ORIGINAL ARTICLE

Pharmacogenetic study of second-generation antipsychotic long-term treatment metabolic side effects (the SLiM Study): Rationale, objectives, design and sample description[☆]

Laura Pina-Camacho [a,b,c,*], Covadonga M. Díaz-Caneja [a,b], Pilar A. Saiz [a,d,e], Julio Bobes [a,d,e], Iluminada Corripio [a,f], Eva Grasa [a,f], Roberto Rodriguez-Jimenez [a,g], Miryam Fernández [a,h], Julio Sanjuán [a,i], Aurelio García-López [a,j], Cecilia Tapia-Casellas [a,b], María Álvarez-Blázquez [a,b], David Fraguas [a,b], Marina Mitjans [a,k], Bárbara Arias [a,k], Celso Arango [a,b]

[a] *CIBER del área de Salud Mental (CIBERSAM), Spain*
[b] *Servicio de Psiquiatría del Niño y del Adolescente, Departamento de Psiquiatría, Hospital General Universitario Gregorio Marañón, Facultad de Medicina, Universidad Complutense, IiSGM, Madrid, Spain*
[c] *Department of Child and Adolescent Psychiatry, Institute of Psychiatry, King's College London, Londres, United Kingdom*
[d] *Área de Psiquiatría, Universidad de Oviedo, Oviedo, Spain*
[e] *Instituto Universitario de Neurociencias del Principado de Asturias (INEUROPA), Oviedo, Spain*
[f] *Servicio de Psiquiatría, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma, Barcelona, Spain*
[g] *Servicio de Psiquiatría, Instituto de Investigación Hospital 12 de octubre (i+12), Universidad Complutense, Madrid, Spain*
[h] *Servicio de Psiquiatría, Hospital Universitario de Santiago Apóstol, Universidad del País Vasco, Vitoria, Spain*
[i] *Departamento de Psiquiatría, Hospital Clínico INCLIVA, Universidad de Valencia, Valencia, Spain*
[j] *Servicio de Psiquiatría, Hospital Universitario Ramón y Cajal, Madrid, Spain*
[k] *Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia e Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*

Abstract
Aim: Weight gain is an important and common side effect of second-generation antipsychotics (SGAs). Furthermore, these drugs can induce other side effects associated with higher cardiovascular morbidity and mortality, such as insulin resistance, diabetes or metabolic syndrome. Preliminary studies show that inter-individual genetic differences produce varying degrees of vulnerability to the different SGA-induced side effects. The Second-generation antipsychotic Long-term treatment Metabolic side effects (SLiM) study aims to identify clinical,

Drug-related side effects and adverse reactions

environmental and genetic factors that explain inter-individual differences in weight gain and metabolic changes in drug-naïve patients after six months of treatment with SGAs.
Materials and methods: The SLIM study is a multicenter, observational, six-month pharmacogenetic study where a cohort of 307 drug-naïve pediatric and adult patients (age range 8.8–90.1 years) and a cohort of 150 age- and sex- matched healthy controls (7.8–73.2 years) were recruited.
Results: This paper describes the rationale, objectives and design of the study and provides a description of the sample at baseline.
Conclusions: Results from the SLiM study will provide a better understanding of the clinical, environmental, and genetic factors involved in weight gain and metabolic disturbances associated with SGA treatment.

PALABRAS CLAVE
Agentes antipsicóticos;
Aumento de peso;
Síndrome metabólico;
Farmacogenética;
Efectos secundarios y reacciones adversas a medicamentos

Estudio farmacogenético del tratamiento a largo plazo con antipsicóticos de segunda generación y sus efectos adversos metabólicos (Estudio SLiM): justificación, objetivos, diseño y descripción de la muestra

Resumen
Objetivo: El aumento de peso es un efecto secundario frecuente e importante de los antipsicóticos de segunda generación (ASG). Además, estos fármacos pueden inducir otros efectos secundarios que están asociados a un aumento de la morbimortalidad cardiovascular, tales como la resistencia a la insulina, la diabetes o el síndrome metabólico. Estudios preliminares indican que las diferencias genéticas interindividuales producen distintos grados de vulnerabilidad a los efectos secundarios inducidos por los ASG. El estudio SLiM (por sus siglas en inglés, *Second-generation antipsychotic Long-term treatment Metabolic side effects*) tiene como objetivo identificar en pacientes no tratados previamente con ASG (pacientes naive), aquellos factores clínicos, genéticos y ambientales que expliquen las diferencias interindividuales en relación con el aumento de peso y los cambios metabólicos generados tras 6 meses de tratamiento con estos fármacos.
Material y métodos: El estudio SLiM es un estudio farmacogenético multicéntrico, observacional, prospectivo, de 6 meses de duración, en el que se ha reclutado una cohorte de 307 pacientes pediátricos y adultos (rango de edad entre 8,8 a 90,1 años) naive a ASG y una cohorte de 150 controles sanos (rango de edad entre 7,8 y 73,2 años) emparejados por edad y sexo.
Resultados: En este artículo se presentan la justificación, los objetivos y el diseño del estudio y se ofrece una descripción de la muestra al inicio del estudio.
Conclusiones: Los resultados del estudio SLiM permitirán una mejor comprensión de los factores clínicos, ambientales y genéticos implicados en el aumento de peso y los trastornos metabólicos asociados al tratamiento con ASG.

