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Article

The Population-Based Hungarian Twin Registry: An Update

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Abstract

Between 2006 and 2021, the Hungarian Twin Registry (HTR) operated a volunteer twin registry of all age groups (50% monozygotic [MZ], 50% dizygotic [DZ], 70% female, average age 34 ± 22 years), including 1044 twin pairs, 24 triplets and one quadruplet set. In 2021, the HTR transformed from a volunteer registry into a population-based one, and it was established in the Medical Imaging Centre of Semmelweis University in Budapest. Semmelweis University's innovation fund supported the development of information technology, a phone bank and voicemail infrastructure, administrative materials, and a new website was established where twins and their relatives (parent, foster parent or caregiver) can register. The HTR's biobank was also established: 157,751 individuals with a likely twin-sibling living in Hungary (77,042 twins, 1194 triplets, 20 quadruplets, and one quintuplet) were contacted between February and March of 2021 via sealed letters. Until November 20, 2022, 12,001 twin individuals and their parents or guardians (6724 adult twins, 3009 parents/guardians and 5277 minor twins) registered, mostly online. Based on simple self-reports, 37.6% of the registered adults were MZ twins and 56.8% were DZ; 1.12% were triplets and 4.5% were unidentified. Of the registered children, 22.3% were MZ, 72.7% were DZ, 1.93% were triplets, and 3.05% were unidentified. Of the registered twins, 59.9% were female (including both the adult and minor twins). The registration questionnaire consists of eight parts, including socio-demographic and anthropometric data, smoking habits and medical questions (diseases, operations, therapies). Hungary's twin registry has become the sole and largest population-based twin registry in Central Eastern Europe. This new resource will facilitate performing world-class modern genetic research.

Keywords: Twin registry; Central Eastern Europe; Genetic research

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History of the Hungarian Twin Registry

The earliest study of Hungarian twin pairs dates to the 1930s (Darányi, 1962). In the 1960s, Malán and colleagues performed studies of height, growth, bone development, goiter, infectious disease, nerve function, intelligence, mental ability, spirituality, and psychological properties of Hungarian twins (Malán, 1962). The lineage of the current Hungarian Twin Registry (HTR) dates to the 1970s and 1980s when Andrew Czeizel and Julia Metneki developed three twin databases: the Hungarian Congenital Abnormality Registry, the Budapest Twin Registry and a national volunteer adult twin registry (Czeizel et al., 2014; Czeizel et al., 1979; Littvay et al., 2013; Metneki, 1996; Metneki & Czeizel, 1989). Building and expanding on these efforts, between 2006 and 2021, the HTR developed into a new volunteer twin registry of all age groups (50% monozygotic [MZ], 50% dizygotic [DZ], 70% female, average age 34 ± 22 years), including 1044 twin pairs, 24 triplets and one quadruplet. *Twin Research and Human Genetics* previously reported on these efforts (Littvay et al., 2013; Tarnoki et al., 2019). For the past decade, our team has also worked to transform the HTR from a volunteer registry into a population-based one (Tarnoki et al., 2019). Slow progress was mainly due to the expulsion of Central European University from Hungary, which initially hosted the project, the severe EU level strengthening of data protection (General Data Protection Regulation [GDPR]) temporarily creating an uncertain research and regulatory environment, and COVID 19. In this article, we report on the conclusion of these efforts.

Research Aims

Through the population-based data collection efforts of the HTR, Hungary's twin registry became the largest in Central Eastern Europe where the registry recruitment was able to focus on the whole population. This new resource allows us to perform world-class modern genetic research. Based on the newly established population-based HTR and its biorepository, disease-discordant MZ twins suffering from certain, partially rare chronic diseases (e.g., cancer, cardiovascular, neurodegenerative, and respiratory diseases) are continuously selected and studied. Phenotypic data and biological samples are acquired and collected during the actual running of studies, after genetic material purification,

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genetic material quality control, molecular zygosity testing and DNA banking. Before each specific research and after adequate information is provided, a separate consent form is signed by the participants. The multidisciplinary approach of the twin registry is accomplished using a combination of imaging, genetic, epigenetic, molecular biology and microbiome procedures. Genetic and epigenetic research includes next-generation sequencing of disease-associated genes and the whole mitochondrial genome, extracellular vesicle and microRNA profiling, methylation analysis, telomere length, and mitochondrial DNA copy number measurements.

