

The Murcia Twin Registry: A Population-Based Registry of Adult Multiples in Spain

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The Murcia Twin Registry (MTR) was created in 2006, under the auspices of the University of Murcia and the regional Health Authority, aiming to develop a research resource in Spain intended to stimulate current research and new investigation on the analysis of genetic factors related to health and health-related behaviors. The MTR development strategy was designed as a step-by-step process. Initially, it was focused on women's health but nowadays it includes males and opposite-sex twins. The database comprises 2,281 participants born between 1940 and 1966 in the region of Murcia, in Spain. There have been three waves of data collection and today the MTR databases include questionnaire and anthropometric data as well as biological samples. The current main areas of research interest are health and health-related behaviors, including lifestyle, health promotion, and quality of life. Future short-term development points to the completion of the biobank and continuing the collection of longitudinal data.

■ **Keywords:** Twin registry, Spain, twin studies, biobank, health promotion, disease prevention

The Murcia Twin Registry (MTR) is a population-based twin registry of adult multiples in the region of Murcia, located in southeast of Spain. The MTR was born in 2006 based on an agreement between the University of Murcia and the Murcia Health Council (Ordoñana et al., 2006). The general goal of this initiative was to constitute a basic research tool for the analysis of the relative contribution of genetic and environmental factors to the development of complex phenotypes, focusing on health and health-related behaviors. Additionally, it was intended to be the starting point for a long-term longitudinal study, centered on traits related to health and their interaction with lifestyle, social environment, and genetic characteristics.

A Short History of the Murcia Twin Registry

In spite of the worldwide implementation of twin research, to our knowledge the MTR is the first established twin registry in Spain, and the only one at present. This lack of experience led us to design a step-by-step process, starting with a relatively small sample of limited geographical dis-

persion and age range, and a narrow research focus which we planned to enlarge in consecutive stages. Hence, the first wave of data collection was carried out in 2007, in a sample of female twin pairs ($n = 874$) living in the region of Murcia, who were born between 1940 and 1966 (age range: 40 to 67). This served as a first contact, and specifically as a trial of the field work and protocol design. These women were again contacted in 2009/2010 in order to collect new data (e.g., 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. Seven out of ten (70.4%) of the original sample provided data in this wave. Additionally, new twins ($n = 124$) were added to the collection at this time.

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**FIGURE 1**

(Colour online) Promotional image of the scientific exhibition *Unique pairs: The science of twins*. (Photo: F. Volkringer).

The third step mirrored the first data collection strategy, but focused on males and opposite-sex twins, keeping the same age range. By following this process, the registry has gradually expanded its population targets and its research methods and topics. Nowadays the MTR includes same- and opposite-sex twins and higher-order multiples, and the dataset holds questionnaire and objective measurements, associated with a biobank.

Since its inception, the MTR has been administered and managed by the Area of Psychobiology of the University of Murcia and its procedures have been approved by the Murcia University Ethical Committee. Inclusion in the MTR is voluntary, subjected to informed consent, not remunerated, and it complies with the Spanish legal requirements on personal data protection. Every participant has full access to his/her data and the right to remove them from the MTR without restrictions.

The Construction of the MTR: Recruitment Procedures

Information about the individuals comes from the computerized databases available at the regional health system. The Murcia Health Service is a public universal health system and it keeps up-to-date databases of all the residents in the area (about 1.4 million citizens). People who were born on the same day and share their surnames are selected

from these databases and contacted as potential twins. This contact includes a presentation letter, and later a phone call designed to present the objectives and characteristics of the registry, request participation, and collect initial basic demographic and health data. Participants are included in the MTR if the two main inclusion criteria are met: both members of the pair are alive at that time and there is no presence of disorders or disabilities which may limit their active and conscious participation.

A parallel communication strategy has been implemented in order to disseminate information about the registry, its characteristics, and research utility as well as to promote participation. This includes the MTR webpage (www.um.es/registrogemelos), notes and interviews in regional newspapers, periodical letters to the participants, and the development of a scientific exhibition composed of informative panels and twin pictures, which has been exhibited to the public in museums and education facilities (Figure 1).

