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Serbian Twin Registry

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SERBIAN TWIN REGISTRY

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Abstract

The first twin study in Serbia began in 2011 as a part of the research project “*Psychological Foundations of Mental Health: Hereditary and Environmental Factors*”. At the same time, the research team from the Faculty of Philosophy and Faculty of Medicine in Novi Sad established the first Serbian twin registry. The registry is intended primarily for the purpose of the research in behavioral genetics, as well as potential future studies in human genetics. It includes information on 1658 volunteers, including twin pairs, their parent and siblings. The behavioral genetic study of adult twins has been focused on the hereditary and environmental sources of variance of different psychological characteristics, such as personality traits, cognitive abilities, executive functions, aggression, as well as some anthropometric measures, and some aspects of mental and physical health. Certain molecular genetic analyses have also been performed. The research team is currently starting the longitudinal twin study of children, which will be focused on different indicators of emotional, cognitive and physical development.

Key words: twin study, personality, cognitive abilities, health, life events, developmental characteristics

Background of the First Twin Study in Serbia

The Serbian Twin Registry (STR) was created in 2011 as a part of the research project *Psychological Foundations of Mental Health: Hereditary and Environmental Factors*, granted by the Ministry of Education, Science, and Technological Development of the Republic of Serbia. Researchers participating in the study are from the Department of Psychology at the Faculty of Philosophy and from the Faculty of Medicine at the University of Novi Sad. This interdisciplinary team is trying to answer some important questions of behavioral genetics. The registry is intended primarily for the purpose of the research in behavioral genetics, as well as studies in molecular human genetics. In 2014, the research team established the Center for Behavioral Genetics (CBG), as an organizational unit of the Faculty of Philosophy in Novi Sad, in which biometric and molecular genetic analyses are conducted, based on the data collected through the STR. The Serbian Twin Registry and the Center for Behavioral Genetics are the first and only institutions of this type in Serbia.

Recruitment of the Twins and Online Platform for Data Collection

The official website of the STR is available at <http://www.blizanci.rs/>. The website provides information on the importance of twin research for science, scientific results of previous twin studies, facts about the project, research team and the CBG. Twins and their parents who are willing to take part in the research can apply for participation through the publicly available application form on the STR website. Invitations for participation are being sent via media, press, newspapers, social networks, promotional posters and leaflets, and through various public campaigns. After the participants submit the application to the STR, we are reaching them through an email or telephone contact to take part in the study. Additionally, a custom web application was developed in order to distribute questionnaires online and collect data directly into the database. Data collection platform is available only to registered twin-pairs having unique security codes, in order to protect potentially sensitive personal information.

Through the web application, twin-pairs can complete all the test material, which significantly facilitates and accelerates the process of data collection.

Ethical Issues

All procedures in twin study were approved by the Ethical Committee of the Faculty of Philosophy, University of Novi Sad, Serbia, which is the Second Instance Commission of the Ethical Committee of the Serbian Psychological Society. The approval certificate can be found at the following link:

http://psihologija.ff.uns.ac.rs/etika/?odobreno=20111020000004_e1b8

Before participation in the study, all twins were informed about main purpose of the study and signed informed consent. Since Competitive Reaction Time Task includes deception about real purpose of the examination, all participants were debriefed immediately after they finished this task. Namely, in this procedure participants were led to believe that they compete with each other in reaction time, but actually there was no real competition.

Sample

The Serbian twin registry contains information on 1658 volunteers, including twins, their parents and siblings (Figure 1). A total of 722 registered twin pairs includes 295 twin pairs who were already registered and tested, 327 youth and 100 adult twin pairs who were registered and scheduled to be tested in the next stage of the study. Additionally, the STR includes 214 family members of the twins. After participation in the research twins were asked to invite their family members to join this research and complete several self-report questionnaires (Table 2).