With the help of the HTR and Semmelweis University, we plan to carry out heritability studies and research on discordant twins on:

(1) Molecular medicine and molecular markers: Expanding the material (pure DNA, serum blood, urine samples) of the established biobank during research, regular maintenance of stored samples, processing of stored samples during follow-up and collaborative research (e.g., tumor development).
(2) Microbiome: Further intestinal microbiological research in various fields (e.g., neurodegenerative, cardiovascular, respiratory disorders, cancers), as well as anaerobic, oral and blood microbiome studies.
(3) Genetic epidemiological studies on — among others — cardiovascular, immunological, respiratory and neurological disorders, as well as psychosocial behavior studies, focusing on epigenetic traits.
(4) Imaging genetics (e.g., radiogenomics, radioepigenetics): Radiomic studies combined with the help of artificial intelligence, where the goal is to recognize imaging biomarkers in the case of tumors (radiogenomics) and other diseases (e.g., atherosclerosis)
(5) Perinatal studies: Our long-term goal is to introduce perinatal (epigenetic) research, together with gynecologists and pediatricians

Setting Up the Population-Based Hungarian Twin Registry

The population-based twin registry was established in the Medical Imaging Centre of Semmelweis University in Budapest, Hungary. Through Semmelweis University's innovation fund (STA-IN-17, STIA 132/2019 10M HUF) we were able to develop information technology, phone bank and voicemail infrastructure, administrative materials, and a new website (https://ikrek.semmelweis.hu) where twins and their relatives (parent, foster parent or caregiver) can register. In anticipation of the need to store genetic samples, we collaborated with the university's Department of Genetics, Cell and Immunobiology, where a dedicated Innova U535 Upright Freezer is now also available for HTR's biosamples.

The Hungarian National Authority for Data Protection and Freedom of Information advised on and approved the initial registration questionnaire. For dissemination, the HTR worked with the Ministry of Interior Deputy State Secretariat for Data Registers, and the Government Office of the Capital City Budapest in collaboration with Budapest's 21st District Government Office to notify all twin pairs via the *Hungarian Post*. In the case of minors, a single letter was sent, addressed to their legal guardian. 'Minor' was defined as children under 18 years of age according to Hungarian legislation. 'Adult' was defined as 18 years or older. This data collection was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Semmelweis University (TUKEB 280-1/2014, 280-2/2014).

The Ministry of Interior Deputy State Secretariat for Data Registers communicated to the team that in January 2021, by matching registry information on birth (family) name, birthday (same ± 1 day), birthplace, mother's maiden name, they were able to identify 157,751 individuals with a likely twin-sibling living in Hungary (77,042 twins, 1194 triplets, 20 quadruplets, and one quintuplet). Without specifics we were informed that around 100,000 of these individuals were above 18 years of age. The search potentially missed twins with twin-siblings who were deceased or living outside of Hungary, and those who explicitly requested denial of all data service from the government (less than 1% of Hungarians).

During February and March of 2021, sealed letters with the letterhead of the Hungarian Government Office were sent to all identified individuals. Contacted individuals were asked to register online, which included a detailed questionnaire, via our voicemail or by post. For those who registered by phone or post, we mailed a paper-and-pencil questionnaire to them (or their legal guardians). Zygosity was assessed by self-reported questionnaires that included questions regarding their overall similarity and the frequency with which they were confused by others (Heath et al., 2003; Jackson et al., 2001). We plan to conduct a latent class analysis in the future for improved identification. Data collection was ongoing throughout the spring and summer of 2021 and has been kept open since then.

Response by Contacted Twins

Due to budget constraints, only one letter was sent to each individual or guardian. Also, due to the government population database not being matched with the respondent database, we were unable to send reminder letters to nonrespondents. For these reasons, the response rate was a relatively low 7.6%. Only 9733 adult individuals or twin parents responded by November 20, 2022. Most twin pairs registered online (6724 adult twins, 3009 parents/guardians), with fewer than 50 via postal letters and fewer than 100 using voicemail messages. Twin-siblings of 2716 registered adults have not contacted us. We explicitly asked these individuals to convince their co-twins to register. Based on simple self-reports, 37.5% of the registered adults were MZ twins and 56.8% were DZ twins, 1.12% (34) were triplets and 4.5% were unidentified. Of the registered children, 22.3% were MZ twins, 72.7% were DZ twins, 1.93% were triplets, and 3.05% were unidentified.

The sex balance was 31.5% males, 68.4% females, with 0.1% nonresponse. The sex balance was better for the minors, with 51.5% males and 49.5% females (see Table 1).

The mean age of adult twins was 42.44 ± 14.84 years, and 11.71 ± 5.16 years for minors (see Figure 1).