Introduction

The prescription of second-generation antipsychotics (SGAs) has dramatically increased in recent years in pediatric and adult population, both for psychotic and non-psychotic disorders.[1,2] Despite a better extra-pyramidal side effect profile than classic antipsychotics, overall, SGAs are associated with a higher prevalence of metabolic and endocrine disturbances, such as weight gain, dyslipidaemia or glycemic abnormalities.[3,4] These disturbances lead to a higher risk of cardiovascular disease and to an increased morbidity and mortality in these patients, as compared with the general population.[5,6] Furthermore, metabolic adverse events have been associated with non-adherence to treatment and poor quality of life.[7–9]

Currently, optimization of antipsychotic treatment is limited by the high variability of response to treatment and tolerability among individuals. This variability is related to clinical heterogeneity, genetic, environmental and social factors[10] and has hindered the use of pharmacogenomic (PGx) testing or genotype-based prescription in clinical practice.[11,12] However, PGx-related techniques could help the identification of patients at higher risk of developing SGA-induced side effects through the identification of PGx biomarkers.[13,14]

The purpose of the second-generation antipsychotic Long-term treatment metabolic side effect study (SLiM study), a multicenter, longitudinal, 6-month follow-up PGx study, was to identify clinical, environmental and genetic predictive factors of weight gain and metabolic changes

Table 1 Included patients and controls per participating site.

	Patients		Controls	
	No.	%	No.	%
Hospital Universitario Gregorio Marañón (HGUGM), Madrid[a]	120	39.1	53	35.3
Oviedo Mental Health Services (OMHS), Oviedo	87	28.3	20	13.3
Hospital de la Santa Creu i Sant Pau (HSCSP), Barcelona	45	14.6	19	12.7
Hospital Universitario Santiago Apóstol (HUSA), Vitoria	19	6.2	21	14.0
Hospital Universitario 12 de Octubre (HU12O), Madrid	18	5.9	25	16.7
Hospital Universitario Ramón y Cajal (HURC), Madrid	10	3.3	8	5.3
Hospital Clínico Universitario de Valencia (HCUV), Valencia	8	2.6	4	2.7
Total	307	100	150	100

[a] Coordinating site.

in a Spanish sample of drug-naïve pediatric and adult patients treated with SGAs, and a cohort of age- and sex-matched healthy controls. The specific goals of the study were: (1) to assess the prevalence of anthropometric and metabolic changes after six months of treatment with SGAs, (2) to assess the association between weight gain/metabolic changes and clinical improvement/non-adherence to treatment/quality of life, (3) to analyze polymorphisms in candidate genes related to appetite control, central nervous system homeostatic regulation, and obesity, (4) to assess the association between the analyzed genotypes, weight gain and metabolic changes, and (5) to identify risk genotypes that explain inter-individual differences in SGA-induced weight gain and metabolic disturbances.

This paper describes the rationale, objectives and design of the SLiM study and provides a description of the sample at baseline.

Subjects and methods

Recruitment procedure

From January 2007 to December 2010, seven sites from the Spanish Psychiatric Research Network (CIBERSAM) (see www.cibersam.es) recruited a sample of 307 pediatric and adult patients consecutively attended in their clinical facilities and prescribed an SGA for the first time or with a prior lifetime exposure to antipsychotics of no more than 10 days. A cohort of 150 age- and sex- matched healthy control subjects was also recruited. Table 1 displays the number of patients and controls included in the study per participating site.

Patients were recruited from emergency rooms, inpatient units, and outpatient clinics. The inclusion criteria for patients were: (i) presence of a psychiatric disorder according to DSM-IV criteria,[15] (ii) capacity to give written informed consent (from participants and from their parents or legal guardians as appropriate), and (iii) being drug-naïve patients (first prescription of an SGA, or, if previously treated, a total lifetime exposure to antipsychotics of no more than 10 days). The initial design of the SLiM study only considered those patients who were firstly prescribed risperidone, olanzapine and quetiapine. Later on, this inclusion criterion was broadened and prescription with other

SGAs was also allowed. Concomitant treatment with stimulants, mood stabilizers, antidepressants, anticholinergic agents, and benzodiazepines/hypnotics was also allowed. Presence of a severe medical condition was the unique exclusion criterion for patients.

Healthy controls were recruited among patients' friends, colleagues and neighbors. Inclusion criteria for controls were (i) absence of any psychiatric diagnosis according to DSM-IV criteria[15] and (ii) capacity to give written informed consent (from participants and from their legal guardians/representatives when needed). Exclusion criteria for controls were (i) presence of a severe medical condition, and (ii) current or past treatment with any antipsychotic drug. The controls were compensated for their time with a 25 € gift card at each visit.

Fig. 1 shows a flowchart of the recruitment procedure. Out of the 415 patients and 153 controls initially identified, 307 patients and 150 controls met the inclusion criteria and completed the baseline assessment. Out of these, 163 patients and 133 controls completed the 6 month follow-up period. Reasons for drop-out were: refusal to continue, loss to follow-up, SGA discontinuation (only for patients), death and other reasons (reason was specified). Shift to or addition of a new SGA during the follow-up was not considered a reason for drop-out.