<< INSERT FIGURE 1 ABOUT HERE >>

Table 1 presents participants of the STR who finished testing as of 2019. We examined 295 twin pairs, reared-together. Twin participants were aged 16 to 60 years old with an average age of 24.49 ($SD = 7.59$). Among the pairs there were 180 monozygotic twins and 93 dizygotic twins. For the remaining 22 twin pairs zygosity has not yet been determined and is currently being analyzed. In total there were 263 male and 541 female participants including twins and their family members. Among the dizygotic twin pairs there were 47 mixed gender pairs and 46 same sex gender pairs.

Family members of 96 twin pairs participated in the study amongst whom there were 76 fathers, 86 mothers and 52 siblings (20 brothers and 32 sisters).

<< INSERT TABLE 1 ABOUT HERE >>

Zygoty Determination

Zygoty was determined by DNA analysis of buccal swabs and is still being determined for new samples. The research was performed according to the Declaration of Helsinki. One part of DNA samples was analyzed at the Institute of Forensic Medicine Novi Sad, and the other part at John Jay College of Criminal Justice in New York, NY. For new samples analyses are still ongoing at John Jay College of Criminal Justice in New York, NY. DNA is tested using short tandem repeat megaplex kits, either Investigator 24plex GO! (Qiagen, Valencia, CA, USA) or GlobalFiler kit (Applied Biosystems, Thermofisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. Both kits detect 21 autosomal STRs. A completely concordant pair with these short tandem repeat is interpreted as a monozygotic twin pair and all the other pairs are designated as dizygotic.

For a small part of sample (1%) the zygosity was determined using the Twins Physical Resemblance Questionnaire (Oniszczenko et al., 1993). This questionnaire includes a series of questions about similarities and dissimilarities between two twins, within twin pairs (e.g., eye colour, body weight, body height, etc.). These items have been shown to be valid indicators of zygosity with zygosity determination being accurate around 95% (e.g., Čolović et al., 2018; Reed et al., 2005).

Measures

The behavioral genetic study of adult twins has been focused on the hereditary and environmental sources of variance of different psychological characteristics, such as personality traits, cognitive abilities, executive functions, aggression, as well as some anthropometric measures, and some aspects of mental and physical health.

Therefore, the measures are divided into several primary areas: personality assessment, assessment of cognitive abilities, assessment of executive functions, assessment of family environment, life events, aspects of mental health, assessment of laboratory induced aggression, medical examination, and DNA sampling.

Personality Measures

Revised NEO Personality Inventory (NEO PI-R; Costa & McCrae, 1992) and *NEO Five-Factor Inventory* (NEO-FFI; Costa & McCrae, 1992; Costa & McCrae, 2019). NEO PI-R is designed to assess Big Five personality traits: Openness to Experience (O), Conscientiousness (C), Extraversion (E), Agreeableness (A), and Neuroticism (N), by 240 items, while NEO-FFI is a short version of the NEO Personality Inventory, comprising 60 items.

Reinforcement Sensitivity Questionnaire (RSQ; Smederevac et al., 2014). RSQ is based on the revised Reinforcement Sensitivity Theory (rRST; Gray & McNaughton, 2000) and contains 29 items with a four-point Likert scale distributed in five scales: Behavioral Inhibition System (BIS), Behavioral Activation System (BAS); Fight, Flight and Freeze. *Sensation Seeking Scale* (SSS V; Zuckerman, 1994) consists of 40 items with a five-point Likert scale, measuring four components of sensation seeking: Thrill and Adventure Seeking, Experience Seeking, Disinhibition and Boredom Susceptibility.

Buss-Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1992, for Serbian adaptation see Dinić & Janičić, 2012) consists of 29 items with a five-point Likert scale, measuring four components of aggressiveness: Physical and Verbal aggression, Anger, and Hostility.