Additional Data Collection During the Registration

Separate registration questionnaires were created for adult twins (18 years or older) and for parents of twins who are minors. One parent (or legal guardian) was able to register both twins on the website. The data of all twins who were registered can only be stored until December 31, 2029, according to the national data protection laws. Thereafter, twins will be requested to reregister in the twin registry. The registration questionnaire consists of eight

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Table 1. Characteristics of twins enrolled in the Hungarian Twin Registry

Information at enrollment		Adults $N = 6724$	Minors (based on the replies of their parents) $N = 5277$
Number of complete twin pair members		3930 (70%)	Number of registered parents: 3009
Number of incomplete twin pair members		2716 (30%)	
Number of triplets		78 members, including 54 (3 × 18) complete set members + 24 noncomplete members	34
Number of quadruplets		0	0
Age at enrollment, years			
Range (age, years)		18-87	0-17
Mean +/− *SD*		42.44 ± 14.84	11.71 ± 5.16
Zygosity (based on the twin's and parent's answers), number of individuals			
MZ	Complete pairs	1667 (44.2% of complete pairs)	1178 (23.5%)
	Twins without registered twin-siblings	858	
DZ	Complete pairs	2125 (55.8% of complete pairs)	3836 (76.5%)
	Twins without registered twin-siblings	1692	
Triplets		78	102
Quadruplets		0	0
Unknown		304	161
Gender			
Male		2121 (31.5%)	2666
Female		4597 (68.4%)	2611
Unknown		6 (0.1%)	
Educational level			
High school diploma, or less		3598 (53.5%)	–
University/college degree of higher		2845 (42.3%)	–
N/A		281 (4.2%)	
Employment status			
Student		1049 (14.9%)	–
Employed		4733 (67%)	–
Unemployed		523 (7.4%)	–
Retired		502 (7.1%)	–
Not mutually exclusive categories*		256 (3.6%)	
Marital status			
Single/divorced		2477 (36.8%)	–
Married		3556 (52.9%)	–
Widowed		173 (2.6%)	–
N/A		518 (7.7%)	
Urbanicity			
Lives in the capital (Budapest)		21.4%	17.4%

Note: *Employment status can, at the same time, be student and employed and for this reason, this does not add up to the total. MZ, monozygotic, DZ, dizygotic.

parts. (1) Contact data, (2) Zygosity and twinning, (3) Personal and socio-demographic data, (4) Marital status, family questions, (5) Smoking habits, (6) Medical questions (diseases, operations, therapies), (7) Anthropometric data, (8) Comments. Table 2 summarizes the collected information.

Due to strict data protection and sensitive personal data, adult individuals were only allowed to provide information about themselves. No questions were asked about their co-twins. Data is stored on a Semmelweis University server that is protected by a virtual private network (VPN). Pairing of co-twins had to be done via the same tools used by the registry as described above.

Other Activities of the Hungarian Twin Registry

We are also eager to report on other activities of the Hungarian twin studies community. The HTR team served as the local host of

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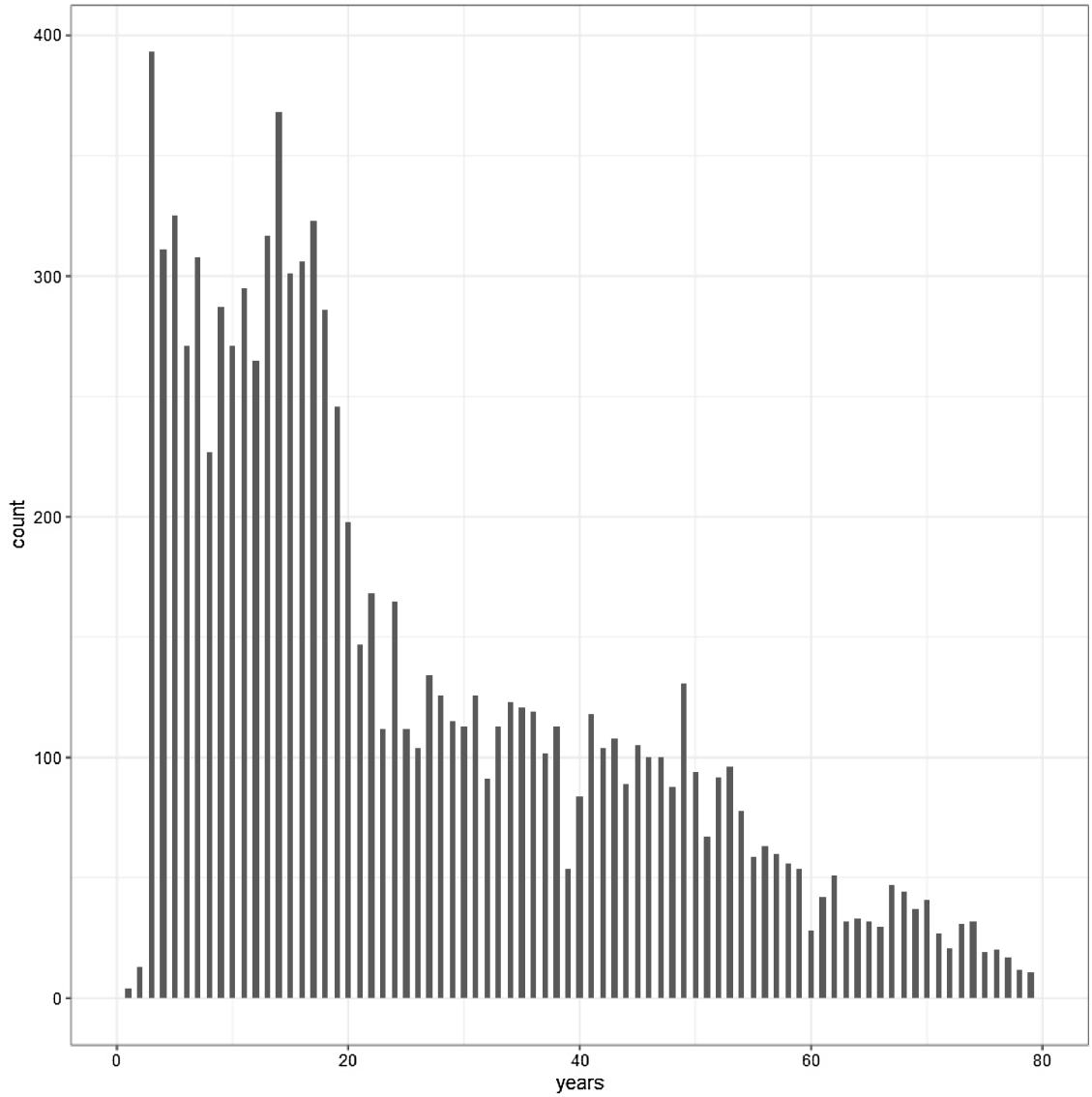


