 months. This exciting new line of research is ongoing, and we are currently examining new cortisol samples collected later in QNTS to see if this pattern is maintained, to document the possible early family mediators of these patterns (i.e., parenting), and to examine the nature of the association between the stress response and peer difficulties.

Parenting and peer relationship are indeed well documented in QNTS. With respect to parenting, we have documented the presence of a gene-environment linking child difficult temperament and harsh-reactive parenting behaviors at 6 months of age (Boivin et al., 2005), but not later, over the 6- to 32-month period (Forget-Dubois et al., 2007).

As indicated earlier, peer relationships are perhaps the best-documented features of the twins' environment in QNTS. Taking advantage of this longitudinal information, we found that peer relationship difficulties, while substantially associated with genetic vulnerabilities through evocative gene-environment (g-e) correlation (Boivin et al., in press), also interact in complex ways with aggressive behaviors and psychological adjustment across development (Brendgen et al., 2008b, 2009, 2011). Gene \times environment interaction processes were also revealed for the moderating role of friendship in behavior problems, peer relation difficulties, and personal adjustment in the early years of school (Brendgen et al., 2008a, in press; van Lier et al., 2007). For example, we found that genetic vulnerabilities and befriending other aggressive children were independently associated to aggressive behaviors in kindergarten, but also that having an aggressive friend contributed more to aggression for children who are genetically at risk for aggression than for children who were not, thus suggesting gene by environment (g \times e) interaction (van Lier et al., 2007). This pattern was also confirmed among first-graders (Brendgen et al., 2008b).

This non-exhaustive review of some of the most important scientific contributions of QNTS illustrates the potential of this database to elucidate the gene-environment processes underlying (1) the early bio-social determinants of various cognitive, behavioral, and social-emotional components of developmental health across childhood, as well as (2) their role in later social-emotional adjustment, school and health outcomes. Hopefully, this work will also lay the foundation for further interdisciplinary and international collaborations around QNTS on these issues.

Future Directions

QNTS will soon open a new chapter with respect to data collection. As indicated previously, we are now planning the next data collection when the twins will be about 17 years old; that is, when most of them will be a few months away from completing high school (i.e., Secondary 5 in Québec). This wave will emphasize academic achievement and aspirations, addictive behaviors and peer relationships in addition to the developmental health data usually collected. After this 14th regular wave, the follow-up (i.e., involving a personal contact with the twins) will be more spaced, and we are now examining the possibility of relying on a Web-based approach to do so. In addition, there will be a strong emphasis on collecting information through administrative

records (e.g., education, health, social welfare, and criminal records) to further document the role of childhood factors in developmental health and human capital.

So far, QNTS has produced more than 40 papers in leading international scientific journals. This production is now expanding as the study reaches its maturity. The challenge in the coming years will be to take full advantage of the wealth in QNTS data to significantly advance our understanding of gene-environment interplay in developmental health.

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