Design

The SLiM study is a prospective, observational, six-month study, in which four assessment visits (at baseline, 6 weeks, 3 months and 6 months) were performed for patients and controls. At baseline, a complete evaluation (which included recording of demographic, diagnostic and clinical data, clinical scales, recording of psychopharmacological treatment and side effects, an anthropometric and cardiovascular study, and collection of fasting blood and urine samples) was performed in the setting where subjects had been recruited. Later on, subjects were contacted by telephone and the same assessments were repeated at 6 weeks, 3 months and 6 months in a psychiatric outpatient setting. A review of the diagnosis and of the ongoing pharmacologic treatment, and an assessment of change in global psychopathology were also conducted. Taking each individual's anthropometric, cardiovascular and blood test data, and based on established
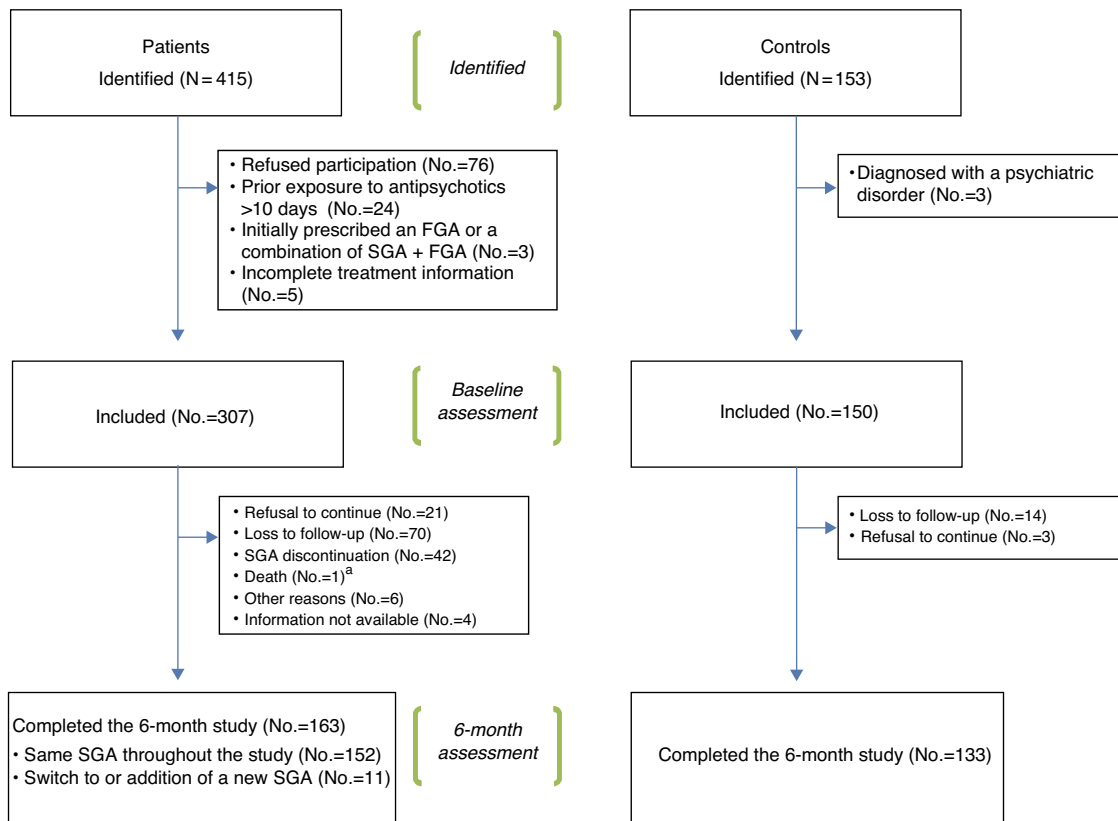
Figure 1 Flowchart for study design.
Abbreviations: FGA: first-generation antipsychotic; SGA: second-generation antipsychotic. [a] Death was caused by complications of a diagnosed dementia disorder.

criteria, presence of insulin resistance[16] and presence of metabolic syndrome[17–19] were determined at each visit. For each participant, after the last completed visit, a completion sheet form was filled out. Date of last visit and reasons for drop-out (where appropriate) were registered. Table 2 summarizes the assessments and their timing throughout the study.

Demographic and clinical assessment

An ''ad hoc'' protocol for recording of demographic data was designed. Age, sex, ethnicity, civil status, educational and occupational level of the participant (or his/her parent or legal guardian if under 18 years of age) were recorded. Diagnosis was made by experienced psychiatrists according to DSM-IV criteria,[15] by means of a clinical interview and a review of medical records of the participant. Also, information on personal medical history (including type 1 and 2 diabetes mellitus), family history of type 1 and 2 diabetes mellitus and/or hyperlipidemia, personal and family history of psychiatric disorder, number and duration of hospitalizations (for patients), pregnancy, and substance misuse was recorded. Data on somatic and psychopharmacological treatment was also recorded, including dosage, dosage changes, and start and discontinuation dates of any previous and/or current treatment. Antipsychotic doses

were derived to chlorpromazine equivalents and current and cumulative doses at each visit were calculated.[20,21]