Competitive Reaction Time Task - CRTT (also known as Taylor Aggression Paradigm - TAP, also known as the Taylor Aggression Paradigm, Taylor, 1967; for details see Warburton & Bushman, 2019) has been used for aggression induction. In this procedure twins were led to believe they were competing with each other in the reaction time tasks. Before each task, each twin had an opportunity to set the “punishment” for his/her twin pair. The punishment was comprised of a settled intensity and duration of an aversive noise. If one twin lost, he/she would receive the punishment determined by the winning twin. There were four blocks in the procedure (each contained 10 tasks), with the first block designed as a practice in which twins only gave the punishment and did not receive it if they lost. In the 2-4 blocks, twins received predetermined punishments which increased during the procedure. In each block it was settled that participants randomly won in 50% of the tasks. The aggression measures were settled intensity and duration of the punishments by twins.

Cognition Measures

Advanced Progressive Matrices (APM: Raven et al., 1998). APM is a non-verbal type of test that measures fluid intelligence. It consists of 48 multi-choice questions, listed in order of difficulty.

Wisconsin Card Sorting Test – WCST (Heaton et al., 1993). The test assesses the possibility of creating and changing the principles of categorization, using the task of classifying a series of cards according to one of the three classification criteria (colour, form, and number of elements).

Trail Making Test – Form A and B (TMT: Reitan, 1955; Spreen & Strauss, 1991). This test consists of two parts, each with a specific aim. The first part aims to measure attention, concentration, visual observation, visuospatial estimation, and visuomotor abilities while the second part assesses complex conceptual monitoring, as a type of executive abilities.

Verbal Fluency Test – Phonemic and Category (VFT; Goodglass & Kaplan, 1983; Lezak, 1995). The test consists of three tasks of phonemic and one task of categorical fluency. Verbal fluency is measured by the number of words produced in the unit of time. Words are usually limited to certain categories. Phonemic fluency is assessed by a test of controlled oral associations, including phonemes S/K/L in language equivalent to the Verbal Fluency Test (FAS) in the English language. In the Category fluency test, the respondents were asked to generate exemplars from a given category. In this study the respondents were asked to indicate, within a minute, as many different animals as possible.

Participants completed a two computer-based tasks of executive functions designed for this research. The first computer-based task (Go/No-Go task) was used to assess the ability of *Inhibition*. The second computer-based task was used to assess Mental Shifting/Flexibility abilities. The computer-based tasks are supported in PsychoPy 1.74 for Windows software, which is in the public domain.

Environment Measures

We used the German version of the *Block Environmental Questionnaire* (BEQ; Riemann & Wagner, 2000) derived from the American version (Hur & Bouchard, 1995). It is the 5-point Lykert questionnaire intended to assess retrospective perception of family environment in twin families. It consists of 6 scales: Acceptance/rejection by mother, Acceptance/rejection by father, Family cohesion, Intellectual orientation of mother, Intellectual orientation of father and Family organization.

Adult Sibling Relationship Questionnaire (ASRQ; Stocker et al., 1995). The ASRQ assesses characteristics of the relationship between twins by self-reported behaviors and feelings toward siblings, as well as perceptions of sibling's behavior and feelings toward them. The questionnaire includes 81 items conceptually grouped into 14 scales: Intimacy, Affection, Knowledge, Acceptance, Similarity, Admiration, Emotional Support, Instrumental Support, Dominance, Competition, Antagonism, Quarrelling, Maternal Rivalry, and Paternal Rivalry. *Life Events List* (Plomin et al. 1990; Saudino et al. 1997) consists of 31 life events, while response categories are (1) never, (2) one-time, and (3) repeatedly experienced within the last 5 years. In addition, twins rated the valence of the experiences on a scale ranging from very negative (-3) to very positive (+3).

Mental Health Measures

Psychiatric Diagnostic Screening Questionnaire (PDSQ; Zimmerman, 2002) consists of 123 items, assessing Axis I psychopathology. The PDSQ is a screening instrument for Major Depressive Disorder, PTSD, Bulimia/Binge-Eating Disorder, Obsessive-Compulsive Disorder, Panic Disorder, Psychosis, Agoraphobia, Social Phobia, Alcohol Abuse/Dependence, Drug Abuse/Dependence, Generalized Anxiety Disorder, Somatization Disorder, and Hypochondriasis.

Satisfaction with Life Scale (SWLS; Diener et al., 1985) consists of five items with a five-point Likert scale and assesses the cognitive component of subjective well-being.