Figure 1. Age distribution of the registered twins.

The Joint 6th World Congress on Twin Pregnancy: A Global Perspective, and the 18th Congress of the International Society for Twin Studies (ISTS) between 15–17 June 2023, organized at the Semmelweis University, Budapest, Hungary (https://www.mcascientificevents.eu/twins/). The ISTS has had a new board since 2020 when Adam D. Tarnoki was elected as the president, David L. Tarnoki serves as the secretary general, and Bianka Forgo became a Board member. Through the efforts of the newly elected leadership, the website of ISTS was renewed (twinstudies.org).

The HTR still collaborates not only with Hungarian institutes but also with twin researchers globally. The most active collaboration is with the Italian, Japanese (Osaka University), South-Korean (Healthy twin study), and the Australian twin registries as well as with The Methodist DeBakey Heart and Vascular Center, Methodist Hospital, Houston, TX, USA (led by Zsolt Garami). A new collaboration is developing with Obafemi Awolowo University in Nigeria, thanks to the support of the Erasmus+, an international credit mobility program of the European Union.

In 2020, Adam Tarnoki and David Tarnoki were awarded the prestigious Bolyai scholarship of the Hungarian Academy of Sciences to support neurological twin research (gut microbiome, epigenetics). For his twin study using data from the United States, Levente Littvay received the 2017 Morton Deutsch Award given to the best article published in the journal *Social Justice Research*. The HTR regularly holds twin studies presentations at the annual Researchers' Night at Semmelweis University, where the public can attend scientific lectures on various subjects. In addition, Adam Tarnoki and David Tarnoki are organizers of the 'Twin research, epigenetics and radiogenomics' PhD course at Semmelweis University. In 2020, the HTR team published a book on *Twin Research and Epigenetics* in Hungarian (Tarnoki et al., 2020) and (together with Jennifer Harris and Nancy Segal) co-edited one of the first comprehensive English language textbooks about twin studies published by Elsevier (Tarnoki et al., 2022).

As a result of the increased interest in the HTR, more than 60 researchers in Hungary of all ranks are engaged in twin studies for their research, of whom 18 are PhD students.

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Table 2. Information collected in initial registration questionnaire

Study field	Variables
A. Contact details and anthropometric data	• Name • Address • Phone number • Email • Sex • Birth name • Place of birth • Date of birth
B. Twinship, zygosity	• Living twin brother/sister • Birth order • Zygosity questionnaire • Birth data (e.g., placenta, DNA information)
C. Personal and socio-demographic data	• Education • Employment • Quality of life
D. Marital status, family	• Marital status • Information about wife/husband, additional children, parents, brothers or sisters
E. Smoking habits	• Past and present smoking habits
F. Health related questions	• Malignancy • Chronic diseases (e.g., pulmonary, neurology, ophthalmology, gastrointestinal, cardiovascular, metabolic, rheumatology, psychiatric, congenital, obstetrics and gynaecological) • Surgeries
G. Anthropometric data	• Height, weight, BMI
H. Other	Additional open comments

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Competing interests. None.

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