The anthropometric study was performed by trained nurses. Weight (in kilograms) and height (in meters) were measured at each visit with the same scale: SECA GMBH & Co., Model 797, in Hospital General Universitario Gregorio Marañón (HGUGM) and Oviedo Mental Health Service (OMHS); Año Sayol, Model Atlantida, in Hospital de la Santa Creu i Sant Pau (HSCSP) and Hospital Universitario Ramón y Cajal (HURC); Soenhle 7831 Digital Scale in Hospital Universitario Santiago Apóstol (HUSA); Body Composition Analyzer BF-350, Tanita Corporation, in Hospital Universitario 12 de Octubre (HU12O); OMROM BF-500 in Hospital Clínico Universitario Valencia (HCUV). Using this data, body mass index (BMI) was calculated as follows: weight (kilograms)/height (square meters). Since BMI varies with age and sex, the BMI value was adjusted using a conversion to a z-score. For children and adolescents, BMI was adjusted for age and sex using Spanish normative charts.[22] For adults, raw scores were transformed to z-scores based on the BMI of the sex-matched control group at each visit. Abdominal circumference was measured using a flexible tape measure placed above the uppermost border of the iliac crests. Total body fat mass was measured at each visit with the same bioelectrical impedance scale: OMRON BF-500 in HGUGM, OMHS, HUSA, HURC and HCUV; OMRON BF-511 in HSCSP; Tanita BF-350 in HU12O). At each visit, participants were asked whether they were on diet and whether they had experienced changes

Table 2 Assessments and timing.

	Baseline	6th week	3rd month	6th month
Demographic and clinical assessments				
Demographic data	X			
Diagnostic (DSM-IV) and clinical interview	X	X	X	X
Medication	X	X	X	X
Family history of diabetes mellitus type 1 and 2	X			
Family history of hyperlipidemia	X			
Anthropometric study	X	X	X	X
Cardiovascular study	X	X	X	X
Physical activity	X	X	X	X
Diet, appetite and intake	X	X	X	X
CGI-S scale	X	X	X	X
CGI-I scale		X	X	X
IWQOL-Lite scale	X	X	X	X
UKU tolerability scale	X	X	X	X
Blood sampling				
Biochemical/metabolic testing	X	X	X	X
Serum levels of SGA (HPLC)	X	X	X	X
Genetic testing	X			
Urine sampling				
Routine urinalysis	X	X	X	X
Pregnancy test (where appropriate)	X	X	X	X
Urine drug testing	X	X	X	X
Metabolic and cardiovascular risk assessments				
Metabolic syndrome	X	X	X	X
HOMA-IR index	X	X	X	X
Completion sheet				
Date of last visit		Last completed visit		
Reasons for drop-out (where appropriate)		Last completed visit		

Abbreviations: CGI-I: Clinical global impression-improvement; DSM-IV: Diagnostic and statistical manual of mental disorders, 4th edition; HOMA-IR: Homeostasis model assessment-estimated insulin resistance; HPLC: High-performance liquid chromatography; IWQOL-Lite: Impact of weight on quality of life; SGA: Second-generation antipsychotic; UKU: *Udvalg for Kiniske Undersogelser*.

in appetite and/or in food intake. Information on levels of physical activity (i.e., hours per week of physical activity during the previous week) was also recorded.

A Cardioline Delta 1 Plus Digital ECG, Versione Base (Renco, Italy) in HGUGM, Cardioline AR600ADV ECG (Cavareno, Italy) in OMHS, Philips M1772A ECG (Andover, MA, USA) in HSCSP, Philips Page Writer Trim I ECG (Eindhoven, The Netherlands) in HUSA, Philips Page Writer TC30 ECG (Andover, MA, USA) in HURC, Cardiovit AT-2 ECG, Schiller AG (Baar, Switzerland) in HU12O and Nihon Kohden Cardio FAX V Ecaps 12 (Rosbach, Germany) in HCUV were used for the ECG assessment. Recording of sitting blood pressure (BP) and heart rate was also performed. For children, raw BP values were derived to percentiles according to the International Task Force for BP.[23]

The Clinical Global Impression-Severity (CGI-S) scale[24] and the Clinical Global Impression-Improvement (CGI-I) scale[24] were completed by experienced psychiatrists. These scales assess severity and improvement, respectively, of global symptomatology on a scale of 1–7. They are particularly helpful in longitudinal studies as they provide an ordinal repeated measure of global clinical status.

The Impact of Weight on Quality of Life (IWQOL-Lite) scale[25] and the *Udvalg for Kiniske Undersogelser* (UKU)

scale[26] were also administered throughout the study by trained psychiatrists. The IWQOL-Lite scale is a 31-item self-report scale for assessment of obesity-specific quality of life which consists of a total score and five subscores, ech one derived from five specific subscales (i.e., physical function, self-esteem, sexual life, public distress, and work). The UKU scale is a comprehensive rating scale including a total of 54 items (divided in four categories) scored 0–3, designed to assess general side effects of psychotropic drugs.

Biochemical determinations

Venous blood samples were collected by nursing staff in 7 polypropylene EDTA-containing and serum tubes in the morning (between 8:00 and 10:00) after fasting overnight and stored initially at 4 °C. One of each extracted blood samples was sent to the Biochemistry Department at each participating site, where biochemical determinations were performed. Determinations included lipid profile, glucose, glycated hemoglobin A1 (HbA1c), and insulin levels. In all the participating sites, blood glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides

(TG) were determined by enzymatic procedures with an Automatic Chemical Analyzer. HbA1c was analyzed by high-performance liquid chromatography (HPLC). Insulin was measured by immunoassay with chemiluminescence detection. The Biochemistry Department at each participating site recorded the biochemical data using pre-established criteria for normality (minimum and maximum) for each parameter. The reference values at each site were recorded and individual values were corrected and homogenized in the common database.

