

## Article

# An Update of Twin Research in Spain: The Murcia Twin Registry

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## Abstract

The Murcia Twin Registry (MTR) is the only population-based registry in Spain. Created in 2006, the registry has been growing more than a decade to become one of the references for twin research in the Mediterranean region. The MTR database currently comprises 3545 adult participants born between 1940 and 1977. It also holds a recently launched satellite registry of university students ( $N = 204$ ). Along five waves of data collection, the registry has gathered questionnaire and anthropometric data, as well as biological samples. The MTR keeps its main research focus on health and health-related behaviors from a public health perspective. This includes lifestyle, health promotion, quality of life or environmental conditions. Future short-term development points to the expansion of the biobank and the continuation of the collection of longitudinal data.

**Keywords:** Biobank; disease prevention; health promotion; Spain; twin registry; twin studies

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During the last decade, the Murcia Twin Registry (MTR) has been steadily growing as a resource for research in the genetics of complex phenotypes. Born in 2006 from an agreement between the University of Murcia and the Murcia Health Council (Ordoñana et al., 2006), the MTR has since continued expanding to become a valued instrument open to collaborations with national and international research and academic institutions. Currently, this research resource manages information from same-sex and opposite-sex twin-pairs and higher order multiples, which includes self-reported variables, objective measurements and a biobank.

The MTR ([www.um.es/registrogemelos](http://www.um.es/registrogemelos)) is a population-based twin registry, with a longitudinal design, aiming to provide a basic research tool focused on, but not limited to, the analysis of the genetic and environmental factors involved in health and health-related behaviors, and quality of life. Despite the proven usefulness of twin research and the increasing development of new registries (Odintsova et al., 2018), the MTR remains the only established registry of its kind in Spain. This makes it a reference for twin research in the Spanish scientific community, where it serves as a platform of interest for collaborations with other national and international research teams.

The target population of the registry comprises all people born in multiple births between 1940 and 1977 in the Region of Murcia,

one of the 17 first-level administrative divisions of Spain. Located in the southeast of Spain, the region of Murcia roughly has 1.5 million inhabitants. There have been five waves of data collection, and currently the registry has information from over 3500 individuals (Table 1), in addition to an associated biobank (<http://www.bancoadn.org/samples/catalog-of-samples-samples-population.html>).

## A Decade Growing

During the last decade, the MTR has kept its original goals, structure and strategy. Recruitment of participants has followed similar procedures since its inception. Basically, potential twins are identified through comparison of surnames and birth date in the computerized databases of collaborating institutions (Murcia Regional Health Council), which hold the information of virtually all residents given that the health care system is public and universal. Potential twin-pairs are contacted first for confirmation of twin status and to provide a description of the goals of the MTR. Upon agreement with the participants, they are incorporated into the registry if they meet two inclusion criteria: (a) both members of the pair are alive at time of contact and (b) there is no presence of disorders or disabilities that could limit their active and conscious participation. Participation in the MTR is always voluntary and subject to informed consent. The registry complies with all European legal requirements regarding personal data protection; it is enlisted with the Spanish Agency for Data Protection, and its procedures have been approved by the Research Ethics Committee of the University of Murcia. Data are stored at a secure

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**Table 1.** Number of twin individuals interviewed and incorporated in the MTR by wave of data collection

Wave	Kind of pair	Collecting dates	Kind of contact	Birth years	Number of interviews	Number of new participants	Biological samples (DNA)
1	Same-sex pairs (female)	December 2006 to December 2007	Telephone	1940–1966	874	874	
2	Same-sex pairs (female)	March 2009 to October 2010	Face to face and telephone	1940–1966	827 <sup>a</sup>	124	743
3	Same-sex pairs (male)	October 2010 to October 2011	Telephone	1940–1966	651	651	
	Opposite-sex pairs			1940–1966	679	679	
4	Same- and opposite-sex pairs	January 2013 to December 2013	Telephone and mail	1940–1966	1631 <sup>b</sup>		78
5	Same- and opposite-sex pairs	June 2018 to December 2018	Telephone and online	1967–1977	1217	1217	
Total core registry						3545	821
1	University students	September 2017 to June 2018	Face to face and online	1981–1999	204	204	186
Total						3749	1007

<sup>a</sup>Longitudinal data collection for participants of Wave 1.

