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Genes, Maternal Smoking, and the Offspring Brain and Body During Adolescence: Design of the Saguenay Youth Study

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Abstract

The search for genes of complex traits is aided by the availability of multiple quantitative phenotypes collected in geographically isolated populations. Here we provide rationale for a large-scale study of gene-environment interactions influencing brain and behavior and cardiovascular and metabolic health in adolescence, namely the Saguenay Youth Study (SYS). The SYS is a retrospective study of long-term consequences of prenatal exposure to maternal cigarette smoking (PEMCS) in which multiple quantitative phenotypes are acquired over five sessions (telephone interview, home, hospital, laboratory, and school). To facilitate the search for genes that modify an

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individual's response to an in utero environment (i.e. PEMCS), the study is family-based (adolescent sibships) and is carried out in a relatively geographically isolated population of the Saguenay Lac-Saint-Jean (SLSJ) region in Quebec, Canada. DNA is acquired in both biological parents and in adolescent siblings. A genome-wide scan will be carried out with sib-pair linkage analyses, and fine mapping of identified loci will be done with family-based association analyses. Adolescent sibships (12–18 years of age; two or more siblings per family) are recruited in high schools throughout the SLSJ region; only children of French-Canadian origin are included. Based on a telephone interview, potential participants are classified as exposed or nonexposed prenatally to maternal cigarette smoking; the two groups are matched for the level of maternal education and the attended school. A total of 500 adolescent participants in each group will be recruited and phenotyped. The following types of datasets are collected in all adolescent participants: (1) magnetic resonance images of brain, abdominal fat, and kidneys, (2) standardized and computer-based neuropsychological tests, (3) hospital-based cardiovascular, body-composition and metabolic assessments, and (4) questionnaire-derived measures (e.g. life habits such as eating and physical activity; drug, alcohol use and delinquency; psychiatric symptoms; personality; home and school environment; academic and vocational attitudes). Parents complete a medical questionnaire, home-environment questionnaire, a handedness questionnaire, and a questionnaire about their current alcohol and drug use, depression, anxiety, and current and past antisocial behavior. To date, we have fully phenotyped a total of 408 adolescent participants. Here we provide the description of the SYS and, using the initial sample, we present information on ascertainment, demographics of the exposed and nonexposed adolescents and their parents, and the initial MRI-based assessment of familiality in the brain size and the volumes of grey and white matter.

Keywords

genetics; isolated population; mri; siblings; heritability; familiarity; morphometry; cognition; cardiovascular; obesity

BACKGROUND

Genetics of Complex Traits

There are two main approaches used to establish a link between a complex genetic trait (phenotype) and genotype: case-control and family-based studies. The case-control approach allows investigators to reveal a trait-genotype association in a population of unrelated individuals based on cooccurrence of a specific gene variant (allele) and a phenotypic value of a trait of interest. Several studies have used this strategy to describe associations between different quantitative measures of brain anatomy and allelic variations of candidate genes in healthy human subjects. These include associations between polymorphisms in the promotor of the 5-HT transporter gene and the grey-matter volume in the subgenual cingulate cortex [Pezawas et al., 2005; $n = 114$], polymorphisms in monoamine oxidase A gene and amygdala volume [Meyer-Lindenberg et al., 2006; $n = 97$], and polymorphism in brain derived neurotrophic factor gene and hippocampal volume [Bueller et al., 2006; $n = 36$]. Such case-control studies, when compared with family-based investigations, have certain limitations. First, they do not allow carrying out a linkage analysis and, as such, they are less suitable for a search for genes influencing a trait of interest throughout the entire genome.

This is despite the fact that massive efforts are currently being made to develop new strategies that would allow investigators to perform a genome-wide search in case-control cohorts as well [International HapMap Consortium, 2005]. Second, case-control studies do not permit distinguishing between allelic identity “by state” and “by descent” (i.e. to define whether an apparently same allele comes from the mother or the father) and, therefore, are associated with reduced fidelity when defining the genotype. Third, case-control studies are more sensitive to biases associated with population admixture and, hence, more prone to spurious results. The latter limitation (population admixture) can also be reduced by studying individuals from a geographically isolated population. Such populations offer several advantages in genetic studies. First, genetic heterogeneity in such populations is lower compared with ethnically mixed populations; this reduces the number of genes influencing a particular complex trait. Second, geographically isolated populations usually have good genealogical records that can improve further the fidelity of defining the genotype. Third, geographically isolated populations typically live in a more uniform environment (e.g. diet, parenting style) that reduces occurrence of confounding factors such as phenocopies. To our knowledge, there are only two large-scale MR-based studies carried out in geographically isolated populations: (1) the Saguenay Youth Study (SYS) described here; and (2) the AGES-Reykjavik study carried out in Iceland in a population cohort of 67 to 93-year-old individuals [Sigurdsson et al., 2006]. The Sagunay Youth Study is carried out in the Saguenay Lac-Saint-Jean (SLSJ) region of Quebec, Canada in a population-based cohort of high-school students (age 12–18 years). It is designed to investigate the interaction between the genetic background of the individual and his/her prenatal exposure to maternal cigarette smoking (PEMCS). Therefore, we will now provide background in- outcomes related to the child’s mental and cardiovascular formation on the known effects of this particular in utero and metabolic health. environment on brain and body.

Prevalence of Maternal Cigarette Smoking During Pregnancy

The prevalence of cigarette smoking in pregnant women varies widely in different countries [Ebrahim et al., 2000; Kendrick and Merritt, 1996]. In the United States, for example, the average prevalence was 16.3% in 1984 and decreased to 11.8% in 1994 [Ebrahim et al., 2000]. In Canada, 25% of women age 15 years and above are current smokers [Health Canada, 1995b]. In Quebec, we found that 25% of expectant mothers smoked cigarettes throughout pregnancy [Japel et al., 2000]. It is important to note that the prevalence rates of cigarette smoking in general, and of cigarette smoking during pregnancy in particular, are not uniform across different groups of women. The strongest predictor of smoking both before and during pregnancy is a woman’s socioeconomic status (SES); for example, a prospective study of 589 preadolescent children found that 52% of their low-SES mothers smoked cigarettes during pregnancy [Cornelius et al., 2000]. Tobacco smoking is particularly frequent in young mothers; in Canada, 40–50% of teen mothers reported that they smoked during their pregnancy [Paquette and Morrisson, 1999]. Similar relationships were observed in the SLSJ region; we found that prevalence of tobacco use is negatively correlated with the level of schooling and with household income [Institute de la statistique du Quebec, 2001].

Cigarette Smoking, the Fetus and the Fetal–Placental Unit

The impact of maternal smoking on the developing fetus is complex. First of all, tobacco smoke may affect the fetus in several ways [Lambers and Clarke, 1996]: (a) inhaled nicotine induces vasoconstriction of the uteroplacental vasculature, leading to uteroplacental underperfusion and, in turn, decreased flow of nutrients and oxygen to the fetus, (b) increased levels of carboxyhemoglobin reduce tissue oxygenation of the fetus, (c) nicotine suppresses the mother's appetite, leading to reduced energy intake by the mother and hence reduced energy supply to the fetus and (d) nicotine causes alterations in the cellular growth and activity of the central and peripheral nervous systems [Slotkin, 1998]. Second, tobacco smoking is frequently associated with epiphenomena, such as risky behaviors, coabuse of other substances, poor prenatal care, and low SES [Slotkin, 1998], which themselves may exert adverse effects on the developing fetus. Finally, interindividual variability in genetic background is likely to modify the response of the fetus to tobacco smoke, i.e. gene–environment interactions [Pausova et al., 1999]. Cigarette smoking during pregnancy has been associated with a number of adverse outcomes related to the child's mental and cardiovascular and metabolic helth.