For both patients and controls, one of the blood tubes was centrifuged ($2000 \times g \times 10$ min, at room temperature) after 1 h of storage. The resultant serum samples were carefully collected in serum tubes and stored at $\leq -20\,^{\circ}$C until they were sent to the Biochemistry laboratory at the coordinating site (HGUGM), where adiponectin and leptin levels were determined (in duplicate) by Enzyme-Linked Immune Sorbent (ELISA) assay (Human Leptin ELISA [ref: RD191001100], Human Adiponectin ELISA [ref: RD195023100]; all Biovendor Laboratory Medicine Inc., Brno; Czech Republic). All these determinations were performed by a trained and experienced biochemist.

Serum SGA determinations

For each patient and at each visit, one of the stored serum samples was also sent to the Biochemistry laboratory at the coordinating site (HGUGM), where HPLC was performed by a trained and experienced biochemist using a Waters 2695 series HPLC system (Alliance HPLC system, Waters Corp. Milford, MA, USA). The analytes were separated on an Agilent Eclipse XDB C-18 column (150×4.6 mm i.d., 5 μm) with a 4.6×12.5 mm Eclipse XDB C-18 guard column, following the protocol described by Zhang et al.[27] This semi-quantitative technique provided an indirect measure of patient's adherence to SGAs at each visit (by means of a 'positive' or 'negative' HPLC result).

Genotype analyses

For each participant, one of each collected blood samples was stored at $\leq -20\,^{\circ}$C until it was sent to the College of Biology at University of Barcelona, where an experienced and trained geneticist performed DNA processing (i.e., coding, storage and extraction, using a commercial extraction kit) and DNA genotyping. Candidate genes of the SLiM PGx study and their selected SNPs and MAFs are summarized in Supplementary Table 1 (Annex). Candidate genes related to mechanism of action of SGAs, appetite control, homeostatic regulation and weight gain or related phenotypes (e.g., obesity) were selected based on previous association and genome wide association studies (GWAS).[28–35] Single Nucleotide Polymorphisms (SNPs) of these candidate genes were selected based on previous literature and the SYSNPS program (www.sysnps.org). SNPs were chosen from the SYSNPS program only if they were TagSNPs of the candidate gene and presented a Minor Allele Frequency (MAF) > 0.2. This MAF criterion was used to increase the statistical power.

Genotyping was blind to group membership (patient vs control) and SGA treatment. The 5-HTTLPR polymorphism (SLC6A4 gene) was genotyped following a standard protocol.[36] The rs6265 (BDNF gene) and rs1049353 (CNR1 gene) were genotyped using Taqman 5'-exonuclease assay. All other SNPs were genotyped by competitive quantitative PCR using allele specific probes with FRET signal detection. A randomly selected subsample of individuals was regenotyped in order to confirm the pattern of reproducibility.

Urine sampling

At each visit and for each participant, urine samples were collected and a routine urinalysis, pregnancy test (where appropriate) and urine drug test were performed. Amphetamines, cannabis, cocaine, opiates, benzodiazepines and barbiturates were determined by immunoassay in HGUGM, HSCSP, HU12O, HUSA, and HURC; by endpoint spectrophotometry with monochromatic light in OMHS; and by thin-layer chromatography in HCUV.

Metabolic and cardiovascular risk assessment

Taking each individual's anthropometric and cardiovascular data, presence of metabolic syndrome was established for each participant at each visit. For adult participants, metabolic syndrome was defined following the American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.[19] For pediatric participants, metabolic syndrome was defined following adapted criteria derived from Cook et al., 2003,[17] and Correll and Carlson, 2006.[18] These criteria include a clustering of the most relevant risk factors for cardiovascular disease: abdominal obesity raised fasting plasma glucose and/or diabetes, abnormal lipid profile and high blood pressure (see Table 3).

Both for pediatric and adult participants, insulin resistance was estimated using the homeostasis model assessment- insulin resistance (HOMA-IR) index,[16] which is easily calculated from a single measurement of fasting insulin and glucose (see Table 3).

Data processing and analysis

In order to integrate all the available information, and to facilitate data management and exploitation, a common database was created. Data entry, database design and management were conducted at the coordinating site.

For the description of the sample at baseline, continuous variables were expressed as means, standard deviations (SD) and ranges, and categorical variables were expressed as frequencies and percentages. Group differences were tested for baseline demographic and clinical data. The comparison groups were patients (whole sample) vs controls (whole sample), pediatric vs adult patients, and pediatric vs adult controls. Chi-square or Fisher exact tests were used for discrete categorical variables. Since all the quantitative variables included in the analysis (age, days of previous SGA exposure, current and cumulative doses of SGA at baseline) showed non-normal distributions, non-parametric tests (Mann–Whitney U) were used. Statistical analyses were carried out with the SPSS 18.0[37] and differences of $p < 0.05$ were considered significant. All tests were two-tailed.

Table 3 Criteria for presence of metabolic syndrome and insulin resistance in pediatric and adult populations.

