

Article

The Hungarian Twin Registry Update: Turning From a Voluntary to a Population-Based Registry

Adam D. Tarnoki^{1,2}, David L. Tarnoki^{1,2}, Bianka Forgo¹, Helga Szabo¹, Dora Melicher^{2,3,4}, Julia Metneki² and Levente Littvay^{2,5}

¹Department of Medical Imaging, Semmelweis University, Budapest, Hungary, ²Hungarian Twin Registry Foundation, Budapest, Hungary, ³Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, ⁴MTA-SE Immune-Proteogenomics Extracellular Vesicle Research Group, Budapest, Hungary and ⁵Department of Political Science, Central European University, Budapest, Hungary

Abstract

Since our last report on the voluntary Hungarian Twin Registry (HTR) in 2012, the number of pairs or multiplets included increased from 310 to 1044. Efforts to turn the registry into a population-based one are on the way. Nearly 128,000 twins living in Hungary (98,500 adults) will be mailed information on how to register on the new HTR website. Twins will be asked to invite their spouses and immediate family members. Meanwhile, strong cooperation through exchange programs has been developed with other foreign twin registries. Current research focuses on radiogenomics, musculoskeletal, cardiovascular and respiratory diseases, gut microbiome as well as basic molecular research and yielded new awards and further publications.

Keywords: Central and Eastern Europe; population-based registry; data collection; radiogenomics; imaging

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The long history of Hungarian twin studies dates back in the 1970s and 1980s when Andrew Czeizel and Julia Metneki developed three different databases: the Budapest Twin Registry, a volunteer adult twin registry, and the Hungarian Congenital Abnormality Registry (Littvay et al., 2013). In 2006, Adam Tarnoki, David Tarnoki and Levente Littvay began an effort to revive the Hungarian Twin Registry (HTR) with the help of Julia Metneki. Based on the previous available twin contact data, classical twin studies have been launched on the active core group of 310 twin-pairs (Littvay et al., 2013). Media reports helped to raise attention toward these studies, which contributed to the significant increase in the membership. Research focused mainly on cardiovascular and respiratory health (Littvay et al., 2013). During the international twin study project, the core team grew as a twin participant, Dora Melicher, became involved in twin research and became a productive contributor and leadership board member of the HTR team. Since the last report on the HTR in *Twin Research and Human Genetics*, this team worked on the expansion of the HTR to a population-based one. The team now reports the progress of this project, the launch of the population-based twin registry in Hungary and additional future plans.

Current Voluntary Registry: Increasing Size

The HTR increased its sample size thanks to the continuous media presence, and currently involves 1044 twin-pairs of all age groups

Author for correspondence: Adam D. Tarnoki, Email: tarnoki2@gmail.com

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(50% monozygotic [MZ], 50% dizygotic [DZ], 70% women, average age 34 ± 22 years), including 24 triplets and 1 quadruplet from the entire country. Zygosity is assessed with multiple questions and latent class analysis in line with the recommendations of Heath et al. (2003).

Since the last review in *Twin Research and Human Genetics*, nearly 50 papers have been published. The largest twin studies in this period included the GLOBAL study (2013 and 2014; Maurovich-Horvat et al., 2015) and sleep study (2014–2019) (Szily et al., 2019), while the analysis of the results of the International Twin Study 2009 (2009–2010) was still in progress (Tarnoki, Baracchini et al., 2012; Tarnoki, Tarnoki et al., 2012; Tarnoki, Tarnoki, Horvath et al., 2013). Since 2013, respiratory health-related studies have also been published (Szily et al., 2019; Tarnoki, Tarnoki, Lazar et al., 2013; Tarnoki, Tarnoki, Medda et al., 2013).

Between 2009 and 2015, a 4-year-old follow-up study of 214 MZ and 154 DZ Hungarian and Italian twins was performed, during which we examined the longitudinal changes of the atherosclerotic and antropometric markers (Pucci et al., 2018; Tarnoki, Tarnoki, Molnar et al., 2013). Among the imaging methods, twins were subjected to state-of-the-art examinations that used brain magnetic resonance imaging (MRI, e.g., diffusion tensor imaging), transcranial Doppler (TCD) tests and ultrasound measurements with new software (liver and thyroid elastography, arterial analysis). Additionally, we carried out the first twin studies examining the telomere-telomerase system and involving the investigation of telomere-mitochondria interplay (Melicher et al., 2018, 2019).

