HealthTwiSt: The Berlin Twin Registry

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The Berlin Twin Registry began as a short-term local project and developed into a resource that now serves partners throughout Germany and Europe. A twin registry as a private company is a different approach with pros and cons. Compared to academic institutions, there are greater flexibilities in collaborations, as well as acquisition and use of research funds. Recruitment is based on invitation in the context of the mass media coverage of scientific results. Phenotyping in normal twin subjects is concentrated on intermediate phenotypes that can bear on common diseases. These phenotypes include proteomic approaches and gene expression. Some results are briefly described to give an impression of the range of research topics and related opportunities for retrospective and prospective collaborative research.

In 1994, a twin study was begun in Berlin that focused on the heritability of blood pressure and response to stress. The original organizers had more in mind than merely a single cross-sectional study. Rather, a longitudinal study on the genetics of health using the combined power of twin study designs and molecular genetics was envisioned. By 2006, this project has evolved into a registry that now includes more than 1700 twin pairs and that operates independently as a privately owned company. This arrangement allows both academic and commercial institutions flexible access to retrospective data, as well as to prospective studies.

Members of the Medical Faculty of the Charité and investigators from the Max Delbrück Center for Molecular Medicine undertook the original twin study. The study was funded by in-house grants. Governmental funding agencies were not amenable to funding twin studies, perhaps in part as a result of the tragic abuse of twin research in German history. Furthermore, reviewers claimed that twin studies were not innovative, outmoded, and unlikely to be applicable for molecular genetic studies. Fortunately and largely as a result of our success, these views were proven to be erroneous. HealthTwiSt was founded largely in response to the twin registry's funding difficulties with the large German scientific granting agencies. The company name was contrived from the project's focus on health rather than a specific disease, and on the 36° turn of the DNA molecule. Since its founding, HealthTwiSt has become more successful in obtaining funding as more sources in support of commercial ventures become available. These sources, coupled with collaborative projects, have led to the company's success.

Recruitment

Recruitment for the Berlin Twin Registry began by inviting twins from the BiLSAT twin registry operated in Bielefeld (Spinath et al., 2002) to take part in medical examinations. During the first years of the study, it became obvious that for practical as well as financial reasons, a more regional focus of recruitment would be needed. The attempt to make use of an address list of potential twins collected in residents' registration offices of the former German Democratic Republic was not successful because of inaccuracies in the database, as well as the extremely high mobility that occurred in eastern Germany after reunification. Western Germany maintained no twin birth information, ostensibly because of German data protection laws. No public records of twin births are available for the same reason at the present time.

Rather than spending money on public relations and advertising agencies, we relied on editorial coverage of our work and established ties to science and technology editors of mass media, such as newspapers, radio, and television stations. Each new scientific journal publication from the group was then used as a 'peg' on which to 'hang' a story. Creating awareness of the twin registry's existence, as well as demonstrating the project's utility, proved to be an effective strategy. Twins correctly view themselves as unique. They are curious about the twinning phenomenon and have been most generous in their participation. In our experience, it is essential to highlight the value of twin studies to the general population as well as the value to the individual twins who participate. Participation provides participants with a free health checkup and free zygosity testing. As added value, the articles provide public education in the field of genetics and genomics. These are 'hot' topics in terms of society, and capture the imagination of the participants.

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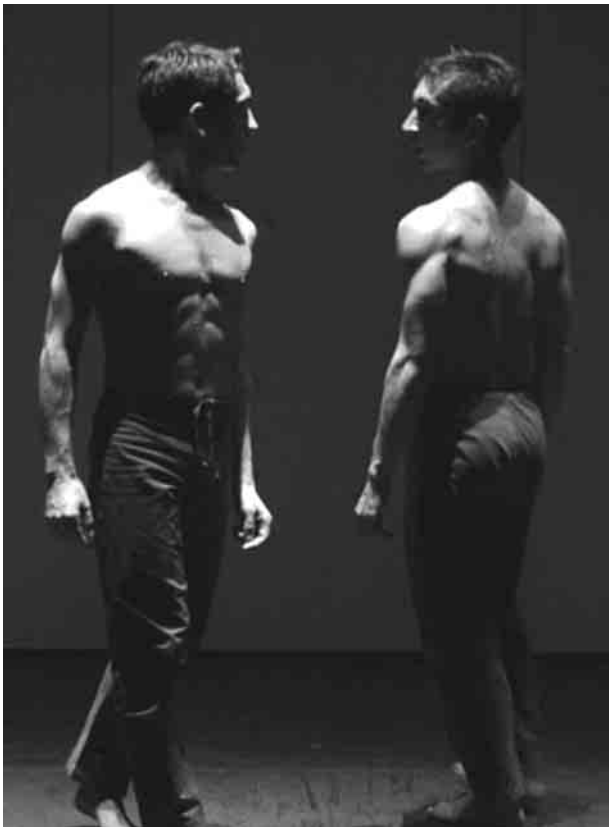