PEMCS and Behaviour

In *human subjects*, there is growing evidence that PEMCS is associated with cognitive sequelae and increased incidence of psychiatric disorders in childhood and adolescence. In a prospective cohort study, Fried [1995] observed systematic differences between children born to "heavy smokers" (>20 cigarettes/day) compared with nonsmoking mothers, in several cognitive domains, including processing of auditory stimuli, attention, and language comprehension. Other [Lassen and Oei, 1998; Obel et al., 1998; Olds et al., 1994] but not all [MacArthur et al., 2001] investigators have observed similar effects. Several studies revealed an increased incidence of externalizing disorders in general [Breslau and Chilcoat, 2000], and attention-deficit hyperactivity [Milberger et al., 1998] and conduct [Wakschlag et al., 1997; Weissman et al., 2000] disorders in particular. Some studies also observed an association between PEMCS and criminal behavior in adulthood [Brennan et al., 1999; Räsänen et al., 1999], and higher rates of aggression in children and adults [Orlebeke et al., 1999; Räsänen et al., 1999]. Furthermore, PEMCS may increase the probability of experimenting with cigarette smoking in childhood [Cornelius et al., 2000] and of developing cigarette-smoking addiction in adolescence [Kandel and Udry, 1999; Weissman et al., 2000].

PEMCS and the Brain

The effect of PEMCS on the *human brain* is largely unknown. A recent study demonstrated subtle differences in the expression of nicotinic and muscarinic acetylcholine receptors in the brains of 5 to 12-week-old fetuses exposed and nonexposed to maternal cigarette smoking [Falk et al., 2005]. Several lines of *indirect* evidence suggest detrimental effects of PEMCS on brain growth and development. Kallen [2000] analyzed the Swedish Medical Birth Registry (1983–1996: 1,362,169 infants) and found significant negative correlation between PEMCS and head circumference at birth. Maternal cigarette smoking was found to

increase the relative risk of periventricular-intraventricular hemorrhage in premature babies [Bada et al., 1990].

PEMCS and the Offspring's Metabolic and Cardiovascular Health

Despite being related to intrauterine growth retardation [Kramer, 2000], PEMCS is associated with obesity in later life. The British National Child Development Study reported that PEMCS significantly increases the risk of obesity beginning in childhood and adolescence [Power and Jefferis, 2002; Toschke et al., 2003; von Kries et al., 2002; Wideroe et al., 2003], and diabetes mellitus in adulthood [Montgomery and Ekbom, 2002]. In prospective cohort studies, the impact of PEMCS on obesity increased with age and remained robust even after adjustment for known confounding factors, such as SES, infant feeding, current diet, and physical activity [Power and Jefferis, 2002; Wideroe et al., 2003].

PEMCS may also increase the susceptibility to hypertension later in life. Significant associations between PEMCS and increased blood pressure in children of different age categories, including neonates and adolescents, have been reported [Beratis et al., 1996; Blake et al., 2000; Morley et al., 1995; O'Sullivan et al., 1996; Williams et al., 1999]. In experimental animals, prenatal exposure to nicotine has been related to a decreased kidney size and increased blood pressure in adolescence [Pausova et al., 2003].

DESIGN

To evaluate long-term consequences of PEMCS in adolescence and to assess how such an adverse intrauterine environment interacts with the individual's genetic background, the design of the SYS has the following features: (1) a family-based (sib-ship) design where only children with one or more siblings and with both biological parents are included; (2) a cross-sectional design with participants' age ranging from 12 to 18 years, and an equal proportion of exposed (n = 500) and nonexposed (n = 500) adolescents of both sexes (1:1 male-to-female ratio); (3) a retrospective-cohort design where *in utero* exposure is assessed retrospectively, while the acquisition of the majority of phenotypes in the offspring is carried out in a prospective fashion; (4) quantitative assessment of brain and behavior and cardiovascular and metabolic phenotypes; and (5) acquisition of DNA samples from the adolescent participants and their biological parents.

SETTING AND PARTICIPANTS

The sib-ships are being recruited from a relatively geographically isolated population with a known founder effect [Grompe et al., 1994] living in the SLSJ region of Quebec, Canada.

History of the SLSJ Population

The initial settlement of the SLSJ region occurred between 1838 and 1911. From a total of 28,656 settlers who moved there during that time, 75% originated from the neighboring Charlevoix region [Gradie et al., 1988]. The settling of the Charlevoix region itself started in 1675 when 599 founders of mostly French descent moved to this region from the Quebec City area. Because of relatively high birth-rates and low immigration into the SLSJ region, today the population represents one of the largest population isolates in North America, with

almost 300,000 inhabitants. As a consequence of the SLSJ population history, the prevalence of several recessive disorders is higher in the SLSJ region than in other populations [De Braekeleer et al., 1991], and a limited allelic diversity exist among patients with these disorders [De Braekeleer et al., 1998; Grompe et al., 1994]. These studies indicate that genetic heterogeneity is reduced in the SLSJ population. Moreover, extensive computerized genealogical records exist in the BALSAC population register maintained at the Inter-university Institute for Population Research. The register contains ascending genealogies going back as far as the 16th century; they have been constructed on the basis of parish records of baptisms, marriages, and deaths of ~1,500,000 individuals [Roy et al., 1991].

The SLSJ Population at Present

According to the 2001 census, the region has a population of 278,279 inhabitants. In 2001, there were 19,114 French-speaking students in 24 secondary schools (20 public and 4 private). A large part of the SLSJ population (60%) is living in the Chicoutimi-Jonquière urban area. There are four school boards in the region, two in Saguenay and two in the Lac Saint-Jan region. According to the 2001 census, there were 80,325 families living in the region. More than half (51,660) of all families have children living at home: 46.5% families have one child; 38.1% two children; 15.4% three or more children.

Selection Criteria

The following selection criteria are used for the exposed subjects: (1) Age 12–18 years; (2) One or more siblings in the same age group; (3) Maternal and paternal grandparents of French–Canadian ancestry; and (4) Positive history of maternal cigarette smoking (>1 cigarette/day in the 2nd trimester of pregnancy). The main exclusion criteria are: (1) Positive history of alcohol abuse during pregnancy; (2) Positive medical history for meningitis, malignancy, and heart disease requiring heart surgery; (3) Severe mental illness (e.g. autism, schizophrenia) or mental retardation (IQ < 70); and (4) MRI contraindications. The nonexposed subjects are matched to the exposed ones based on the level of maternal education and the school attended. In the case of mothers of nonexposed offspring, we require negative history of maternal cigarette smoking during pregnancy and during the 12-month period preceding the pregnancy. The full list of exclusion-inclusion criteria is presented in Supplementary Table I.

Recruitment

Subjects are recruited through regional high schools; the number of students in each school varies between 600 and 1,900. Following a briefing of the teachers, the team *visits individual classrooms* and presents the project. Before the visit, we mail *a letter to all parents* of students attending the visited school; the letter contains an information brochure, a letter from the principal, a consent form for a telephone interview and a self-addressed and stamped response card. We ask the parents to mail *the response card* back to the team and indicate whether or not they are interested in participating in the project, how many children aged 12–18 years they have and to provide the home phone number for a follow-up call by a research nurse. During *the follow-up call* to the interested families, the nurse verifies basic eligibility for the project (number of children and their age), solicits consent to a telephone interview and, if agreed, proceeds with the interview. Given the nature of the interview (see

later), the respondent should be the children's biological mother whenever possible. The *telephone interview* is carried out on a laptop computer using software written for the project, providing the nurse with the script and allowing her to record all responses on line; the software navigates the nurse through the interview by skipping "not-applicable" questions. The interview covers the following areas: demographics of the parents (French Canadian origin, age, level of education), pregnancy (smoking, drinking, drug use, medical complications), and medical history of the children and parents. If the nurse deems the family eligible, she sets up an appointment for a home visit; this can be done immediately only for the exposed subjects, whereas nonexposed subjects are called back once selected by matching to the exposed subjects from the same school. The *home visit* that concludes the recruitment phase begins with the signing of the consent (parents) and assent (adolescents) forms.

MEASUREMENTS

Data collection begins with the telephone interview, continues with a *home visit*, neuropsychological testing during *a laboratory visit*, *a hospital visit* for the cardiovascular and metabolic phenotyping and magnetic resonance imaging (MRI), and it concludes with *a school visit*; hospital visits always take place on Saturdays. In this section, we will describe the various instruments by types of data sets rather than by place/time of their collection (Supplementary Table II).