<sup>b</sup>Longitudinal data collection for participants of Waves 2 and 3.

server, and identity data, such as name, address, phone and identity codes, are in a separate database. Researchers working with survey data cannot access personal data that could be used to identify the individual. Additional description of the recruitment and management methods is available elsewhere (Ordoñana *et al.*, 2013).

### Participants

To date, the MTR has focused mainly on adults aged 40 or older. The registry has periodically incorporated volunteers who have provided self-report and objective data, as well as a biological sample donation. Table 1 displays a description of the five completed waves of data collection, including the number of DNA samples stored in the biobank.

**Wave 1.** The first wave was carried out during 2007 with a sample of female twin-pairs ( $N = 874$ ) born between 1940 and 1966. This served as a pilot study where the protocol for future waves was developed.

**Wave 2.** Between 2009 and 2010, the same women were contacted again to collect additional data (including blood/saliva sample), improving the quality of the information gathered (e.g., objective measurements of anthropometric data), and widening the range of phenotypes available for study. The conditional response rate for Wave 1 respondents at Wave 2 was 70.4%. Additionally, new twins ( $n = 124$ ) were added to the registry at this time.

**Wave 3.** The third wave (2010–2011) expanded the registry by collecting data on male and opposite-sex twin-pairs from the same birth years as the women from Waves 1 and 2 (i.e., 1940–1966). By the end of this wave, an additional 1330 twins were incorporated in the MTR.

**Wave 4.** Longitudinal data from the full sample became available at Wave 4. In 2013, 70.1% ( $n = 1631$ ) of the twins participated in a follow-up survey, providing both repeated measures and new data on the evolution of twins' health and their quality of life.

**Wave 5.** Finally, during 2018, the population-based MTR was extended to recruit new cohorts of same-sex and opposite-sex twins born between 1967 and 1977. By following the previously successful procedures and incorporating new strategies, such as the use of online questionnaires, the MTR registered 1217 additional participants.

Altogether, the main database of the registry currently contains information from 3545 individuals in 1458 complete pairs, 601 twin individuals without a co-twin and 28 individuals in triplets, who have participated at any stage, with longitudinal information for 1631 participants. The MTR is representative of the population in the region of Murcia and Spain, as evidenced by the distribution of sociodemographic characteristics and prevalence of key health traits (Ordoñana *et al.*, 2018).

Besides the core data collection, other investigations involving objective ambulatory or laboratory data collection for more specific phenotypes have been carried out with the participation of restricted subsamples of the registry. Specifically, 50 and 65 twin-pairs collaborated in studies about chronobiological health and optics and visual health, respectively.

### The University Students Sample

As an additional resource, the MTR wanted to broaden its scope to encompass satellite samples with ages outside the age range of the core registry. University students are an accessible and convenient sample, in particular, for the development of experimental or intervention studies, where representativeness may not be a determinant issue. Therefore, in 2017, the MTR started the process of identifying and contacting twin-pairs from the University of Murcia student database.

A similar procedure as for the MTR core recruitment was followed: surnames and birthdate were matched for the students enrolled in any of the university schools. Then, potential twins were contacted for verification of twin status; they were provided information about the registry and were invited to participate. Data collection involved face-to-face interviews, laboratory assessments and online questionnaires. This process is repeated every year to contact with new students. Currently, 204 individual twins have contributed with data to this satellite sample.

### The MTR Biobank

As previously mentioned, the MTR has developed a biobank with samples from participating twins. The MTR biobank is managed through collaboration with the DNA National DNA Bank – Carlos III (NDNAB), a public technological platform that supports biomedical research and promotes genomic research in Spain. Collection of biological samples consisted of whole blood (10 ml of EDTA and 6 ml of ACD) or complete saliva (Oragene DNA collection kit). In later phases, saliva has been collected through mouthwash with chlorhexidine (10 ml), which is a cost-effective and safe method of obtaining DNA of adequate quality (Pinto et al., 2018). Upon collection, samples are posted to the NDNAB, where they are processed, and DNA extracted and stored. More information about the technical procedures and storage of DNA is available at the bank's website ([www.bancoadn.org/en](http://www.bancoadn.org/en)). The samples are available to the researchers of the MTR and other interested groups ([www.bancoadn.org/en/samples](http://www.bancoadn.org/en/samples)) with the aim of facilitating, promoting and developing national and international scientific research on human evolution, genetic/genomic diversity and risk factors focused on health or the etiology and treatment of illnesses. Currently, DNA samples from 1007 participants are stored at the NDNAB, of which 623 (91% females) have been genotyped using the Illumina GSA Beadchip and imputed to 1000G Phase 3 Version 5. Researchers interested in using the samples can contact the NDNAB through its website, and their proposals will be evaluated by a committee of members of the MTR and the NDNAB.