With our current twin study, accurate measurements of carotid and femoral plaque volume, automatic intima-media thickness

(IMT) and local stiffness parameters are performed to characterize the relationship between different phenotypes of atherosclerosis with molecular biology markers (telomere length, mitochondrial DNA copy number, extracellular vesicles, gut microbiome), to find out about their epigenetic mechanism. Examination of MZ twins discordant for cardiovascular disease can help our understanding of how genetic and environmental effects contribute to their radiological appearance. Imaging genetics is an integrated research method that combines imaging and genetics to assess the impact of genetic variations on various phenotypes. MZ twins discordant for a chronic disease are unique tools for genomic, epigenomic, and transcriptomic studies combined with imaging (MRI, computed tomography) in an integrative manner from this perspective. This is referred to as radiogenomics or imaging genomics in cancer discordant twins, that is, the study of relationship between imaging phenotypes and genomics (Mazurowski, 2015). In addition, imaging epigenetics — which is a multidisciplinary field combining imaging and epigenetics — is capable of answering whether different imaging phenotypes covary and predict epigenetic modifications that are related to the structure, function and metabolism of organs (e.g., vascular), which affect the risk and progression of disease (Lancaster *et al.*, 2018).

There are many Hungarian institutes and research groups working together with us, among which the examination of dental, writing analysis and linguistics are of particular importance.

The list of the currently available parameters collected from the Hungarian twins is shown in Table 1.

Professional and Academic Activities

The HTR served as a local host of the Joint 3rd World Congress on Twin Pregnancy & 15th ISTS International Congress in Budapest, November 16–19, 2014. Moreover, we hosted the ISTS Twin Registries' Networking Meeting between 15 and 16 April, 2019, at Semmelweis University, Budapest, which served as a platform for discussion about future collaborations on retrospective and prospective studies among twin registries.

The HTR collaborated not only with Hungarian institutes but also with foreign twin researchers. The most active collaboration is with the Italian, Osaka, South Korean (Healthy Twin Study) and the Australian twin registries. The Italian Twin Registry was helpful in providing support with statistical analyses. To acknowledge their help, Maria Antonietta Stazi was recommended to be elected the Doctor Honoris Causa of Semmelweis University, which was approved in 2018.

In the past years, active exchange programs and joint research activities have been conducted with the financial support of the bilateral Erasmus+ Exchange Program. The program provided a platform to collaborate with Seoul National University (Prof Dr Joohon Sung; 2-2 instructors, 4-4 PhD students, 2016–2018) and the Methodist DeBakey Heart and Vascular Center, Houston, Texas, USA (Dr Zsolt Garami; 1-1 instructor, PhD student 2-2, 2016–2018) and the Osaka University (Ass Prof Chika Honda, Prof Dr Norio Sakai; 2-2 teachers, 2-2 instructors, 4-4 PhD students exchange, 2018–2020), as well as with the University of Sydney (Ass Prof Dr Paulo Ferreira, 2-2 instructors, 2018–2020).

The increasing interest in the HTR resulted in dozens of student scientific researchers pursuing twin studies for their diploma work and seven PhD students (Bianka Forgo finished in 2019) working in twin research on Hungarian twins.

Population-Based Twin Registry

The HTR is undergoing transformation to a population-based twin registry, which provides a unique opportunity to become the largest twin research center in the Central and Eastern European region. The large database will allow us to perform world-class, modern genetic research. Comparing the imaging traits, molecular biology markers, genome, epigenome and microbiome of the discordant MZ twins for a chronic disease can unravel the specific biomarkers.