Questionnaires

Demographics and measures of SES: Supplementary Table IIIA provides a summary of datasets acquired in these domains, together with the instruments used for their collection. Note that a large number of SES measures, as well as stressful life events, are recorded for the following four periods of each child's life: birth, 3 years and 10 years of age and present. *Medical and psychiatric history*: Supplementary Table IIIB summarizes measures collected in this domain. In addition to the medical history of each child from conception to present (pregnancy, delivery, breastfeeding, concussions, etc), we also collect data on the parental mental health (depression and anxiety) and, in the adolescents, evaluate the likelihood of psychiatric diagnosis with DISC Predictive Scales. *Cigarettes, alcohol, and drugs*: Supplementary Table IIIC shows that several instruments are used to collect information about the use of cigarettes, alcohol, and illegal substances by the adolescent, as well as his/her mother and father. Maternal reports of the use of these substances during pregnancy are checked against medical records. In utero exposure to second-hand cigarette smoke is also documented. As seen in Supplementary Table IIID, *anti-social behavior* is documented extensively in both adolescents (self-report and parental report) and in their parents (maternal and paternal self-report). *Lifestyle* measures provide information about the adolescents' engagement in physical activities, their food habits, sleep pattern, sexuality, extracurricular activities, attitudes towards school, as well as their academic/vocational aspirations (Supplementary Table IIIE). A number of *self-attributes and personality traits* (Supplementary Table IIIE) are evaluated using two instruments, namely the 5C (Competence, Connection, Character, Confidence, and Caring/Compassion) questionnaire

and the NEO-Personality Inventory. In addition, we employ two body-esteem and body-image instruments [Aleong et al., 2007], and a peer-pressure questionnaire.

Neuropsychological Assessment

This assessment (see Supplementary Table II: Section 3) consists of two types of tests: (1) standardized psychological instruments and (2) domain-specific tests. Total testing time is 6 h, divided into two 3-h blocks separated by a lunch break. To characterize the two groups of subjects and collect clinically relevant information, we administer several standardized tests including the WISC-III (except Mazes), Woodcock-Johnson Achievement subtests (reading comprehension and arithmetic and a spelling test) and Children's Memory Scale (Dot Locations and Stories). We have also included an extensive battery of domain-specific tests based on neuropsychological and cognitive-neuroscience research. The following domains are assessed: (1) executive functions, including working memory (self-ordered pointing, [Petrides and Milner, 1982]), word fluency (phonetic and semantic), and resistance to interference (Stroop test [Stroop, 1935]), (2) phonological skills and frequency-modulation (FM) auditory threshold [Talcott et al., 2000], delayed auditory feedback, phonological learning, (3) fine motor skills (Grooved Pegboard, Simple tapping) and motor coordination (bimanual coordination [Stephan et al., 1999]), (4) number sense [Galistel and Gelman, 2000] and (5) emotion and motivation (Pollak faces [Pollak and Kistler, 2002], repeated failure test, and card playing task [Newman et al., 1987]).

Magnetic Resonance Imaging—Acquisition

MRI data are collected on a Phillips 1.0-T superconducting magnet; the session lasts between 45 and 60 min. (1) *Structural MRI of the brain*: High-resolution anatomical MR images are acquired using the following three sequences: T1-weighted images (T1W; 3D RF-spoiled gradient echo scan with 140–160 slices, 1-mm isotropic resolution, TR = 25 ms, TE = 5 ms, flip angle = 30°), T2-weighted images (T2W; 2D multi-slice fast spin echo scan with 70–80 2-mm contiguous slices with a 1 mm in-plane resolution, TR = 3,300 ms, TE effective =11 ms) and proton-density weighted images (PDW; same as for T2 scan, but with TE effective = 105 ms). *Magnetization-transfer ratio*: Magnetization transfer (MT) imaging [Wolff and Balaban, 1989] is a quantitative MRI technique that provides information on the macromolecular content and structure of tissue; it has been shown to reveal subtle white matter abnormalities that are undetected by conventional MRI, in diseases such as multiple sclerosis [Dousset et al., 1992; Gass et al., 1994; Pike et al., 2000]. In the context of this study, MT data should provide a more sensitive and accurate measure of myelination as compared with white matter (WM) density maps obtained via the methods discussed below. MT data are acquired using a dual acquisition (3D RF-spoiled gradient echo scan with ~50 slices of 3 mm thickness and 1 mm in-plane resolution, TR = 41 ms, TE = 7.9 ms, flip angle = 20°) with and without an MT saturation pulse (Gaussian RF pulse of 7.68 ms duration, 1.5 kHz off resonance, and 500° effective pulse angle). Magnetization transfer ratio (MTR) images are calculated as the percent signal change between the two acquisitions [Pike, 1996]. (2) *MRI of abdominal fat*: Imaging of adipose tissue using MRI is relatively straightforward, as T1 relaxation time of adipose tissue is much shorter than that of most other tissues [Snijder et al., 2002]. Thus, in the present project, the MR images of abdominal fat are acquired with a heavily T1-weighted, single breath-hold, multi-slice (10 axial slices

of 10-mm thickness) spin-echo (TR/TE = 200 ms/20 ms) measurement that is centered on the L4–L5 region (Fig. 3). (3) *Kidney MRI*: The kidneys can be well visualized on T1-weighted MRI acquisitions, providing that respiratory motion can be controlled for. Therefore, anatomical MR images of the kidneys are obtained with a multi-slice T1-weighted gradient recalled echo acquisition (3D Gradient Echo, TR/TE = 4 ms/1.9 ms; flip angle 10°), collected within a single breath-hold. Approximately, 27 coronal slices of 3-mm thickness and 3-mm in-plane resolution are acquired.

Magnetic Resonance Imaging—Analysis

Computational Analysis of the Brain MR images is used to assess differences in brain structure between exposed and nonexposed adolescents, as well as to identify more general effects of age and sex, structure-function relationships, and genetic/familial influences on brain structure. A typical image-processing "pipeline" (Fig. 1) begins with the *linear registration* of a T1W image from the acquisition ("native") space to standardized stereotaxic space, namely that of the average MNI-305 atlas [Evans et al., 1993], aligned with Talairach and Tournoux space [Talairach and Tournoux, 1988]. We use as a registration target the average brain (SYS333) computed from our adolescent population ($n = 333$), and aligned with the average MNI-305 atlas [Guimond et al., 2000]. Note that there is a negligible difference in the overall brain size between adolescent brains and those of young adults (23.4 ± 4.1 years) who constitute the MNI-305 atlas. The next step involves *brain-tissue classification* into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF); an automatic classification is achieved by combining information from different types of MR images, namely T1W, T2W, and PDW acquisitions [Cocosco et al., 2003; Zijdenbos et al., 2002]. The tissue-classification step yields three sets of binary 3D images (i.e. GM, WM, and CSF). Each of the binary images can be smoothed to generate *probabilistic "density" images*. These maps are used in voxel-wise analyses of age- or group-related differences in GM or WM density [see Ashburner and Friston, 2000 for methodological overview]. Another important processing step is a nonlinear registration of the subject's image to a template brain; local differences between the subject and the template are captured in a *deformation field*. The template brain contains information about anatomical boundaries; this anatomical information can be "projected" onto each subject's brain using the corresponding deformation field and fine-tuned by combining it with the tissue-classification map [Collins et al., 1994, 1995]. In this way, the pipeline provides automatic estimates of *regional volumes*. Another voxel-wise approach has been developed to quantify individual differences in the 3D anatomy of the cerebral cortex. These include estimates of the *cortical thickness* [Fischl and Dale, 2000; Lerch and Evans, 2005; MacDonald et al., 2000] and the quantification of individual differences in the position, depth, and length of the *cerebral sulci* [Le Goualher et al., 1999; Mangin et al., 2004].

Computational Analysis of Fat and Kidneys

Fat is well contrasted on T1W images and therefore amenable to semi-automated or automated segmentation (Fig. 2). To this end, we developed a histogram-based classification algorithm where the combination of local intensity measures and geometrical constraints enabled the segmentation and volumetric measurements of subcutaneous and intra-abdominal (visceral) adipose tissues. We are also developing deformable-model based

techniques for the automated segmentation of the kidneys to provide an overall volume of each kidney, and semi-automated classification approaches to partition the kidney into the cortex and medulla to calculate cortex/medulla ratio.