### Zygoty Assessment

Initially, the MTR developed a questionnaire to determine the zygosity of the participants. This instrument was adapted from the questionnaire used by the Netherlands Twin Registry, consisting of 12 items about physical similarity and difficulties in telling the twins apart. Accuracy of the classification was assessed by comparison with DNA testing in a subsample (342 pairs/triplets). The zygosity questionnaire correctly classified zygosity in 95.5% of the cases (Ordoñana et al., 2013). As of today, 50.1% of participants from same-sex twin-pairs registered in the MTR are monozygotic (MZ).

### Record Linkage

The MTR database can be linked with the regional health record system with the objective of collecting or validating health-related data. Providing that participants give informed consent, data from their health records, both in the primary and the speciality health care systems, can be matched with self-reports in order to validate and incorporate additional data regarding clinical, laboratory and treatment records.

In the case of twins recruited from the University of Murcia, the MTR can also access their university entrance exam records, after obtaining informed consent from the students. These records include their scores in a standardized universal general exam comprising all major subjects (e.g., maths, history and literature), which is used to prioritize their admittance to specific university degrees.

### Feedback to Participants

During the last decade, the MTR has been developing a process to communicate with the participants in the MTR about the results of their collaborating effort, based on their interest and willingness to know. All twins have access to the general results of the registry's

research activities through the MTR web page, which is open access. For those who have donated biological samples, personal feedback includes the result of genotyping regarding zygosity. Additionally, when twins collaborate in specific studies, the MTR provides them with basic reports about individual results that may be of interest for them, such as results of ocular exams, chronobiological patterns or dietary information. Moreover, the MTR collaborates with health promotion activities of the regional health authority by providing participants with accurate health information and specialist referrals to improve health and quality of life, during the research process.

### Core Phenotypes and Research Interests

The health-related phenotypes collected by the MTR include the presence of diseases and disorders, health-related behaviors and perceptions, lifestyles, demographic and environmental conditions, as well as important processes affecting these outcomes, such as aging or menopause. Additionally, data about specific psychological constructs (i.e., fusion with the twin, reaction to life events or personality assessment) and laboratory instrument data (i.e., actimetry, eye structure or hormonal measures) have been gathered in restricted subsamples. The main phenotypes and data collected in each wave are summarized in Table 2.

### Collaborations

The MTR was designed as a collaborative resource and, accordingly, it is open to cooperation with other research groups from national or international institutions. Consequently, the registry has joined efforts with multiple research teams and has participated in several consortia and networks, thus widening its horizons and multiplying its impact potential. These initiatives are mainly focused on health-related specific issues: Genetic Research into Quality of Life (GeneQoL; Sprangers et al., 2009); nausea and vomiting during pregnancy (Colodro-Conde et al., 2017); anthropometric measures for the Cohort Description of Collaborative Project of Development of Anthropometrical Measures in Twins (CODATwins; Silventoinen et al., 2015); twin research development (e.g., International Network of Twin Registries [Buchwald et al., 2014] and EuroDiscotwin [Willemsen et al., 2015]) and applied behavioral genetics (e.g., Enhancing Psychiatry Genetic Counselling, Testing and Training in Europe [EnGage]).

### Main Areas Explored

Because the MTR focuses on issues of relevance from a public health standpoint, the main results so far are in the areas of health-related lifestyles and behaviors, health-related quality of life and health promotion and disease prevention behaviors. There are multiple physiological, psychological and social factors that can influence these variables, and twin research is instrumental in increasing scientific knowledge in these areas. Hence, the MTR has provided insights into the genetic factors related to anthropometric traits by, for instance, comparing body mass index (BMI) heritability between two European countries of different cultural backgrounds (Ordoñana et al., 2007), analyzing how the number of childbirths moderates BMI variance distribution (Rebollo-Mesa & Ordoñana, 2009), exploring BMI association with chronic low back pain or sleep quality through co-twin designs (Dario et al., 2016; Madrid-Valero et al., 2017) or participating in large studies of genetic and environmental effects on anthropometric traits (CODATwins Project).