	Adult participants	Pediatric participants
Metabolic syndrome	[a]≥3 of the following: - Waist circumference ≥ 102 cm in males or ≥ 88 cm in females - Glucose ≥100 mg/dl - Triglycerides ≥150 mg/dl - HDL cholesterol <40 in males or <50 in females. - Blood pressure ≥ 130/85 mmHg	[b,c]≥3 of the following: - BMI ≥ 95th percentile or waist circumference ≥ 90th percentile - Glucose ≥ 100 mg/dl - Triglycerides ≥110 mg/dl - HDL cholesterol <40 mg/dl - Blood pressure ≥ 90th percentile
HOMA-IR[d]	Fasting glucose (mg/dl) * Insulin (μU/ml)/405	

Abbreviations: BMI: body mass index. HDL: high density lipoprotein, HOMA-IR: homeostasis model assessment-insulin resistance.
[a] Grundy et al.[19]
[b] Cook et al.[17]
[c] Correl and Carlson.[18]
[d] Matthews et al.[16]

Results

Sample description at baseline

The demographic and clinical characteristics at baseline of the 307 (71 pediatric and 236 adult) patients and 150 (18 pediatric and 132 adult) healthy controls who were included in the SLiM study are shown in Table 4. Data on SGA and concomitant treatments, both for the whole sample and for each age group, are also shown in Table 4.

A significantly higher proportion of patients (47%) than healthy controls (11%) dropped out of the study through the follow-up. The main reasons for drop-out are shown in Fig. 1 and Table 4.

Discussion

The SLiM study is the first 6-month prospective, observational, multicenter, pharmacogenetic (PGx) study including a large cohort of SGA drug-naïve patients across a large age span (8–90 years) and with a variety of diagnoses, and a cohort of healthy controls matched by age, sex and ethnicity. In our sample, the group of pediatric patients had a significantly higher proportion of males, a higher proportion of subjects diagnosed with bipolar disorder and a higher proportion of severe patients (defined as having a CGI-S score ≥ 6) than the group of adult patients. Irrespective of age, almost 40% of patients were completely naïve to SGAs at baseline. Risperidone was the most commonly prescribed SGA across the entire age span. Pediatric patients had higher prescription rates of risperidone, anticholinergic agents and stimulants at baseline than their adult counterparts. Conversely, olanzapine and antidepressants were more commonly prescribed in adults. Although not statistically significant, mean daily doses of prescribed SGAs were slightly lower in the pediatric than in the adult group.

SGAs other than risperidone, olanzapine and quetiapine were scarcely prescribed in our sample. This may be partly due to the study design. At the beginning of this study, only those patients who were firstly prescribed one out of these three SGA drugs were included; further on, the inclusion criteria were broadened and prescription of any SGA was allowed. This may have biased the results regarding prescription rates in both age groups. Nonetheless, our results were congruent with previous observational studies in similar settings, in which those were the most prescribed SGAs, both in paediatric[38,39] and adult samples.[40] Except for a patient that had been prescribed risperidone for aggression in the context of a diagnosed conduct disorder and low intelligence quotient, the rest of pediatric patients enrolled in the SLiM study were taking SGA drugs for off-label uses. That said, the fact that olanzapine was poorly prescribed in the pediatric group, points toward the increasing awareness of the metabolic profile of this drug in child and adolescent psychiatrists.[41] Regarding concomitant treatments, even though antidepressants are increasingly co-prescribed with antipsychotics in children and adolescents,[42,43] co-prescription rates appear to be higher in adult population.[40,44]

The SLiM study has a number of limitations that are inherent in its naturalistic design. Prescribed SGA treatment was not controlled and doses were chosen by the treating psychiatrists, based on clinical criteria. Also, the sample was quite heterogeneous in setting provenance, diagnoses, use of concomitant medications or presence of alcohol and substance misuse. This will be taken into account when carrying out further analyses on the clinical, environmental, and genetic factors that might be associated with weight gain and metabolic disturbances. The main difficulties that we encountered were the recruitment of drug-naïve patients and the control group matching strategy. That said, a relatively large cohort of drug-naïve patients and healthy controls was recruited and followed-up during 6 months. Through the follow-up, one of the main difficulties that we encountered was the large drop-out rate (around 50% of patients and 11% healthy controls dropped out of the study). The main reason for drop-out both in adult and pediatric patients was the loss to follow-up (19.9% and 32.4%, respectively), while refusal to continue was more frequent in adults than in young people (8.5% vs 1.4%, respectively). Discontinuation of the prescribed SGA was a reason for drop-out in around 14% of patients (both in adult and pediatric subgroups). Nonetheless, discontinuation rates may have

Table 4 Demographic and clinical characteristics at baseline of pediatric and adult patients and controls.