Blood Pressure and Heart Rate Measurements at Rest and in Response to Postural and Mental Challenges

The cardiovascular protocol is carried out in a hospital setting and lasts 56 min (Fig. 3). It includes posture and mental-stress tests [McAdoo et al., 1990]. During the cardiovascular protocol, blood pressure and heart rate are measured (1) *with an automated blood pressure monitor* (Omron) at two time points and (2) with the *Finometer* (Finapres Medical Systems, Arnhem, Netherlands) continuously, i.e. beat-by-beat, throughout the protocol (Fig. 3). Using Beat-scope software (Finapres Medical Systems, Arnhem, Netherlands), the Finometer provides the following parameters at each beat: upstroke, systolic, diastolic, and mean blood pressure, heart rate, inter-beat interval, stroke volume, cardiac output, ventricular ejection time, peripheral resistance, aortic impedance, and aortic compliance. During the entire protocol, respiration rate is recorded with a sensor (Model 1132 Pneumotrace II Respiration Transducer, UFI, Morro Bay, CA, USA). In addition, salivary cortisol is measured in response to mental stress and posture changes [Fig. 3, Roy et al., 2001].

Assessments of the Quantity and Distribution of Body Fat, Energy Intake, and Energy Expenditure

The quantity and distribution of body fat is measured with *anthropometry, bioelectrical impedance* (Xitron Technologies, San Diego, CA, USA) and *MRI* (described above). Energy intake and expenditure are assessed with a 24-h food-recall interview, and food-frequency and physical activity questionnaires. The 24-h food recall, conducted by a trained nutritionist, is employed to collect quantitative and qualitative information on foods and drinks that the subject has consumed during the past 24 h. The recall is complemented by *food-frequency and physical activity questionnaires* used by Santé Québec in its survey of Quebec children aged 6–16 years.

Biochemical Analyses of a Fasting Blood Sample

In all subjects, a fasting blood sample is drawn between 8 a.m. and 9 a.m. This sample is used to evaluate (1) *glucose and lipid metabolism* (glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, free fatty acids, glycerol, and apolipoproteins A, B, C, and E), (2) *pro-inflammatory and pro-thrombotic states* (C-reactive protein and PAI-I), (3) the *hypothalamic-pituitary-adrenal axis and sympatho-adrenal system* (cortisol, epinephrine, and norepinephrine), (4) *sexual maturation* (total and bio-available testosterone, estradiol, and leptin) and (5) *current cigarette smoking* (cotinine). The sample is also used for DNA extraction.

WEB-BASED PROJECT MANAGEMENT SYSTEM

To manage smooth communication across the various project sites, we have implemented a number of web-based tools. The entire *procedure manual* is available on a password-

protected web site. The manual contains a detailed version of the study flowchart (Supplementary Table II) and electronic versions (Word, PDF) of all instruments; a total of 107 individual documents are available in this electronic manual.

The key element for the management of recruitment, screening and scheduling is the *field module*. This is a web-based system, developed with MySQL/PHP programming tools; it allows a research nurse to track a subject through the different stages of the study, from recruitment to study completion. When the team receives the response card from an interested family, the family name, and the telephone number are entered into the field module, and the family is assigned automatically an identification code (FAM9999). To ensure confidentiality, nominal information is restricted to local access (nurse) and is not accessible through the web. In order to proceed with the screening of families, this information is transferred electronically to the *telephone interview software* installed on stand-alone personnel computers employed by research nurses. The software guides the nurse through a structured interview with the parent; the generic text is tailored to each particular family by inserting automatically family-specific text (e.g. children's names) and skipping irrelevant questions based on the recorded answers. The first data-collection step in the interview identifies all biological children of the mother and their biological father. At this point, a six-digit identification code is assigned automatically to each family member; this code is combined with the family identification number assigned in the field module (see above). This combination of the family and subject identification numbers (e.g. FAM9999_888888) is carried together throughout the study. Information recorded during the telephone interview, together with the family and subject identification codes, is transferred electronically back to the field module. At this point, all nominal information is stripped away and denominalized data are released to the web-based part of the field module that is accessible to all members of the research team. Tracking of data and data entry is an integral component of the system; this is described later.

DATA ENTRY, DATA TRANSFER, AND QUALITY CONTROL

The project database is a relational database developed with MySQL/PHP programming tools. Each participant (i.e. biological mother, biological father, and all their biological children) is identified using a unique six-digit code assigned automatically by the telephone-interview software (see earlier). This combination of the family and subject identification (ID) number (e.g. FAM9999_888888) is entered on all paper forms (e.g. questionnaires and testing forms) and in the subject field of the various computer-based instruments (e.g. Finometer, MRI, neuropsychological tests). Given the variety of instruments, there are several modes of data entry (see Supplementary Table II). *Web-based manual data entry* is employed for the majority of standardized neuropsychological tests and some other data-entry forms. Individual data-entry modules are available on a password-protected data-entry web site. Graphical user interface (GUI) is designed (PHP/Java script) to mirror the actual layout of a given form; this facilitates data entry and reduces possible errors due to spatial incompatibilities between the GUI and the hardcopy. Each data-entry form begins with the subject identification; the operator is requested to enter both subject and family ID and the computer validates whether the combination of the two numbers (e.g. FAM9999 and 888888) is correct. This system minimizes the possibility of entering data under an incorrect

ID number (e.g. FAM9999_898888). In addition to data entry, this module allows investigators to view and export (in text format) all entered data. Most of the questionnaires are entered using the *Remark Office Optical Mark Recognition* (OMR) software (www.PrincipiaPro-ducts.com; Paoli, Pennsylvania, USA). The software works in conjunction with an image scanner to collect the data (Remark Office OMR, Version 5.5 for Windows, User's Guide, May 2002). The output forms generated by the Remark Office OMR software are uploaded to the project database, raw data stored in questionnaire-specific tables and relevant scales calculated and stored.

Magnetic Resonance Images are stored at the MR console in DICOM (**D**igital **I**maging and **Co**mmunication in **M**edicine; http://medical.nema.org) format (12 bits) and copied onto a compact disk. Using a secure file transfer protocol, DICOM images are then transferred into a dedicated MR database and converted into MINC (Medical Imaging NetCDF; http://www.bic.mni.mcgill.ca/software/minc/) format. All other *electronic files* collected using various computer programs (e.g. computer-based neuropsychological tests) or specialized hardware (e.g. Finometer, Bioimpe-dance) are stored in their proprietary format, copied onto a compact disk and uploaded to the project database using again a secure file transfer protocol.

STATISTICAL ANALYSES

Here we highlight only the main principles that will guide *statistical analyses* of the entire dataset. These analyses will rely on the Generalized Estimating Equations (GEE) extension of the multiple linear regressions for clustered data [Liang and Zeger, 1986]. This methodology will allow us to account for: (1) quantitative (continuous) measurement scales of primary and intermediary variables; (2) potential imbalances between exposed and non-exposed subjects in the distribution of characteristics related to the variables, such as duration of breast feeding and secondhand smoke; and (3) a likely correlation between the variables within sib-pairs. Point (1) calls for statistical analyses designed for continuous, normally distributed data, such as linear regression analysis. Point (2) requires multivariable linear regression methods that will adjust the estimated PEMCS effect for potential confounding factors. Point (3) requires the GEE extension of the conventional multiple linear regression to account for the dependence of observations within sib-pairs. In GEE, we will assume the exchangeable covariance structure of the residuals, with just two observations per cluster, i.e. a pair of siblings [Jennrich and Schluchter, 1986].

The sibship-based design of the SYS allows us to evaluate the degree of "familiality" of the various phenotypes; familiality is defined here as the proportion of variance attributable to the combination of shared family environment and shared genes. Familiality estimates were derived from intraclass correlations (ICC) computed with the FCOR program (version 5.3.0, S.A.G.E. package, Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio; http://darwin.epbi.cwru.edu/sage/). Within-trait and cross-trait estimates of familiality were computed; the former reflects the proportion of variance of a single trait determined by familial factors shared between siblings (e.g. brother–brother), whereas the latter indicates the proportion of variance between two traits determined by familial factors shared between siblings.