Figure 1

Twins Giuseppe and Michele de Fillipis performing 'A.Null — Copie Umane in Movimento'.

Aside from recruiting new twin pairs, keeping in contact with current registry members is the second important part of maintaining a vibrant twin registry. While some twins have been participating in ongoing studies, a larger portion may either not be suitable for current projects or may not be available. It is crucial to keep ties and maintain involvement even if no immediate participation is possible. Regular newsletters, including research news as well as other twin-related news like the twin pair now running Germany's immediate eastern neighbor Poland, are one tool being employed. Other measures, such as automated but personalized birthday greetings, still await realization. Requests for questionnaire studies are always welcome as they generally have less limited inclusion criteria. An interactive website will be developed to allow twins to communicate, exchange experiences, as well as twin strollers and other objects that twins and their parents may need.

In 2005, recruitment efforts culminated in a local twin festival in Berlin-Buch, hosted by the Max Delbrück Center in its conference facility. While research grants cannot directly be used for buffet meals, lanyards, toll-free numbers, and artists' fees, the set-up allows for such expenses to be covered. Given the appeal of twin pairs dressed up for such an event, it was not unusual to see 6 television stations on site, along with scores of reporters from other media.

This celebration of more than 10 years of twin research in Berlin-Buch combined popular science and art, including twins Giuseppe and Michele de Fillipis performing 'A.Null — Copie Umane in Movimento' (Figure 1), and health checks which proved to be a resounding success. As an additional highlight, the twins were offered a hands-on crash course in molecular genetics. The course took place in the educational genetics laboratory operated on the campus (Figure 2). The twins were given the opportunity to extract their own DNA and make it visible on a gel. There was an overwhelming interest in the switching of sides from proband to researcher. 'Twins as researchers' will certainly be used as a recruitment tool in the context of further studies.

Whenever possible, parents of twins are involved in studies as well. Parental DNA is of great value in linkage as well as in the detection of genotyping errors; furthermore, the twinning phenomenon itself can only be studied and understood by looking at the parents of twins. Furthermore, the parental DNA samples have been a valuable source of information in the detection of genotyping errors, and of course parental DNA permits identity-by-descent linkage analysis. While the current approach of twin-pair 'self-recruitment' has resulted in a substantial number of twins, future efforts will be directed towards population-based samples by targeting residents' registration offices, pending due approval.

Phenotyping Strategy

Data is stored in a MySQL database with PHP-based web front-end. This includes all personal as well as phenotypic information. A major portion of internal resources are devoted to continuing database development.

Once a twin pair has agreed to come to the hospital for the particular investigation, including additional measurements is easier than arranging a second visit. In the first study, blood was drawn for DNA extraction. The same samples were suitable for determining, in addition, lipid levels or other serum parameters without additional inconvenience to the twins. In addition to blood pressure measurements, electrocardiograms were performed. As long as a balance is found between research interest and burden to the twins, the combination of studies is a suitable strategy to maximize the power of phenotyping in twins.

To address disease phenotypes, population-based registries with direct links to the respective national health care systems are the most reliable source of information. As this twin collection is rather a self-selected healthy adult sample with a wide age range, phenotyping was concentrated on intermediate traits, and the influence of genetic variability on normal physiological variation studied. Direct measurements of traits like blood pressure were extended by

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Figure 2
Genetics laboratory.

functional assays to determine venous function, for example (Brinsuk et al., 2004). Molecular phenotyping has been included in one study in which the percentage of neutrophils expressing Proteinase 3 on their cell surface was counted (Schreiber et al., 2003). Gene expression assays measuring gene activity as an intermediate phenotype are currently under way.

While past studies were based on status variables, intervention studies looking at changes in response to nutriceuticals have been started. Using the monozygotic (MZ) co-twin control design, blood pressure changes to an olive tree leaf extract were determined over an 8-week period.