General Medical Examination

General medical examination included general physical and neurological exam, as well as the measurement of the body height, weight and subsequent anthropometric measurements (skinfolds: chest, subscapular, midaxillary, biceps, triceps, abdominal, suprailiac, supraspinal, thigh, calf; circumference: head, neck, forearm, upper arm relaxed and flexed, chest, waist, hip, quadriceps and calf; diameter: femur, humerus, forearm, forearm and hand, thigh). Body height and weight were used to obtain the body mass index (BMI).

General physical exam consisted of acquiring personal medical history of birth, childhood and adolescence; family history regarding twin pregnancies and the history of neurological disorder and physical illnesses, and a structured physical exam with an evaluation of the heart rate, blood pressure and respiratory rate. Neurological exam evaluated the status of cranial nerves, pyramidal, extrapyramidal, cerebellar, sensory, and peripheral nerves, as well as the walking and speaking ability.

<< INSERT TABLE 2 ABOUT HERE >>

Data Collection and Procedures

Data collection is mostly done at the Faculty of Philosophy in Novi Sad, while a small part of the sample was collected in other Serbian cities: Belgrade, Niš, Zrenjanin and Novi Pazar. The session lasts from 3 to 5 hours, with a break for a meal and refreshments. The examination itself is conducted in several stages:

1. The twin pair undergoes a general medical examination
2. Buccal swabs is taken from the twin pair for DNA analysis
3. The twin pair participates in a comprehensive interview, in which both twins provide basic information about their similarities and differences, pre-existing illnesses, schooling, family opportunities while growing up, etc.
4. The twin pair completes personality and intellectual assessment tests. Participants performed executive function tests individually with trained researchers.
5. A twin pair participates in computer-based testing of executive functions
6. A twin pair participates in computer-based testing of laboratory induced aggression

After the examination process, every twin pair receives information about the test results by email, or at their home address, in the form of the report on personality structure and cognitive performance.

Molecular Genetic Analysis

Until now, molecular genetic analyses were performed for Human Catechol-O-methylTransferase (COMT), dopamine D2 receptor (DRD2) and Human Brain-Derived Neurotrophic Factor (BDNF) genes, while all necessary preparations were made for the tryptophan hydroxylase 2 (TPH2) and Gene 5-hydroxytryptamine receptor 1A (HTR1A) genes.

COMT gene, located at position 22q11, consists of six exons, of which the first two are non-coding. One of the most-studied polymorphisms is Val158Met (rs4680) in the fourth exon that is involved in biosynthesis and metabolism of dopamine, norepinephrine, and epinephrine (Salunkhea et al.,2019).

DRD2 gene is located at 11q22.3-11q23.1 position, consists of eight exons, codes for a G-protein-bound receptor, expressed predominantly in the hippocampus, which is considered to

have a protective effect on memory. One of the most frequently studied polymorphisms is rs1800497 and SNP (rs1800497) which has been associated with many behavioral phenotypes (Klaus et al., 2019).

BDNF gene is located on chromosome 11p13 and consists of 11 exons (Pruunsild et al., 2007). One of its most frequent single nucleotide polymorphisms is rs6265 at position 196 of exon 2, which results in valine (val) to methionine (met) substitution at codon 66 (Val66Met). Consequences of this polymorphism involve alterations of BDNF intracellular packaging, its axonal transport and activity-dependent secretion at the synapse (Chen et al., 2004).

TPH2 gene located at chromosome 12q21.1 encodes a member of the pterin-dependent aromatic acid hydroxylase family. The encoded protein catalyzes the first and rate limiting step in the biosynthesis of serotonin, an important hormone and neurotransmitter. One of TPH2 gene frequent polymorphisms is rs4570625, also known as G-703T which has been linked to several psychiatric and/or behavioural phenomena (Gao et al., 2012).

HTR1A gene, which encodes a G protein-coupled receptor for 5-hydroxytryptamine (serotonin), is located at chromosome 5q12.3. One of the most investigated polymorphisms of this gene is rs6295, that leads to the replacement of C with G (Drago et al., 2007).