FINDINGS

As of October 2006, we have collected full data-sets in 408 adolescents and 195 sets of their biological parents. In this section, we provide information on ascertainment, demographics, PEMCS, and the initial data on the degree of familiarity of brain-MRI phenotypes.

Ascertainment

Between November 2003 and October 2006, the research team has approached a total of 5,033 students. An overall response rate (i.e. number of returned response cards) was 19% and varied between 8 and 36% across schools. Of the number of returned response cards, 64% of families agreed to being contacted by the research nurse. Based on the telephone interview (n = 768), 37% families were excluded for a variety of reasons: cigarette smoking 12 months before but not during pregnancy (n = 104), one of the two adolescents not interested (n = 73), child not of eligible age (n = 75), MR contraindications (braces: n = 59), other than French Canadian origin (n = 46), medical reasons (n = 42 [most common: heart abnormalities: 12, epilepsy: 6, diabetes: 4]), one parent not available (n = 30),twins (n= 28),premature birth (n = 14), and placental detachment/rupture (n = 5). In the total of 423 eligible families, there were 229 (26%) exposed and 657 (73%) nonexposed adolescents eligible for the study. Note that the ratio of the exposed and nonexposed adolescents is similar to the population average of maternal cigarette smoking during pregnancy established in this region previously [Japel et al., 2000]. From the pool of all eligible families, we have matched (maternal education, school attended) 199 exposed and 209 nonexposed adolescents belonging to a total of 198 families. These subjects and families have completed the entire protocol and are considered below.

Prenatal Exposure to Maternal Cigarette Smoking

The exposure status and the number of cigarettes smoked during each pregnancy rely on information provided by the mother during the telephone interview. For all subjects born in the two main hospitals in the region (Chicoutimi [159 adolescents] and Jonquiere [140 adolescents]), we have verified the mother's report by extracting relevant antenatal information from the medical chart completed during pregnancy (1st trimester [73%], 2nd trimester [7%], and 3rd trimester [20%]). We were able to obtain relevant information in a total of 260 adolescents (87%), with an equal distribution across the exposed and non-exposed adolescents. To assess the overall agreement between the exposure statuses ascertained antenatally from the medical records and by the maternal report during the telephone interview, we calculated Kappa statistics (range: −1 to +1) and found that, with a value of 0.69 ± 0.04, it indicates a "good" strength of agreement [Landis and Koch, 1977; good agreement: >0.6 to ≤0.8].

By definition, mothers of exposed adolescents smoked during pregnancy whereas the mothers of nonexposed subjects did not (Table IA). As a group, the mothers reported smoking an average of 11 ± 6.7 cigarettes/day during the 2nd trimester (range: 1–42; quartiles: 1–6, 7–10, 11–15, >15 cigarettes/day). A significant number of mothers of nonexposed adolescents never smoked in their life (51%). On the other hand, many more mothers of exposed (vs. nonexposed) adolescents smoke at present (x^2 =52.2, P < 0.0001).

When smoking, the number of cigarettes smoked per day at present is higher in the mothers of exposed versus mothers of nonexposed adolescents ($F(3,61) = 5.6$, $P = 0.002$, Effect size defined as Cohen's $d = 1.23$) and, in the mothers of exposed subjects, it is similar to the number of cigarettes smoked during pregnancy ($r^2 = 0.47$, $P < 0.0001$).

Demographics

The exposed and nonexposed groups do not differ in their mean age and sex distribution (Table IB). All subjects are of French–Canadian origin and live within the region (98,709 km^2). The two groups do not differ in the different measures of SES, including the level of maternal education (used for matching nonexposed adolescents to the exposed ones), level of employment of both parents and household income (Table IB).

Pregnancy, Birth Weight, and Breastfeeding

Duration of pregnancy did not differ between the two groups; but note that pregnancy shorter than 35 weeks was exclusionary (Table IC). As expected, birth weight of the exposed offspring was lower compared with the non-exposed offspring by 291 g, or 8.5% (Table IC; $P < 0.0001$; $d = 0.6$). When comparing the difference between exposed and nonexposed subjects, the effect of exposure was similar in males ($d = 0.57$) and females ($d = 0.61$). In the exposed males, we observed a small but significant dose effect in that the number of cigarettes smoked during the 2nd trimester of pregnancy correlated negatively with the birth weight ($r^2 = 0.05$, $F(1,90) = 4.6$, $P = 0.04$). This was not the case in the exposed female subjects. Exposed adolescents were less likely to have been breastfed ($x^2 = 25.7$, $P < 0.0001$); when breastfed though, there were no group differences in the total duration of breastfeeding (Table IC).

Medical History, Psychiatric Symptoms, and Antisocial Behavior

The two groups were comparable in the overall frequency of positive answers to various questions related to the children's medical history (Table ID). As for the mental health of the parents at the time of the home visit, we found no significant group differences on the scales of depression and anxiety reported by the mothers and fathers in the Mental Health and Antisocial Behavior questionnaire. We have observed no differences in the current antisocial behavior (Table ID) of the mothers and fathers. But we found a slightly higher score on the scale of antisocial behavior during adolescence in mothers of exposed versus nonexposed adolescents (Table ID; $F(3,188) = 2.95$, $P = 0.03$, $d = 0.38$).

Familiality of Brain Size and Volumes of Grey and White Matter

Using a fully automatic processing pipeline (see earlier), we have derived the following measures: brain size (based on the combination of X, Y, and Z scaling factors derived from the nine-parameter linear registration of each brain to the SYS333 template aligned with the MNI305 atlas), and the volumes of GM and WM constituting each of the four lobes (frontal, parietal, temporal, and occipital) in the left and right hemispheres. The initial analysis included 337 adolescents who passed our quality control of the registration (linear for brain size, nonlinear for volumes) and tissue classification (GM, WM, CSF) steps of the processing pipeline. This sample gives rise to a total of 171 sibships consisting of 38

brother–brother, 75 brother–sister, and 58 sister–sister pairs. Given the significant effect of age on GM and WM volumes, intra-class correlations were computed using age-adjusted residuals.

Table II lists ICC computed for brain size, as well as for the relative (i.e. brain-size corrected) volumes of GM and WM constituting each of the four lobes in the left and right hemispheres; GM and WM volumes were age-adjusted using linear regressions carried out separately for male and female adolescents. Pooled across the different types of siblings (171 pairs), familiarity indexed by ICC varied between 0.141 (occipital WM volume) and 0.337 (temporal GM volume). Overall, familiarity appeared somewhat higher for GM than WM but comparable for the left and right hemispheres. Familiality (ICC) values were highest in brother–brother sibpairs (0.454–0.543 for GM), as compared with sister–sister (0.221–0.399 for GM) and brother– sister (0.324–0.429 for GM) sibpairs.

Figure 4 illustrates cross-trait familiarity in brother– brother and sister–sister pairs. In the former group of pairs, for example, GM volumes in the temporal lobe show relatively high cross-trait correlations across the two hemispheres. In sister–sister pairs, such a pattern is present for the frontal and parietal volumes.

DISCUSSION

The main purpose of this paper is the description of the SYS. Nonetheless, the few initial findings mentioned here are largely consistent with the existing literature. For example, birth weight of offspring born to mothers who smoked during pregnancy, as compared with the nonexposed control group, is lower by 8.5% (291 g). Note, however, that we have not adjusted our data using any of the possible confounding variables and corrected for within-sibling similarity; this will be done in the upcoming detailed reports of our findings. One of the possible limitations of the SYS is the retrospective nature of the questionnaire-based assessments of perinatal events, most importantly the exposure to maternal cigarette smoking during pregnancy. But as concluded by Rice et al. [2006b] in their recent paper on the reliability of maternal reports of various perinatal events, “mothers can provide accurate reports in comparison to information from medical records.” In their study, which compared medical records with questionnaire-based maternal reports obtained 4–9 years later, Kappa statistics varied between “very good” for smoking during pregnancy ($\kappa = 0.81$) and “poor” for alcohol during pregnancy ($\kappa = 0.17$). For the birth weight, the correlation between the two time-points was excellent ($r = 0.99$). In our study, Kappa statistics for smoking during pregnancy was comparable to the Rice et al. findings ($\kappa = 0.69$).