	Patients				Controls				
	Total (N = 307)	Pediatric (N = 71)	Adult (No. = 236)	Paed vs adults*	Total (No. = 150)	Pediatric (No. = 18)	Adult (No. = 132)	Paed vs adults*	Patients vs controls*
Age (years), mean (SD), [range]	37.5 (19.9) [8.8–90.1]	15.9 (1.6) [8.8–17.9]	44.0 (18.4) [18.0–90.1]	**0.001**	34.1 (13.7) [7.8–73.2]	15.2 (2.8) [7.8–17.8]	36.7 (12.5) [18.4–73.2]	0.001	0.556
Male sex, No. (%)	169 (55.0)	49 (69.0)	120 (50.8)	**0.007**	71 (47.3)	11 (61.1)	60 (45.5)	0.212	0.121
Ethnicity, No. (%)[a]									
Caucasian	260 (85.5)	53 (75.7)	207 (88.5)	0.052	137 (91.9)	13 (72.2)	124 (94.7)	**0.001**	0.172
Hispanic	25 (8.2)	8 (11.4)	17 (7.3)		10 (6.7)	3 (16.7)	7 (5.3)		
Other	19 (6.2)	9 (12.9)	10 (4.3)		2 (1.3)	2 (11.1)	0 (0)		
Diagnosis (DSM-IV criteria), N (%)[b]									
Schizophrenia spectrum disorders	42 (14.0)	9 (12.9)	33 (14.3)	0.779	–	–	–	–	–
Bipolar disorder	28 (9.3)	14 (20.0)	14 (6.1)	**0.001**					
Other psychotic disorders	108 (36.0)	26 (37.1)	82 (35.7)	0.772					
Depressive disorders	35 (11.7)	1 (1.4)	34 (14.8)	**0.003**					
OCD	1 (0.3)	0 (0)	1 (0.4)	0.999					
Other anxiety disorders	9 (3.0)	0 (0)	9 (3.9)	0.124					
Substance disorders	9 (3.0)	0 (0)	9 (3.9)	0.124					
Adjustment disorders	14 (4.7)	3 (4.3)	11 (4.8)	0.999					
Dissociative disorders	2 (0.7)	1 (1.4)	1 (0.4)	0.410					
Somatoform disorders	3 (1.0)	2 (2.9)	1 (0.4)	0.135					
Eating disorders	1 (0.3)	0 (0)	1 (0.4)	0.999					
Sleep disorders	3 (1.0)	0 (0)	3 (1.3)	0.999					
Personality disorders	16 (5.3)	5 (7.1)	11 (4.8)	0.541					
Mental retardation	3 (1.0)	0 (0)	3 (1.3)	0.999					
Autism spectrum disorders	2 (0.7)	1 (1.4)	1 (0.4)	0.410					
ADHD	5 (1.7)	5 (7.1)	0 (0)	**0.001**					
Conduct disorder/ODD	2 (0.7)	2 (2.9)	0 (0)	0.053					
Other infancy/childhood/adolescence	1 (0.3)	1 (1.4)	0 (0)	0.231					
Delirium	10 (3.3)	0 (0)	10 (4.3)	0.124					
Dementia	6 (2.0)	0 (0)	6 (2.6)	0.342					

Table 4 (*Continued*)

	Patients				Controls				
	Total (N = 307)	Pediatric (N = 71)	Adult (No. = 236)	Paed vs adults[*]	Total (No. = 150)	Pediatric (No. = 18)	Adult (No. = 132)	Paed vs adults[*]	Patients vs controls[*]
CGI- severity, No. (%)[c]					–				–
Mild (1–3)	26 (9.9)	11 (15.9)	15 (7.7)	**0.001**					
Moderate (4–5)	201 (76.4)	36 (52.2)	165 (85.1)						
Severe (6–7)	36 (13.7)	22 (31.9)	14 (7.2)						
Treatment status at baseline, No. (%)[d]									
Completely naïve	121 (39.4)	27 (38.0)	94 (39.8)	0.785	–	–	–	–	–
Prior exposure to SGA ≤ 10 days	186 (60.6)	44 (62.0)	142 (60.2)						
Before baseline visit	184 (59.9)	43 (60.6)	141 (59.7)						
In the past	2 (0.7)	1 (1.4)	1 (0.5)						
Prescribed SGA at baseline, No. (%)[e]									
Risperidone	126 (41.0)	51 (71.8)	75 (31.8)	**0.001**	–	–	–	–	–
Olanzapine	77 (25.1)	3 (4.2)	74 (31.4)	**0.001**					
Quetiapine	88 (28.7)	15 (21.1)	73 (30.9)	0.109					
Other[f]	16 (5.2)	2 (2.8)	14 (5.9)	0.378					
Previous SGA exposure (days), mean (SD), median, [range]	2.4 (2.7), 2.0 [0–10]	2.1 (2.4), 2.0 [0–10]	2.5 (2.8), 1.5 [0–10]	0.372	–	–	–	–	–
Current dose of SGA at baseline (mg), mean (SD), [range][g]	145.7 (185.7) [0–1200]	123.5 (142.1) [0–530.3]	152.2 (196.5) [0–1200]	0.736	–	–	–	–	–
Cumulative dose of SGA at baseline (mg), mean (SD), [range][g]	560.9 (859.7)[0–6105.3]	377.4 (545.8)[0–2727.3]	614.8 (926.1)[0–6105.3]	0.330	–	–	–	–	–
Concomitant treatment, No. (%)[h]									
Antidepressants	78 (25.6)	5 (7.2)	73 (30.9)	**0.001**	–	–	–	–	–
Lithium	3 (1.0)	0 (0.0)	3 (1.3)	0.999					
Other mood stabilizers	25 (8.2)	3 (4.3)	22 (9.3)	0.185					
Stimulants	5 (1.6)	5 (7.0)	0 (0)	**0.001**					
Benzodiazepines	142 (46.4)	26 (37.1)	116 (49.2)	0.077					
Anticholinergic agents	12 (3.9)	8 (11.3)	4 (1.7)	0.001					

Table 4 (*Continued*)