DNA was extracted using standard DNA extraction techniques (QIAgen, Hilden, Germany) as recommended by the manufacturer. The genotyping of the DRD2 gene (rs1800497), COMT gene (rs4680) and BDNF (rs6265) is carried out using TaqMan assays (TaqMan SNP, Applied Biosystems, Warrington, UK), as recommended by the manufacturer at the Faculty of Medicine, University of Novi Sad, Serbia. The TaqMan single nucleotide polymorphism (SNP) Genotyping Assays uses TaqMan 5' -nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples and takes advantage of minor groove-binding probes for superior allelic discrimination. The SNP Genotyping Assays

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contain a VIC-dye-labelled probe, FAM-dye-labelled probe with two target-specific primers. PCR was performed using 10 ng of genomic DNA together with 1 µl TaqMan Genotyping assay and 12.5 µl of genotyping master mix in final 25 µl reaction on a 96-well plate using ABI Prism 7500 Fast PCR device (Applied Biosystems, Foster City, California, USA). DRD2, COMT and BDNF alleles with the specific fluorescence curves were detected and analyzed using the 7500 System SDS program, integrated into the ABI Prism 7500 Fast PCR device.

The genotyping of the TPH2 and HTR1A will begin by the end of 2019.

Collaborations

Our research team collaborates with Professor Rainer Riemann from the University of Bielefeld, who helped design the study at the very beginning. A great help in determining zygosity via DNA analysis is provided by Mechthild Prinz from John Jay College of Criminal Justice, City University of New York and Zoran Budimlija from Department of Neurology, NYU School of Medicine, New York.

Preliminary results of the first behavioral genetic study in Serbia have been published in the special issue of *Primenjena psihologija*, dedicated to behavioral genetics (Dinić et al., 2018; Jovanov & Zgonjanin Bosić, 2018; Milovanović et al., 2018; Nikolašević et al., 2014; Sadiković et al., 2018). *Primenjena psihologija* (Applied Psychology) is an open access journal of high importance in Serbia.

Future Plans

Our future activities are organized in three directions. First, we intend to conduct further data collection from already tested participants in order to examine the relationship between epigenetic change and the development of different psychological phenomena across the life span. Second, we are preparing the assessment of the children twins, from the earliest period

of their childhood through the end of adolescence, and further. In our register, there are 327 pairs of young twins (101 pairs aged 0 – 5; 163 pairs aged 6 – 10; 42 pairs aged 11 – 14; and 21 pairs aged 15 – 17) whose parents had applied to participate in our study. Our medical team has organized the systematic examination of psychomotor and socioemotional development of the youngest twins through standard medical procedures (medical history, measurement of weight, height, body mass index, comparison with the World Health Organization Growth Standards for children, complete physical examination) (WHO, 2016), but also using the parent reports by Ages and Stages Questionnaires (Squires & Bricker, 2009; Squires et al, 2015). Our medical team had also organized the systematic examination of the school twin cohort through the same medical procedures above mentioned and comparison with the World Health Organization Growth Reference Data for 5-19 years (WHO, 2013). School twin cohort will be examined partly through parent reports by our online platform, and partly by individual testing, depending on twins age. Parents will respond to a set of questionnaires which are primarily intended to evaluate personality (Kodžopeljić et al., 2019) and executive functions (Thorell & Nyberg, 2008) in children. Individual testing will include assessment of intelligence by Individual testing will include assessment of intelligence using the Wechsler Intelligence Scale for Children form IV (WISC-IV) (Wechsler, 2003, adapted by Matešić, 2009), emotional regulation by ERQ – child version (Gullone & Taffe, 2012), satisfaction with life by SWLS – child version (Gadernann et al., 2009) etc., but also assessment of some new constructs in children twin studies, such as math anxiety (Hart et al., 2017). We are also planning to collect information about parents and siblings through time in order to conduct longitudinal research and nuclear family twin study. The third direction of our plans is aimed to investigate the molecular genetic basis of different psychological phenomena in children and adults mapping the genetic factors which are related to serotonin - and dopamine - induced behaviors or

psychological traits. Overall, our intention in the future is to provide an extensive set of data that could be used for improving not only the knowledge about behavior genetic foundations of human characteristics but also the quality of life, using the biopsychosocial twin research framework.