The sibship-based design of the SYS allows us to examine familiarity of the range of phenotypes collected in our sample, from questionnaire-based assessment of personality, through cognitive performance, to brain structure. Although we cannot distinguish between the effect of shared environment and genes, respectively, and calculate true heritability of a given phenotype, familiarity values are informative in different respects. First of all, we can compare familiarity of different phenotypes, such as the volumes of grey and white matter, both within and across different types of siblings (brother–brother vs. sister–sister); in the initial sample, familiarity appears higher for grey than white matter especially for male

adolescents. This may reflect continuing maturation of white matter in boys, compared with girls, during this period of human development. Wallace et al. [2006] provides comparable data for monozygotic and dizygotic twins in the age range between 5 and 18 years; the number of same-sex pairs varied between 52 male MZ pairs and 15 female DZ pairs. Overall, values of ICC in the DZ twins (male and female pairs combined) varied between 0.29 (parietal GM) and 0.65 (temporal WM). After splitting the groups into “younger” and “older” subgroups, Wallace et al. observed lower ICC values in older versus younger DZ twins for GM volumes and vice versa for WM volumes, with no changes for the MZ twins. If we consider sex-differences in the stage of sexual maturation, that is female adolescents being ahead of male adolescents, this pattern is consistent with our observation of sex differences in familiarity: lower ICC in sister–sister versus brother–brother pairs for GM and vice versa for WM. Note, however, that the above pattern of differences in ICC is not statistically significant and may not be stable given relatively low number of pairs (38 brother–brother and 58 sister–sister pairs). As the study progresses, the number of available pairs will increase thus providing more statistical power and reliability for the estimates of familiarity. Finally, the access to a wide range of quantitative phenotypes both within the brain and behavior .domain and across the brain and body (i.e. cardiovascular and metabolic phenotypes) will provide unique opportunities for estimating cross-trait familiarity. This approach is illustrated using cross-lobe familiarity estimates for brother– brother and sister–sister datasets (Fig. 4).

Inclusion of the *genetic component* in the SYS is motivated conceptually by the emerging view in which the expression of a given phenotype is determined by a combination of environmental and genetic factors [Caspi et al., 2003; Pausova et al., 1999; Rice et al., 2006b; Thapar et al., 2005; Wang et al., 2002]. Identifying genes that modulate individuals’ response to PEMCS should also provide the initial pointers vis-à-vis possible molecular pathways, and hence possible mechanisms, mediating such effects. The family-based design involving sibships (full phenotyping and genotyping) and their parents (partial phenotyping and genotyping) will allow us to carry out genome-wide linkage and association analyses, and identify regions of the genome not necessarily predicted by previous knowledge. At the same time, candidate-gene studies can also be used to test specific hypotheses. The reduced genetic heterogeneity of a geographically isolated population should increase the power of these analyses, as the number of genes involved in determining individual complex traits is expected to be lower in this population than in multiethnic populations (e.g. Montreal metropolitan area). The overall advantage of the genetic approach is the control of yet another variable—the genome—that may explain some of the variance in the studied phenotypes. Disadvantages of this approach, other than its additional cost, include organizational demands associated with the siting of the study in a specific geographical location and a selective recruitment (siblings only).

The full phenotype is being acquired in adolescents 12– 18 years of age. Adolescence is a highly dynamic period of human development during which the children’s brains and bodies adopt adult roles. It is also a time when adoles cents begin to make their own choices affecting their health: sexual behavior, eating habits, physical activity, consumption of alcohol, cigarette smoking, and the use of recreational drugs, to name but a few. What are the factors determining such choices? The relatively comprehensive design of the SYS and

its large sample size (n = 1,000) should provide a suitable platform for predicting variance in a particular phenotype, such as blood pressure, cardiovascular reactivity, and school performance, using multiple independent variables capturing the individual's environment and life habits, his/her genetic background and the state of other organs (e.g. fat metabolism, brain morphology). The structure and function of the adolescent's organs are less likely to be affected by a disease process that requires treatment, a situation quite common in adults; this minimizes possible confounding effects of medication. Furthermore, recent epidemiological studies suggest that processes underlying the development of common disorders, such as the metabolic syndrome, may be emerging as early as in adolescence [Weiss et al., 2004]. Thus, adolescence may be a critical period during which meaningful prevention strategies are still possible.

The *inter-disciplinary* design of the SYS requires input from many disparate fields, such as medicine, computer science, genetics and statistics, physics, psychology, and sociology. Although the different conceptual background of investigators working in the respective fields often hampers the initial communication and the setting of common goals, the richness of the various approaches and tools benefits the program at the end. To succeed, individual investigators must be willing to adapt their approaches to fit the overall design. For example, questionnaires must be designed in a way that maximizes collection of quantitative data based on an individual (e.g. a composite index of academic achievement) rather than a group (e.g. frequency of individuals with a particular grade in math). Or, MR acquisition protocols must be robust to allow for a consistent collection of images over a long period of time (e.g. 5 years) at the expense, perhaps, of fine-tuning the acquisition as new sequences are developed. As the data become available, the biggest challenge is faced by colleagues working at the interface between data processing (bioinformatics, computer science, and statistics) and their biological interpretation (genetics, psychology, sociology, and medicine). In our view, this challenge can be best met by having a core group of investigators working in the same physical location for an extended period of time.

Overall, we hope that the above description of the SYS will be helpful to colleagues interested in similar studies of environmental and genetic factors influencing human health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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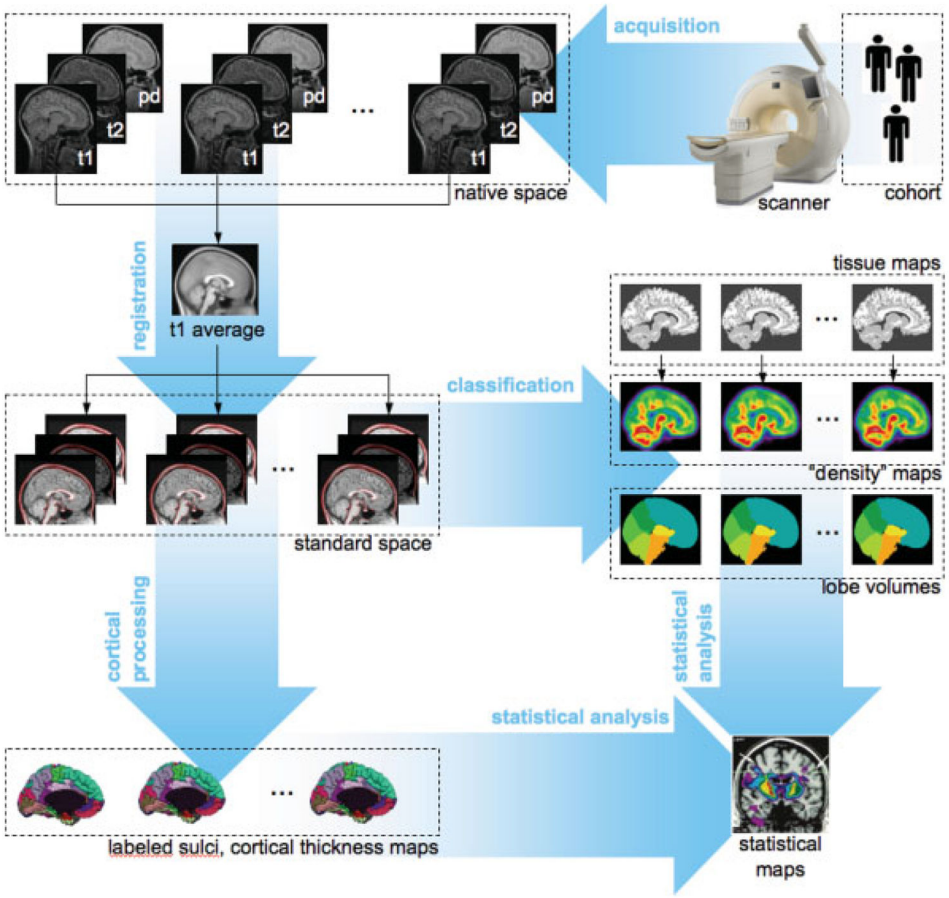

Figure 1.