The population-based twin registry will be established with the support of the Semmelweis University. All twin-pairs living in Hungary (ca. 128,000 individuals) will be contacted by mail in 2019 (Figure 1), requesting them to register online by filling out a detailed questionnaire. The infrastructure (e.g., refrigerators, document recognition, data capture, language processing softwares, document scanners, telephone system for twin registrations) is ready to use. Thanks to Semmelweis University's innovation application (Semmelweis Tudományos és Innovációs Alap, STIA), we have finalized the data protection licenses after lengthy negotiations with the Hungarian National Authority for Data Protection and the Freedom of Information Authority. The administrative material related to twin research and a new website (www.ikrek.semmelweis.hu; Figure 2) have been completed, and the twins and their relatives can register through the new website. The infrastructure of the Twin Registry (IT and other devices) has also been set up, including a telephone and voicemail system, a computer network (e.g., laptops, scanner software), and a freezer (Innova U535 Upright Freezer). The Central Office for Administrative and Electronic Public Services of the Ministry of the Interior is ready to release the data of twins (name and postal addresses) shortly. According to the legal procedure, the data of the twins (i.e., name and postal addresses) will be released within 3 months from the date of the request, and around 120,000 letters will be mailed in a closed envelope. For twins who do not have internet access, we will post the survey sheets by Hungarian Post. Data collection is expected to begin in the second half of 2019 (Figure 3).

With the help of the population-based twin register in Semmelweis University, we will be able to conduct high-level twin research in the following areas: (1) molecular medicine and molecular markers; (2) microbiome; (3) cardiovascular, immunological, brain research; (4) imaging genetics (e.g., radiogenomics, radiopigenetics).

Accordingly, the HTR aims to become one of the largest European twin research centers and in the top 10 largest population-based twin registries in the world. Based on the newly established registry and its biorepository, disease-discordant MZ twins suffering from certain chronic diseases (e.g., cancer, cardiovascular, neurodegenerative and respiratory diseases) will be selected and studied. Phenotypic data and biological samples will be acquired and collected after genetic material purification, genetic material quality control, molecular zygosity testing and DNA banking. The detailed registration through our website will allow us to build a nationwide biobank and select the appropriate disease concordant and discordant twin-pairs for further specific epigenetic studies. Using radiogenomics, imaging phenotypes will be associated with gene expression patterns, gene mutations and genome-related characteristics, as well as molecular biology patterns, enabling deeper insights into tumor and disease biology by means of integration of genomic and imaging data. So far, no radiogenomics twin study has been published, making this field innovative worldwide.

Table 1. Variables collected from the Hungarian twins in the past and plans for future data collection

Study field	Variables	Sample size	Plan for future data collection
Cardiovascular	Arterial stiffness (tonometry, oscillometry), blood pressure and its components, carotid and femoral IMT/plaques/stiffness (US), TCD, coronary CTA, Abdominal aortic diameter, jugular and femoral venous distensibility, cardiac echo (including strain, speckle tracking), brain white matter hyperintensities-microvascular lesions (MRI, FLAIR)	<ul style="list-style-type: none"> - Arterial stiffness tonometry 105 pairs: 70% MZ, 30% DZ - Oscillometry 200–250 pairs: 70% MZ, 30% DZ) - Blood pressure and its components 300 pairs: 70% MZ, 30% DZ - Carotid and femoral IMT/plaques/stiffness (US) 300–350 pairs: 70% MZ, 30% DZ - TCD 100 pairs: 70% MZ, 30% DZ - Coronary CTA 105 pairs: 70% MZ, 30% DZ - Abdominal aortic diameter 160 pairs: 70% MZ, 30% DZ - Femoral venous distensibility: 100 pairs 70% MZ, 30% DZ - Cardiac echo 105 pairs: 70% MZ, 30% DZ - Brain MRI: 70 pairs 	Yes
Epigenetics	DNA methylation	105 twin-pairs	Yes
Microbiome	Fecal microbiome (16s RNA)	90 MZ twin pairs	Yes
Genetics	Fractions: whole blood, thrombocyte-free plasma for upcoming extracellular vesicle studies, PBMC sample for upcoming immunological studies, mitochondrial DNA copy number and telomere length results	<ul style="list-style-type: none"> - 260 MZ and DZ sample, Fractions: whole blood, thrombocyte-free plasma for upcoming extracellular vesicle studies - 108 MZ and DZ PBMC sample for upcoming immunological studies - 110 MZ and DZ mitochondrial DNA copy number and telomere length results - 142 MZ and DZ mitochondrial DNA copy number and telomere length results 	Yes
Anthropometric	Self-reported and measured BMI and body composition (BIA)	<ul style="list-style-type: none"> - Self-reported BMI 800 pairs: 70% MZ, 30% DZ - Measured BMI and body composition 250 pairs: 70% MZ, 30% DZ 	Yes
Mental health	<ol style="list-style-type: none"> 1. Neurocognitive tests: MoCA, Brigg's and Nebe's test, TMT A and B, STROOP test, WF-L and WF-C, RAVLT, WAIS — Digit Symbols, BVRT, STAI (Spielberger-type status), BDI 2. Psychological tests: Zuckerman-type sensor Experience search Questionnaire, Barrat Impulsivity Scale, Tangney: Self-Control Scale, Rosenberg's Self-Assessment Questionnaire, Keyes: General Well-Being questionnaire, Derogatis: Short Symptom, Reward Symptom Questionnaire, 'mindfulness' questionnaire, creativity, positive thinking 	<ul style="list-style-type: none"> - Neurocognitive tests 120 twin-pairs: 70% MZ, 30% DZ - Mindfulness and creativity questionnaire 105 twin-pairs: 70% MZ, 30% DZ - Positive thinking 160 twin-pairs: 70% MZ, 30% DZ 	Yes
Metabolic	Laboratory tests (normal laboratory test with cardiometabolic variables)	150–200 twin-pairs: 70% MZ, 30% DZ	Yes
Respiratory	Spirometry, SpO2, e-nose, sleep apnea (polysomnography, questionnaire), circulating biomarkers (e.g., survivin), secondhand smoke, smoking, COPD (HRCT)	<ul style="list-style-type: none"> - Spirometry 160 pairs: 70% MZ, 30% DZ, - SpO2 160 pairs: 70% MZ, 30% DZ - E-nose: 30 pairs, 70% MZ, 30% DZ - Sleep apnea (polysomnography, questionnaire) and circulating biomarkers (e.g., survivin) 75 pairs: 70% MZ, 30% DZ - Secondhand smoke 160 pairs: 70% MZ, 30% DZ - Smoking 160 pairs: 70% MZ, 30% DZ - COPD (HRCT) 5 pairs: 2 DZ, 3 MZ 	Yes