Following these procedures, the MTR currently comprises (August, 2012) a total of 2,281 participants (998 people in female pairs/trios, 651 people in male pairs/trios, and 632 people in opposite-sex pairs/trios) who are representative of the general population of the region (Table 1).

TABLE 1
Number of Adult Twin Subjects in the MTR
by Sex and Estimated Zygosity

	MZ	DZ	Total
Male-Male	289	362	651
Female-Female	493	505	998
Opposite-sex		632	632
Total	782	1499	2281

Phenotypes and Data Collection

As mentioned above, the MTR has undergone three major waves of data collection. The first wave was focused on female twins, in 2007. Information about demographics, zygosity, self-reported anthropometric data, a basic health history, and health-related questionnaires were collected through telephone interview. Two years later (2009/2010) these women were again contacted and a face-to-face interview was scheduled. The interviews took place in the facilities of the regional health system (i.e., the subject's primary care center). This interview gathered again self-reported health-related information and self-assessed variables from standardized questionnaires. Additionally, objective anthropometric measures, and biological samples (i.e., blood or saliva) were collected. Finally, the first contact with male and opposite-sex twins took place in 2010/11. Following a similar procedure, a questionnaire based on those used before was administered by phone. A brief summary of the main phenotypes with available data is shown in Table 2.

The Biobank

Following a similar step-by-step strategy, the MTR launched a biobank to store and make available to interested researchers biological samples of the twins participating in the registry. Biological samples have been donated by 743 women from the registry. These samples included whole blood (10 mL EDTA and 6 mL ACD) or whole saliva (using an Oragene DNA collection kit), when blood extraction was not possible. These samples have been processed by the National DNA Bank at the University of Salamanca. The

National DNA Bank receives, processes, and stores DNA, plasma, and cell samples from voluntary donors along with relevant information on health and lifestyle habits related to the samples. Information about the technical procedures for sample processing and storage is available at the website (www.bancoadn.org). The collection from the MTR is included among the samples that are made available to the scientific community with the aims of facilitating, promoting, and developing national and international scientific research on human evolution, genetic/genomic diversity with regard to health, origins, and treatment of illnesses (www.bancoadn.org/en/muestras1.htm).

Zygosity Assessment

Twin zygosity was ascertained initially by a questionnaire, which was specifically developed for this purpose, adapting the 12 items used by the Netherlands Twin Registry. This questionnaire-based zygosity corresponds well with zygosity as determined by DNA testing with an agreement in nearly 97% of the cases (Willemsen et al., 2005). Thanks to the biological samples, the zygosity of female twins has been established and a validation process of the Spanish zygosity questionnaire adaptation has been carried out.

Current approaches for zygosity ascertainment are based on minisatellite DNA fingerprinting analysis, highly multiplexed SNPs, or STRs typing (Christiansen et al., 2003; Hannelius et al., 2007; Hill & Jeffreys, 1985; Yang et al., 2006). The samples available at the MTR were analyzed by the BIOMICs Research Group from the University of the Basque Country, using a STRs approach based on 14 autosomal loci plus amelogenin gender determination; an analytical procedure with a good power of discrimination. In addition, ten X-STRs were also typed. The procedures for these analyses have been described previously (Illescas et al., 2012b; Odriozola et al., 2011).

The analysis is based on 338 pairs of twin sisters and four sets of triplets. Of these, 168 pairs shared autosomal and X STRs profiles showing that they were monozygotic twin sisters, and 170 pairs were dizygotic twins because they did not share their autosomal STR profiles. Finally,