Acquisition and analysis of brain magnetic resonance images. T1W, T2W, PDW, T1-weighted, T2-weighted, and proton-density weighted images, respectively.

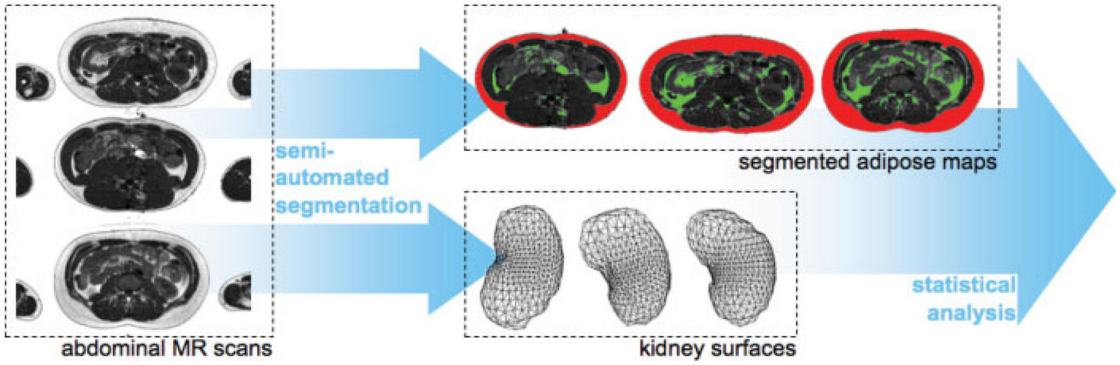

Figure 2.
Acquisition and analysis of abdominal magnetic resonance images.

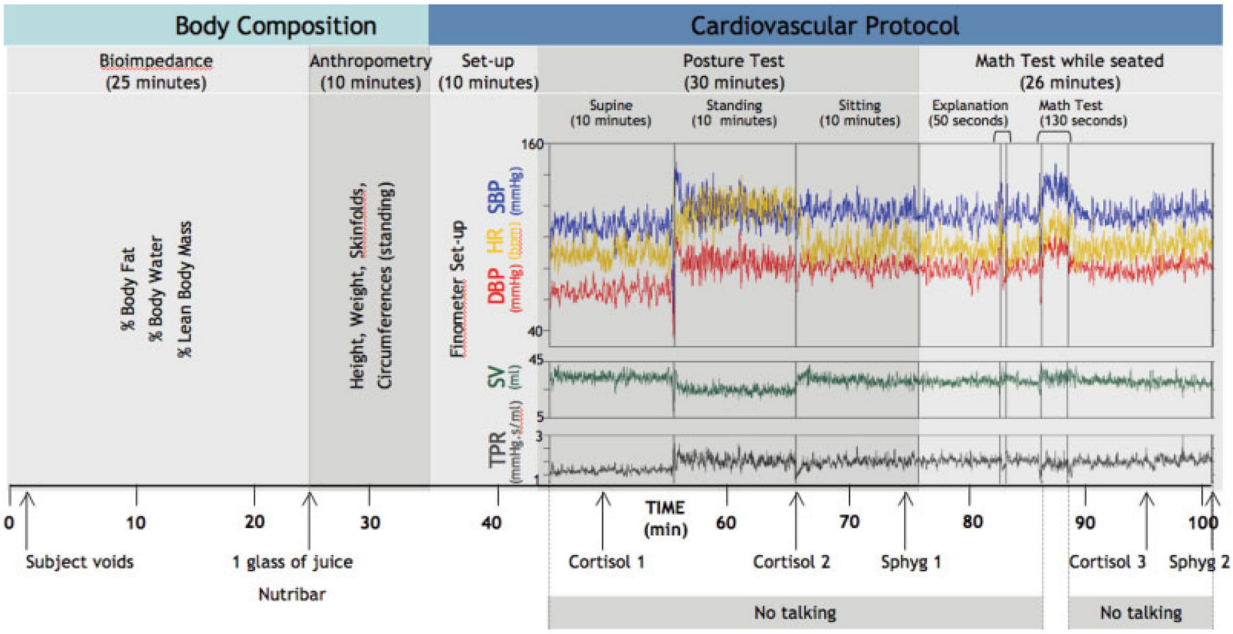

Figure 3.

Cardiovascular and body-composition protocol. Subjects undergo a 101-min protocol that includes body composition measurements (bioimpedance and anthropometry), and continuous beat-to-beat monitoring of blood pressure and other cardiovascular parameters (see later) using a Finometer™. The cardiovascular component involves two main challenges: a posture test and a math stress-test. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance; Sphyg 1 and 2, additional measurements of blood pressure using sphygmomanometer; Cortisol 1, 2, and 3, samples of saliva obtained for cortisol measurements.

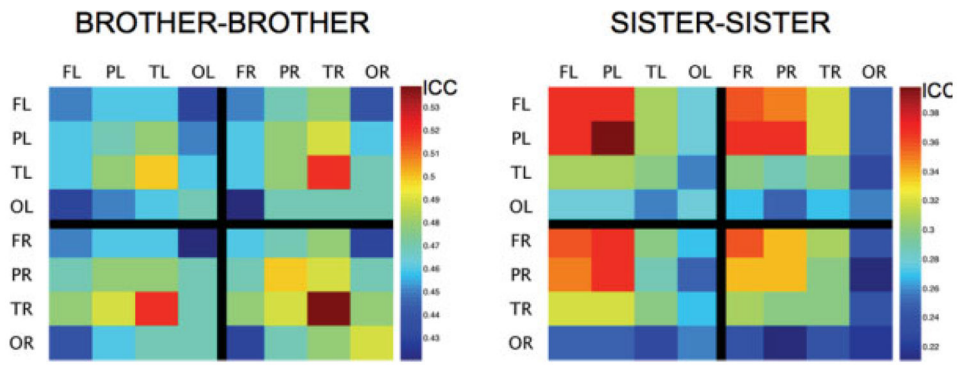

Figure 4.

Familiality of brain-size corrected grey-matter volumes. Estimates of intra-trait (e.g. FL vs. FL) and cross-trait (e.g. FL–PL) familiality indexed by intra-class correlation coefficients computed for brother-brother (left; 38 pairs) and sister-sister (right; 58 pairs) pairs. F, P, T, and O denote, respectively, Frontal, Parietal, Temporal, and Occipital lobes. L and R denote the Left and Right hemispheres.

Results

TABLE I.