(Continued)

Table 1. (Continued)

Study field	Variables	Sample size	Plan for future data collection
Imaging	US (carotid artery, femoral artery and vein, thyroid gland, jugular vein, liver, abdominal aorta, kidneys, liver and thyroid elastography, TCD), coronary CTA, lung HRCT, BMD (DEXA and calcaneal ultrasound), brain MRI, sella MRI, cervical MRI, lumbar spine MRI	<ul style="list-style-type: none"> - Carotid artery US 200–300 pairs: 70% MZ, 30% DZ - Femoral artery US 200 pairs: 70% MZ, 30% DZ - Femoral vein US 150 pairs: 70% MZ, 30% DZ - Thyroid gland US 250 pairs: 70% MZ, 30% DZ - Jugular vein US 150 pairs: 70% MZ, 30% DZ - Liver US 150 pairs: 70% MZ, 30% DZ - Abdominal aorta US 150 pairs: 70% MZ, 30% DZ - Kidney US 150 pairs: 70% MZ, 30% DZ - Liver and thyroid elastography 120 pairs: 70% MZ, 30% DZ - TCD 100 pairs: 70% MZ, 30% DZ - Coronary CTA 105 pairs: 70% MZ, 30% DZ - Lung HRCT 5 pairs: 3 MZ, 2 DZ - BMD (DEXA and calcaneal ultrasound) 100 pairs: 70% MZ, 30% DZ - Brain and sella MRI 100 pairs: 70% MZ, 30% DZ - Cervical and lumbar spine MRI 120 pairs: 70% MZ, 30% DZ 	yes
Neurology	Imaging (brain, sella MRI, cervical and lumbar spine MRI, TCD, carotid US), back pain questionnaires, neurocognitive and psychological tests (see mental health)	<ul style="list-style-type: none"> - Brain MRI 100 pairs: 70% MZ, 30% DZ - Cervical and lumbar spine MRI 120 pairs: 70% MZ, 30% DZ - TCD 100 pairs: 70% MZ, 30% DZ - Carotid US 200–300 pairs: 70% MZ, 30% DZ - Back pain questionnaires 100 pairs: 70% MZ, 30% DZ - Neurocognitive and psychological tests (see mental health) 	Yes
Chronic pain	Cervical pain, back pain questionnaires, general health questionnaires	<ul style="list-style-type: none"> - Cervical pain, back pain questionnaires approximately 100 pairs: 70% MZ, 30% DZ - General health questionnaires 100–200 pairs: 70% MZ, 30% DZ 	Yes
Cancer	Questionnaire data, imaging (CT, US)	Approximately 20–30 pairs	Yes
Biomarkers/OMICS	Fractions: whole blood, thrombocyte-free plasma for upcoming extracellular vesicle studies; PBMC sample for upcoming immunological studies; mitochondrial DNA copy number and telomere length results, circulating biomarkers (e.g., survivin)	<ul style="list-style-type: none"> - 260 MZ and DZ sample Fractions: whole blood, thrombocyte-free plasma for upcoming extracellular vesicle studies - 108 MZ and DZ PBMC sample for upcoming immunological studies - 110 MZ and DZ mitochondrial DNA copy number and telomere length results - 142 MZ and DZ mitochondrial DNA copy number and telomere length results; - Circulating biomarkers (survivin, etc) 75 pairs: 70% MZ, 30% DZ 	Yes
Endocrinology	Sella MRI, thyroid US and elastography, adrenal and pancreas CT	<ul style="list-style-type: none"> - Sella MRI 70 pairs: 70% MZ, 30% DZ - Thyroid US 250 pairs: 70% MZ, 30% DZ - Thyroid elastography 120 pairs: 70% MZ, 30% DZ - Adrenal and pancreas CT 95 pairs: 70% MZ, 30% DZ 	Yes