Open Science and Science Communication

Relying on the attitude that “the field is imbued with an ethos of building a progressive science based on replicable findings” (Plomin et al., 2016. p.17), our research team is dedicated to replicating previous findings in behavioral genetics and respecting the basic principles of open science.

Data regarding CRTT task, zygosity, polymorphisms, AQ, and RST as well as CRTT procedure in PsychoPy software can be found at OSF: <https://osf.io/j4vqx/>. Data regarding behavioral genetic study of NEO-FFI personality traits in German, Croatian and Serbian culture can be found at OSF: <https://osf.io/5shdy/>

In order to popularize STR, raise awareness about the importance of twin studies, and engage community members in research, STR and CBG activities are continuously being promoted through various public campaigns, events, lectures, and TV appearances. One of the key events of this kind is the National Twins Day, organized annually by the CBG team at the University of Novi Sad in the first week of June. The event features assorted activities, such as presenting research results, organizing workshops for parents and children, art performances, prize games, as well as recruiting new participants. Twins who had participated in the research, receives the results of their personality profiles, cognitive abilities, general health and zygosity. National Twins Day gathers not only twins and their families, but also the members of the academic and public community. Another relevant science communication event is the annual National Science Festival where the CBG team

presents their current research and popularize STR through various popular science projects, such as the “Who is your movie hero twin?” online questionnaire.

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Table 1: Participants (N = 804) who completed tests in the Serbian Twin Registry as of 2019

		Male			Female			OSDZ
		SSMZ	SSDZ	unknown	SSMZ	SSDZ	unknown	
Twins	N	84	28	8	276	64	36	94
	Age (SD)	23.4 (7.13)	22.92 (3.60)	30.50 (8.23)	25.51 (8.11)	22.84 (6.68)	26.78 (10.33)	23.00 (5.84)
Parents	N	76			86			
	Age (SD)	56.04 (8.81)			54.06 (8.79)			
Siblings	N	20			32			
	Age (SD)	23.08 (7.82)			29.95(10.01)			

Note. N = individuals, SS = same-sex, MZ = monozygotic twins, DZ = dizygotic twins, unknown = zygosity unknown, Age = age in years.

OSDZ = opposite-sex dizygotic twins

Table 2: List of measures

Assessment	Adult twins	Parents	Siblings
<i>Personality</i>			
NEO PI-R (Costa & McCrae, 1992)	+	+	+
NEO-FFI (Costa & McCrae, 1992; Costa & McCrae, 2019)	+	+	+
RSQ (Smederevac et al., 2014)	+	+	+
SSS V (Zuckerman, 1994)	+	+	+
AQ (Buss & Perry, 1992)	+	+	+
CRTT (Tylor, 1967)	+		
<i>Cognition</i>			
APM (Raven, Raven, & Court, 1998)	+		
WCSt (Heaton et al., 1993)	+		
TMT (Reitan, 1955)	+		
VFT (Goodglass & Kaplan, 1983; Lezak, 1995)	+		
<i>Family environment</i>			
BEQ (Riemann and Wagner, 2000)	+		
ASRQ (Stocker, Lanthier & Furman, 1995)	+		
<i>Mental health</i>			
PDSQ (Zimmerman, 2002)	+		
SWLS (Diener et al., 1985)	+	+	+
Life events list (Plomin et al. 1990; Saudino et al. 1997)	+		
<i>Medical examination</i>	+		
<i>Anthropometrical measures</i>	+		
<i>DNA sampling</i>	+		
<i>Genes</i>			
COMT	+		

DRD2	+
BDNF	+
HTR1A	+
TPH2	+

Figure 1. The participants of the Serbian Twin Registry as of 2019