Major Findings

The main focus has been on the cardiovascular system. The heritability analysis of blood pressure response to stress (Busjahn et al., 1996) was extended to linkage and association with candidate genes (Li et al., 2001). For resting blood pressure with its well-established genetic variance, a number of candidate genes were tested based on findings from animal studies, cell-based assays, or case-control studies in hypertension (Busjahn, Aydin, et al., 1999, 2002; Busjahn et al., 2000; Firouzi et al., 2006; Gollasch et al., 2002; Nagy et al., 1999).

Other cardiovascular traits of interest included baroreflex sensitivity (Gollasch et al., 2002; Tank et al., 2001), the electrocardiographic QT interval (Aydin et al., 2005; Bezzina et al., 2001, 2003; Busjahn, Knoblauch, et al., 1999; Busjahn et al., 2004), lipid metabolism (Al Kateb et al., 2002; Knoblauch et al., 1997, 1999, 2000), and cardiac function as well as morphology as determined by echocardiography (Busjahn et al., 1997, 2000).

The obesity epidemic is increasingly affecting Germany, with noticeable changes mostly in the former East Germany. Accordingly, obesity-related measurements were included as well. New heritability estimates have been obtained for free and bound leptin concentrations, together with the leptin receptor (Jordan et al., 2005). Candidate genes for obesity have also been studied (Dieter et al., 2004; Knoblauch et al., 1999).

Psychological traits are not our main focus; however, we have studied 'stress coping' as stress has been implicated as a risk factor. Heritability estimation and gene identification studies for stress coping have been carried out accordingly (Busjahn, Faulhaber, et al., 1999; Busjahn, Freier, et al., 2002).

Collaborations

The structure of a private company hosting a twin registry has the main advantage of allowing greatest

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Table 1

Number of Twin Subjects by Age and Zygosity

	Zygosity			
Age group	MZ	DZ	Ambiguous	Total
Under 11	11	21	100	132
11–20	108	113	138	359
21–30	234	339	627	1200
31–40	313	245	361	919
41–50	108	75	155	338
51–60	66	48	70	184
61–70	72	19	69	160
Over 71	45	25	48	118
Total	957	885	1568	3410

collaboration flexibility. The registry has close ties with the Medical Faculty of the Charité, where most of the clinical phenotyping takes place, and the Max-Delbrück Center for Molecular Medicine. Other collaborations have included the University Hospital Eppendorf in Hamburg, the Tübingen University, the Max-Planck Institute for Psychiatry in Munich, the Dr. Margarete Fischer-Bosch Institute for Clinical Pharmacology, Stuttgart, and the Academic Medical Center of the University of Amsterdam.

Collaborative research may include access to clinical data and DNA from earlier studies in the context of candidate gene studies, as well as prospective studies to obtain new data and biological samples. Prospective studies may include intervention studies in the context of nutriceuticals/cosmeceuticals, but not clinical trials in phase I or II.

Ethical Responsibilities

An Institutional Review Board, or Ethics Committee, must give approval for any investigation involving human subjects. The Ethics Committee of the Charité has approved all projects. There is no direct access to nonanonymized data or twin addresses in the studies. The subjects are able to withdraw at any time, and under these circumstances, the guarantee that their DNA samples will be destroyed. No subject has ever made such a request, as they are aware of the purpose of the research. Studies have focused on genetic variation that could influence the development of cardiovascular disease. Should the focus change in the future, informed consent statements would be modified accordingly.

Closing commentary

Depending on the background of the collaboration, development and fine-tuning of the research methodology could be done either by the external partner, ourselves, or in close collaboration. If necessary, local partners are involved in phenotyping and/or genotyping.

Table 2

Number of Twin Pairs by Sex and Zygosity

Zygosity	Sex		
	Male/Male	Female/Male	Female/Female
MZ	174	0	311
DZ	41	330	76
Ambiguous	257	0	522
Total	472	330	909

In conclusion, the Berlin Twin Registry HealthTwiSt has revived interest in twin research in Germany and continues to serve a broad range of research interests. We believe that twin research is a novel approach to complicated problems. Moreover, since the healthy are the harbingers of traits that cause disease, studies of the healthy are an underutilized resource. The purpose of HealthTwiSt is to find answers to fundamental questions regarding complex, common diseases. It is our view that commercialization has contributed rather than hindered that end.

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