BDI = Beck Depression Inventory, BIA = bioelectrical impedance analysis, BMD = bone mineral density, BMI = body mass index, BVRT = Benton Visual Retention Test, COPD = chronic obstructive pulmonary disease, CT = computed tomography, CTA = computed tomography angiography, DEXA = dual-energy X-ray absorptiometry, DNA = deoxyribonucleic acid, DZ = dizygotic, FLAIR = Fluid Attenuated Inversion Recovery, HRCT = high-resolution computed tomography, IMT = intima-media thickness, MoCA = Montreal Cognitive Assessment, MRI = magnetic resonance imaging, MZ = monozygotic, OMICS = radiomics, genomics, epigenomics, metabolomics, transcriptomics, PBMC = peripheral blood mononuclear cell, RAVLT = Rey-Osterreich Auditory Verbal Learning Test, RNA = ribonucleic acid, SpO2 = oxygen saturation, STAI = State-Trait Anxiety Inventory, TCD = transcranial Doppler, TMT = Trail Making Test, US = ultrasound, WAIS = Wechsler Adult Intelligence Scale, WF-L = word fluency with the letter, WF-C = word fluency with category naming.

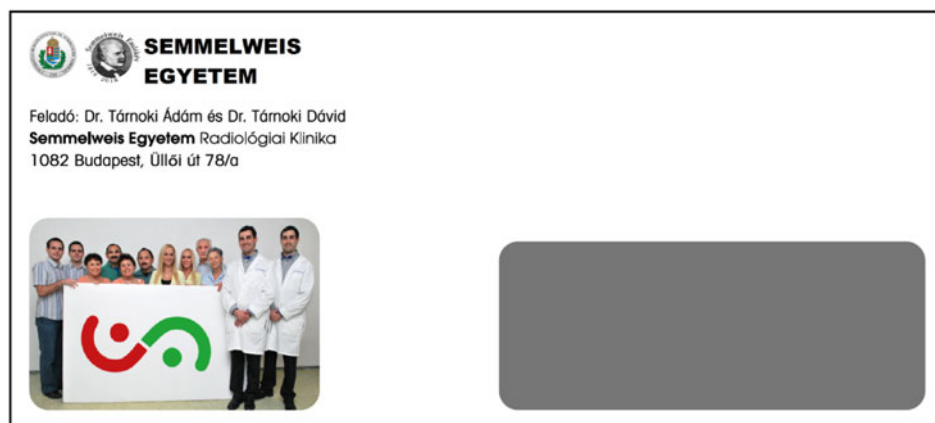


Fig. 1. Envelope which will be sent to the twins requesting them to register in the population-based Hungarian twin registry.