TABLE 2
Summary of the Main Variables in the MTR

<i>Twins and family characteristics</i>	Zygosity screening, ancestors' origin, family members, birth order, life-time residence.
<i>Sociodemographic characteristics</i>	Sex, year of birth, marital/partner status, education, occupation, employment
<i>Anthropometric data</i>	Self-reported: eye color, natural hair color, laterality, birth weight, height, weight, BMI. Objectively measured (only females): height, weight, BMI, proportion of fat, fat mass, fat free mass, bone mass, visceral fat ratio, waist and hip circumferences
<i>Reproductive history</i>	Age at menarche, number of childbirths, breastfeeding, use of contraceptives, menopause, hormonal replacement therapy, menopause-related quality of life
<i>Health status and quality of life</i>	Self-rated health, health-related quality of life (EQ-5D), medical history, use of medicines, functional status, use of health services, hospital admissions.
<i>Health-related lifestyles, behaviors, and perceptions</i>	Smoking, alcohol use, coffee and tea consumption, physical activity, physical exercise, treatment adherence, disease prevention and detection activities, overweight perception, eating behavior (TFEQ), sleep quality (PSQI), depression (STD1).

two trios were monozygotic triplets and the other two trios were composed of two monozygotic twins and one dizygotic sister.

In our sample, the zygosity questionnaire correctly classified zygosity in 95.5% of the cases (Sanchez-Romera, 2011) as compared to DNA analyses. Hence the questionnaire appears to have a validity close to the standards in the field (Ohm Kyvik & Derom, 2006). As of today, 47.4% of the same-sex twin pairs registered are MZ.

Major Interest and Research Results

The conception of the MTR revolves around issues of relevance from a public health standpoint. This includes health-related lifestyles and behaviors, health-related quality of life, or health promotion and disease prevention behaviors as well as important processes affecting these outcomes like aging or menopause. Many psychological and social factors can influence these variables and the MTR has the goal of enlarging our knowledge, incorporating the perspective of behavior genetics, on this field. This endeavor has produced some results on classical phenotypes, such as BMI, comparing its heritability between two European countries of different cultural background (Ordoñana et al., 2007) or analyzing the moderating effect of number of child-births on this measure (Rebollo-Mesa & Ordoñana, 2009). On the other hand, estimations of heritability on phenotypes that are relevant from a public health perspective, but have received less attention in the genetic literature (e.g., breastfeeding initiation and duration, adherence to health recommendations, psychoactive medication use, or health related quality of life), have been reported preliminarily at different conferences (Ordoñana et al., 2010a, 2010b, 2011), or are under review for publication. Additionally, the MTR dataset has also been used to analyze the effect of environmental variables on relevant phenotypes such as breastfeeding duration (Colodro-Conde et al., 2011) or for forensic population genetics studies (Illescas et al., 2012a).

Finally, the MTR collaborates with other groups and initiatives, participating in research (Ordoñana et al., 2008), meta-analyses (Nan et al., 2012), and in research consortia like GeneQol (Genetic Research into Quality of Life) (Sprangers et al., 2009, 2010).

Future Development and Concluding Remarks

The results reported here are part of the research that has been carried out since the implementation of the MTR. However, the registry is still at the developmental stage. There is much work to be done before we can say that the MTR is well established and reaches its full research potential.

Within this frame, the main short-term objectives are completing the biobank with biological samples from male and opposite-sex twins, and improving the quality of the

data already collected, adding to the database new objective measures and self-assessed variables. Methodological goals are continuing with classical quantitative genetic analyses of relevant phenotypes, while incorporating new methodological advances in molecular genetics to our analytical procedures. Other objectives are the widening of the age range of the registry and incorporating new phenotypes.

The MTR is the only population-based registry in Spain and one of the few twin registries in the mediterranean area. Its phenotyping strategy is oriented to relevant phenotypes from a public health standpoint, some of which have not yet received much attention from the field. As such, it is currently a reference for researchers from different disciplines (e.g., behavioral genetics, epidemiology, psychology, or forensic and population genetics) interested in the use of twins for their research aims.

The planning and implementation of the MTR was intended to facilitate all possible forms of collaboration with national and international research groups. Keeping this idea in mind, a final, long-term goal of the MTR is to serve as a starting point and pilot project that might help in the setting up of similar and more ambitious projects that could stimulate the progress of this research area in Spain.

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