	Patients				Controls				
	Total (N = 307)	Pediatric (N = 71)	Adult (No. = 236)	Paed vs adults[*]	Total (No. = 150)	Pediatric (No. = 18)	Adult (No. = 132)	Paed vs adults[*]	Patients vs controls[*]
Drop-out before 6th month assessment, N (%)[i]	144 (46.9)	36 (50.7)	108 (45.8)	0.464	17 (11.3)	3 (16.7)	14 (10.6)	0.432	**0.001**
Refusal to continue	21 (6.8)	1 (1.4)	20 (8.5)	**0.034**	3 (2.0)	2 (11.1)	1 (0.8)	**0.038**	**0.027**
Loss to follow-up	70 (22.8)	23 (32.4)	47 (19.9)	**0.034**	14 (9.3)	1(5.6)	13 (9.8)	0.999	**0.001**
SGA discontinuation	42 (13.7)	10 (14.1)	32 (13.6)	0.950	–	–	–	–	–
Death[j]	1 (0.3)	0 (0)	1 (0.4)	0.999	0 (0)	0 (0)	0 (0)	–	0.999
Other reasons	6 (2.0)	2 (2.8)	4 (1.6)	0.628	0 (0)	0 (0)	0 (0)	–	0.185
Unknown	4 (1.3)	0 (0)	4 (1.6)	0.577	0 (0)	0 (0)	0 (0)	–	0.308

In all cells, % refers to percentages (within columns) of participants for whom information was available. Abbreviations: CGI: clinical global impression; Paed: pediatric; SGA: second-generation antipsychotic.

[*] Statistically significant *p*-values in bold. For qualitative variables, Chi-square tests (χ^2) (Fisher exact test when needed). For quantitative variables, Mann–Whitney *U* tests.

[a] Information available for 304 patients and 149 controls.

[b] Information available for 300 patients. Diagnosis refers to the psychiatric condition for which the SGA was prescribed. Forty-five patients had a co-morbid Axis I or Axis II diagnosis (data available upon request). ADHD: Attention deficit and hyperactivity disorder. Depressive disorders: major depressive disorder, dysthymia, and depressive disorder not otherwise specified. OCD: Obsessive-compulsive disorder. ODD: Oppositional defiant disorder. Other anxiety disorders: generalized anxiety disorder, anxiety disorder not otherwise specified, and posttraumatic stress disorder. Other infancy/childhood/adolescence: other disorders of infancy, childhood, or adolescence. Other psychotic disorders: brief reactive psychosis, psychotic disorder not otherwise specified, major depressive disorder with psychotic symptoms, substance-induced psychosis, and delusional disorder. Schizophrenia spectrum disorders: schizophrenia, schizophreniform and schizoaffective disorder.

[c] Information available for 263 patients.

[d] Information available for 307 patients. Completely naïve refers to those patients who were prescribed an SGA for the first time just at the time the baseline visit was performed. Prior exposure to SGA ≤ 10 days refers to those patients who had a prior lifetime exposure to antipsychotics of no more than 10 days, either because they had been prescribed an SGA right before the baseline visit or in the past.

[e] Information available for 307 patients.

[f] Other SGAs: paliperidone (n = 6 adult patients), aripiprazol (n = 2 pediatric and 4 adult patients), amisulpride (n = 2 adult patients), asenapine (n = 1 adult patient), combination of olanzapine + sulpride (n = 1 adult patient).

[g] In chlorpromazine equivalents: 100 mg chlorpromazine = about 1.5 mg risperidone/5 mg olanzapine/150 mg quetiapine.

[h] Information available for 305 patients.

[i] Information available for 303 patients and 150 controls.

[j] Death was caused by complications of a diagnosed dementia disorder.

been higher, since discontinuation might have happened for some of the patients who were lost to follow-up or refused to continue participation. Furthermore, the controls were compensated with a gift card for their participation in the SLiM study. This may have led to a 'volunteer bias' (i.e., the bias resulting from the fact that a particular sample can contain only those participants who are actually willing to participate in the study or experiment[45]). Nonetheless, the authors tried to control for this by means of a careful control group matching strategy. Another relevant limitation is that serum SGA determinations at each visit only provide an indirect measure of treatment adherence. Nonetheless, these determinations were carried out at one site, where a trained and experienced biochemist performed the analyses following a standardized protocol.

The main strengths of this study include the prospective design of the study, with a 6-month follow-up, the homogeneity of the patient and control sample, the large age span of the recruited sample and the relatively large sample size considering the above mentioned characteristics of the sample. This strategy will increase the power of the PGx study with few confounding factors and carry-over effects.

Results from the SLiM study will provide a better understanding of the clinical, environmental, and genetic factors involved in weight gain and metabolic disturbances associated with SGA treatment. Additionally, we will be able to explore the effect of age in such changes and the impact of weight gain in clinical outcome and quality of life of drug-naïve patients treated with SGAs. Finally, the study will enable the identification of potential PGx biomarkers as predictors for SGA tolerability. In the near future, this could potentially assist the individualization of antipsychotic treatment and facilitate the development of new therapeutic tools with better safety and tolerability profiles.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Declaration of Helsinki.

Confidentiality of Data. The authors declare that there is no confidential data appearing in this article. Privacy and confidentiality of data were warranted throughout the study.

Right to privacy and informed consent. The authors obtained the informed consent of the participants and/or their parents or legal guardians as appropriate mentioned in the article. The author for correspondence is in possession of this document.

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Conflict of interest

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Appendix A. Supplementary data

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