A screenshot of the Hungarian Twin Registry (HTR) website. The header includes the HTR logo (Hungarian TWIN REGISTRY) and the word "Magyar". Navigation links include "Front page", "About us", "Research", "Contact", "Publications", and "Bejelentkezés / Regisztráció". A search icon is also present. The main content area features a large blue banner with the text "Welcome to the website of the Hungarian Twin Registry!" and a sub-header "Welcome to the website of the Hungarian Twin Registry!". Below this is a paragraph of introductory text and a "REGISTER" button with a link to "or login". To the left of the banner is a photograph of several young women on bicycles. Below the banner are two article teasers: "ICTS Satellite Meeting Brisbane June 20th 2016" and "Conjoined sisters undergo rare separation surgery in Texas". A sidebar on the right lists various content categories like "Events", "This+That", "International News", etc.

Fig. 2. New website of the population-based Hungarian twin registry.

The HTR biorepository will facilitate and support medical and scientific studies by providing a valuable resource for investigating these complex phenotypes and their underlying biology. A multi-disciplinary approach of the project will be accomplished by the combination of imaging, genetic, epigenetic, molecular biology

and microbiome studies. Genetic and epigenetic research will include next-generation sequencing of the disease-associated genes and the whole mitochondrial genome, extracellular vesicle and microRNA profiling, methylation analysis, telomere length and mitochondrial DNA copy number measurements. Incorporating

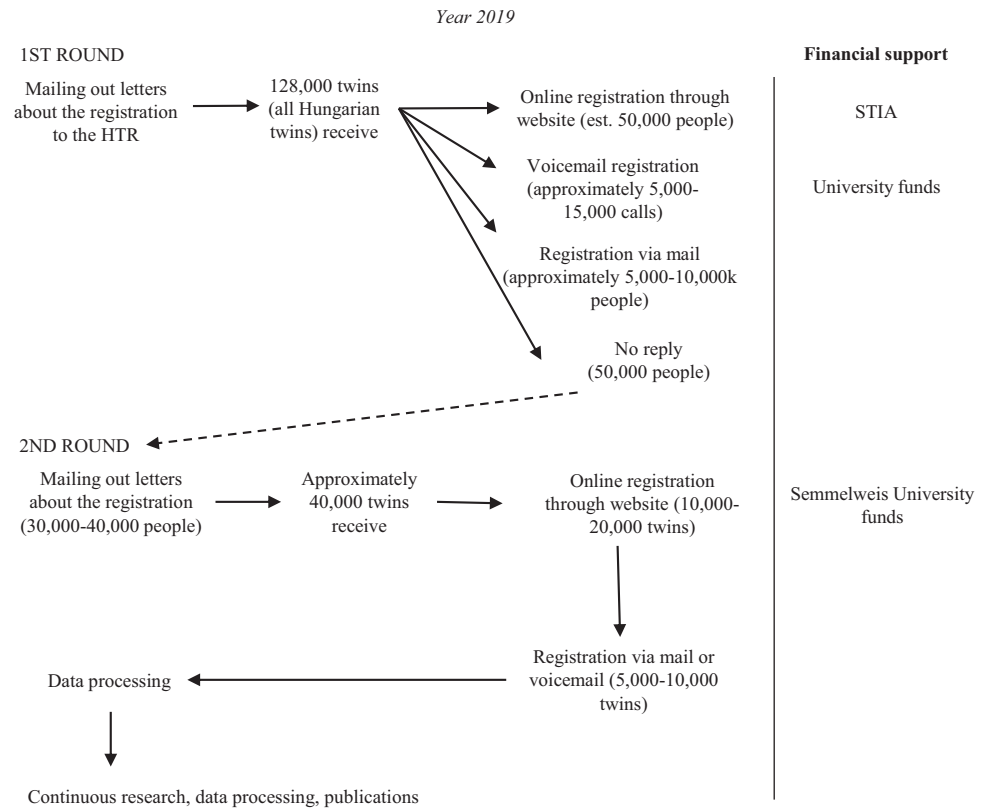


Fig. 3. Flowchart of the foundation of the population-based registry.

the results and finding synergies of the molecular biological and imaging techniques will be the focus of the project, with the aim of finding clinically relevant biomarkers. In the modern genomic era and evolving field of personalized medicine, such twin studies will lead to the discovery of disease liability and novel targets for individualized treatment.

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Conflict of interest. None.

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