	Nonexposed	Exposed	Mixed
A. Maternal cigarette smoking			
Mother: Cig smoking during pregnancy [No cig/day in 2nd trimester; M ± SD (*n*)]	0 (209)	11 ± 6.7 (199)	N/A
Mother: Cig smoking during breastfeeding [No cig/day; M ± SD (*n*)]	0	7.5 ± 7.5 (65)	N/A
Mother: Age of onset for cig smoking [years; M ± SD (*n*)]	**12.4 ± 5.2 (35)**	**14.3 ± 2.6 (59)**	**15.4 ± 2.9 (14)**
Mother: Negative life long history of cig smoking (No of mothers)	**45 (88)**	**0(82)**	**0(23)**
Mother: Cig smoking at present (No of mothers)	**8(88)**	**48 (82)**	**5(23)**
Mother: Cig smoking at present [No cig/day; M ± SD (*n*)]	**7 ± 4.2 (8)**	**16.8 ± 7.98 (48)**	**12.4 ± 3.6 (5)**
B. Demographics			
Ado: Age (M ± SD)	14.5 ± 1.87 (209)	14.7 ± 1.86 (199)	N/A
Ado: Sex (F:M)	103:106	107:92	N/A
Female Ado: Menstruation (Percent menstruating)	84% (83/99)	83% (87/105)	N/A
Female Ado: Menarche [M ± SD; years; (*n*)]	11.8 ± 1.4 (83)	12.1 ± 1.4 (87)	N/A
Puberty [M ± SD; Tanner stage; (*n*)]	3.75 ± 0.9 (191)	3.86 ± 0.85 (173)	N/A
Sibship Size [M ± SD (*n*)]	2.2 ± 0.45 (89)	2.1 ± 0.39 (82)	2.2 ± 0.4 (24)
Mother: Age [M ± SD (*n*)]	42.01 ± 3.65 (89)	41.6 ± 3.6 (82)	41.7 ± 3.8 (24)
Father: Age [M± SD (*n*)]	44.8 ± 3.8 (88)	43.96 ± 3.3 (82)	44.4 ± 4.5 (24)
A07f: Mother: Edu [M ± SD (*n*)]	3.3 ± 0.9 (78)	3.3 ± 0.8 (67)	3.7 ± 0.9 (20)
A07f: Father: Edu [M ± SD (*n*)]	3.6 ± 0.9 (74)	3.3 ± 0.8 (66)	3.5 ± 0.96 (19)
B09f: Mother: Edu [M ± SD (*n*)]	4.7 ± 1.5 (82)	4.5 ± 1.45 (71)	5.5 ± 1.4 (19)
B09f: Father: Edu [M ± SD (*n*)]	5.3 ± 1.7 (77)	4.7 ± 1.6 (66)	5.1 ± 1.4 (19)
Mother: Employment [M ± SD (*n*)]	2.5 ± 1.8 (86)	2.7 ± 1.9 (81)	2.3 ± 2.1 (24)
Father: Employment [M ± SD (*n*)]	1.6 ± 1.6 (83)	1.7 ± 1.6 (75)	1.3 ± 1.3 (23)
Household income [in thousands CAD, M ± SD (*n*)]	54.9 ± 21.4 (87)	51.7 ± 25.4 (81)	59.2 ± 18.5 (24)
Perceived economic situation [M ± SD (*n*)]	1.9 ± 0.6 (84)	1.9 ± 0.6 (75)	1.7 ± 0.5 (23)
Number of children in household [M ± SD (*n*)]	2.46 ± 0.7 (84)	2.3 ± 0.6 (75)	2.4 ± 0.8 (23)
Number of persons in household [M ± SD (*n*)]	4.57 ± 0.9 (84)	4.3 ± 0.8 (74)	4.5 ± 1.03 (23)
C. Pregnancy, birth, and breastfeeding			

	Nonexposed	Exposed	Mixed
Number of pregnancies [M ± SD (*n*)]	3.3 ± 1.2 (89)	3.2 ± 1.4 (81)	3.58 ± 1.5 (24)
Number of spontaneous abortion and stillbirths [M ± SD (*n*)]	0.48 ± 0.8 (89)	0.54 ± 0.9 (81)	0.79 ± 1.14 (24)
Pregnancy duration [weeks; M ± SD (*n*)]	39.1 ± 1.6 (206)	39.2 ± 1.5 (198)	N/A
Pregnancy and birth complications [M ± SD (*n*)]	0.28 ± 0.5 (208)	0.26 ± 0.5 (195)	N/A
Birth weight (g; M ± SEM)	**3530 ± 468 (205)**	**3239 ± 486 (195)**	**N/A**
Breastfeeding: Frequency of yes (total *n*)	**116 (206)**	**61 (195)**	**N/A**
Breastfeeding: Total duration [age in weeks; M ± SD (*n*)]	18.6 ± 16.3 (116)	15.2 ± 12.3 (61)	N/A
Breastfeeding: First other milk [age in weeks; M ± SD (*n*)]	13.8 ± 12.5 (116)	11.3 ± 8.7(61)	N/A
Breastfeeding: First other liquid [age in weeks; M ± SD (*n*)]	**12.6 ± 9.3 (208)**	**10.45 ± 8.6 (193)**	**N/A**
Breastfeeding: First solids [age in weeks; M ± SD (*n*)]	**14.02 ± 9.7 (208)**	**12.2 ± 8.5 (191)**	**N/A**
D. Medical History, psychiatric history and antisocial behavior			
Child's medical history: Positive answers [M ± SD (*n*)]	0.09 ± 0.3 (208)	0.1 ± 0.32 (195)	N/A
Ado: History of head trauma (Frequency)	27/200	19/191	N/A
Ado: Psychiatric symptoms (Sum of DPS symptoms)	**15.5 ± 11.5 (201)**	**18.8 ± 12.8 (188)**	**N/A**
Family's Medical History, Mother's side: Positive answers [M ± SD (*n*)]	3.7 ± 2.01 (88)	3.2 ± 2.2 (81)	3.04 ± 2.2 (24)
Family's Medical History, Father's side: Positive answers [M ± SD (*n*)]	3.98 ± 2.3 (88)	3.3 ± 2.2 (81)	3.5 ± 2.00 (24)
Mother's depression [M ± SD (*n*)]	16.25 ± 4.0 (84)	17.1 ± 4.0 (77)	17.04 ± 5.4 (23)
Mother's anxiety [M ± SD (*n*)]	19.3 ± 5.98 (84)	19.1 ± 4.9 (77)	18.3 ± 4.1 (23)
Father's depression [M ± SD (*n*)]	16.5 ± 4.1 (82)	15.8 ± 4.3 (73)	16.7 ± 5.6 (23)
Father's anxiety [M ± SD (*n*)]	18.1 ± 5.9 (82)	17.3 ± 4.96 (73)	17.7 ± 5.4 (23)
Mother's antisocial behavior in adolescence [M ± SD (*n*)]	**0.13 ± 0.4 (84)**	**0.36 ± 0.6 (77)**	**0.17 ± 0.4 (23)**
Mother's antisocial behavior in aduthood [M ± SD (*n*)]	0.05 ± 0.26 (84)	0.05 ± 0.28 (77)	0.04 ± 0.21 (23)
Father's antisocial behavior in adolescence [M ± SD (*n*)]	0.39 ± 0.86 (82)	0.7 ± 0.9 (73)	0.4 ± 0.9 (23)
Father's antisocial behavior in adulthood [M ± SD (*n*)]	0.16 ± 0.46 (82)	0.24 ± 0.6 (73)	0.13 ± 0.46 (23)
Ado's physical aggression [M ± SD (*n*)]	3.34 ± 0.9 (200)	3.3 ± 0.9 (181)	N/A
Ado's indirect aggression [M ± SD (*n*)]	6.3 ± 1.6 (200)	6.3 ± 1.5 (181)	N/A
Ado's fighting [M ± SD (*n*)]	5.2 ± 0.8 (200)	5.2 ± 0.8 (181)	N/A
Ado's stealing [M ± SD (*n*)]	4.6 ± 1.4 (200)	4.7 ± 1.4 (181)	N/A
Ado's vandalism [M ± SD (*n*)]	2.1 ± 0.4 (200)	2.2 ± 0.6 (181)	N/A

M, Mean; SD, Standard deviation; F, Female; M, Male; CAD, Canadian dollars; Exposed family = all children exposed to maternal cigarette smoking during pregnancy; Non-exposed family = no children exposed; Mixed family = some children exposed, some non-exposed.

Bold indicates statistical significant differences between the groups (see results for details).

TABLE II.

Familiality (intraclass correlation coefficients)

Lobe, Tissue	Brother-Brother (*n* = 38)	Sister-Sister (*n* = 58)	Brother-Sister (*n* = 75)	Siblings (*n* = 171)
Left hemisphere				
Frontal, Grey	0.45	0.37	0.39	0.33
Frontal, White	0.44	0.38	0.29	0.25
Parietal, Grey	0.47	0.40	0.37	0.34
Parietal, White	0.30	0.38	0.34	0.23
Temporal, Grey	0.50	0.30	0.43	0.33
Temporal, White	0.16	0.32	0.33	0.21
Occipital, Grey	0.47	0.28	0.32	0.31
Occipital, White	0.37	0.09	0.21	0.15
Right hemisphere				
Frontal, Grey	0.46	0.36	0.36	0.30
Frontal, White	0.41	0.39	0.30	0.24
Parietal, Grey	0.50	0.34	0.35	0.31
Parietal, White	0.31	0.35	0.33	0.23
Temporal, Grey	0.54	0.30	0.42	0.33
Temporal, White	0.23	0.33	0.30	0.20
Occipital, Grey	0.49	0.22	0.34	0.32
Occipital, White	0.30	0.19	0.13	0.14
Brain size	0.42	0.33	0.35	0.27