**Table 2.** Summary of the phenotypes collected in the MTR

Twins and family characteristics	Zygosity screening, ancestors' origin, number and sex of siblings, birth order, town of residence throughout life, parental education, parental smoking and alcohol use, fusion with the twin.
Sociodemographic characteristics	Sex, year of birth, marital/partner status, number of children, education, occupation, employment, religiosity.
Anthropometric data	Self-reported: eye color, natural hair color, laterality, birth weight, height, weight, BMI. Objectively measured (only females and university students): height, weight, BMI, body composition (bioimpedance), waist and hip circumferences.
Reproductive history (only females)	Age at menarche, number of childbirths, contraception, breastfeeding, nausea and vomiting during pregnancy, postpartum depression, use of contraceptives, menopause, hormonal replacement therapy, menopause-related quality of life.
Health status and quality of life	Self-rated health, health-related quality of life (EQ-5D), medical history, musculoskeletal pain, use of medicines, functional status, use of health services, hospital admissions.
Health-related lifestyles, behaviors and perceptions	Smoking, alcohol use, drug use and abuse, coffee and tea consumption, physical activity, physical exercise, treatment adherence, disease prevention and early detection activities, overweight perception, eating behavior (TFEQ), timing of meals, sleep quality (PSQI), sleeping patterns, depression (STDI, IDER, CES-D, PHQ8), anxiety (GAD7), life satisfaction, subjective happiness, social support (OSLO-3).
Chronobiological measures	Circadian rhythmicity (temperature, activity and position), hormonal variation, chronotype (M-EQ).
Ocular measures	Ocular biometry, peripheral optics, optical quality (corneal and ocular aberrations), refractive error, visual quality (visual acuity, contrast sensitivity), intraocular scattering.
Cognitive functions (only university students)	Intelligence (K-Bit), working memory (WAIS), creativity (CREA).
Vital events	Stressful life events.

The MTR has also produced some analyses about the role of genetic factors on phenotypes that are relevant from a public health perspective but that have received less attention in the genetic literature, such as breastfeeding initiation and duration (Colodro-Conde et al., 2013, 2018), unintentional injuries (Ordoñana et al., 2008) and psychoactive medication use (Rosagro et al., 2013). In a similar line, the registry reported some of the first estimations of heritability for anatomical and functional traits, such as human eye optics (Taberner et al., 2017), chronobiological rhythmicity (López-Mínguez et al., 2016, 2019) or siesta (López-Mínguez et al., 2017). Likewise, genetic and environmental associations among those phenotypes and with mental health have been investigated, in particular, the relationship between symptoms of depression, poor sleep quality and chronic musculoskeletal pain (Pinheiro et al., 2015; Pinheiro, Morosoli, Colodro-Conde

et al., 2018; Pinheiro, Morosoli, Ferreira et al., 2018). Last but not least, the registry data have been used to examine the role of specific social and cultural characteristics of the MTR sample on the genetic and environmental architecture of phenotypes such as sleep quality (Madrid-Valero et al., 2018), smoking (Mezquita et al., 2018), altruistic behavior (Tornero et al., 2018) or educational attainment (Colodro-Conde et al., 2015).

### The Near Future in the MTR

After a decade of being a reference for twin research in Spain, the MTR still has room to grow before reaching its full potential. Objectives for the near future continue in the same roadmap as in previous years: increasing sample size by incorporating new subjects, widening the age range of the registry and introducing new objective measures, more longitudinal data and additional phenotypes. In addition, we aim to increase the number of collaborations with other researchers and increase the size of the biobank with samples from as many participants from the MTR as possible.

With those objectives in mind, and keeping the focus on a public health perspective, the MTR expects to continue with classical quantitative genetic analyses of relevant phenotypes, while incorporating new advances from molecular genetics to our studies. In summary, following the strategy that has been useful to date, the MTR keeps its goals of contributing to scientific knowledge and serving as a reference for twin research in Spain.

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