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Establishing a Twin Registry in Guinea-Bissau

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Twins traditionally retain a special status in many African societies. In Guinea-Bissau, twins are often well regarded yet still suffer from a very high mortality, especially in the perinatal and infant period. At the Bandim Health Project, a health and demographic surveillance site, we have recently established one of the first twin registries in Sub-Saharan Africa. Our short-term aim is to describe twin mortality and morbidity in order to design appropriate health interventions. Our long-term goal is a large-scale database to explore the pathogenesis of prevalent diseases; for example, diabetes mellitus, metabolic syndrome, and infectious diseases such as HIV, tuberculosis, and malaria. A major focus area is also the etiology of low birth weight and how epigenetic processes might modulate the consequences of low birth weight in Sub-Saharan Africa. For this, monozygotic twin studies represent a powerful tool. Though twin studies have been carried out by the Bandim Health Project for more than 30 years, the renewed registry described here was officially established in 2009 and includes both a cohort of newborn twins and a cohort of young and adult twins. Currently more than 1,500 twins are being followed in the two cohorts combined. We believe that the registry holds exciting possibilities and will encourage the establishment of further twin registries across the region.

■ **Keywords:** twins, Africa, mortality, low birth weight, cohort study, epigenetics

Background

Twin data from Sub-Saharan Africa is scarce (Pison, 1992). Available studies confirm that twinning is common in the region (Pison, 1992), but also that early twin mortality is markedly higher than in other parts of the world (Jaffar et al., 1998; Jahn et al., 2006; McDermott et al., 1995; Pison, 1992). Long-term follow-up studies are almost entirely lacking, hence little is known whether twins continue to be more vulnerable in childhood and beyond (Justesen & Kunst, 2000).

From high-income countries it is well established that twins are at increased risk at time of birth and in the neonatal period (Kiely, 1990; McCulloch, 1988). This is mainly attributed to obstetrical complications, low birth weight (LBW) and prematurity. It has also been debated whether twins may be at a higher risk of metabolic and cardiovascular disorders as adults due to adverse conditions in fetal life (Petersen et al., 2011; Poulsen et al., 2009).

Currently, a large number of twin registries exist around the globe, yet with the exception of a Nigerian registry almost none from Africa (Hur et al., in press). In light of this paucity, we have established a twin registry in Guinea-Bissau. Our primary aim is to assess the consequences of being born a twin in Guinea-Bissau, both in the short and long term.

Second, the registry can be used in heritability studies, including metabolic conditions and communicable diseases. As 60% of the twins are born with LBW (Bjerregaard-

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Andersen et al., 2012) our cohort at the same time represents an opportunity to examine how epigenetic factors might affect the consequences of LBW in Sub-Saharan Africa.

Third, twins are a natural form of infant and childhood crowding, thereby facilitating studies of the impact of crowding (Aaby et al., 1983) and cross-sex transmission (Aaby & Molbak, 1990; Aaby et al., 1992) on disease severity. Twin studies also offer unique possibilities for studying interventions with possible sex-differential effects (Aaby et al., 1995, 2004).

Methods

Bandim Health Project (BHP)

The twin registry was established in Guinea-Bissau with the Bandim Health Project (BHP). Guinea-Bissau is a small and low-income country in West Africa. The total population is approximately 1.6 million. The official language is Portuguese, though most people speak the common language Creol or local languages.

The BHP is a Health and Demographic Surveillance Site (HDSS) and a member of the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH). The demographic surveillance covers approximately 30% of the capital Bissau and registers all pregnancies, births, migration, and deaths in the study area. All inhabitants have a personal ID number and censuses are carried out on a regular basis. Children below 3 years of age are visited every 3 months to collect information on vaccination, morbidity, breastfeeding, and anthropometry.

Previous Twin Studies by the BHP

Since the foundation of the BHP in 1978, registration of twins has been a central feature, mainly to examine the effects of crowding and gender on mortality (Aaby & Molbak, 1990; Aaby et al., 1983, 2004) and to ensure that neonatal mortality is correctly estimated. If a mother delivers twins and one dies she may be unlikely to report the co-twin and its death unless asked specifically whether the child is a twin. Hence, twin status is included as a variable in the HDSS database. This makes identification of twins in the study area possible.

Twin Registry

Due to the pre-existing twin studies (Aaby et al., 1983, 2004), the well-established research infrastructure at the BHP, and the fact that twin data from Sub-Saharan Africa is very limited, it was decided to resume the twin studies in Guinea-Bissau by creating a formal twin registry. The new twin registry was initiated in September 2009 and is intended to be ongoing. It comprises both a *prospective* cohort of newborn twins born at the National Hospital Simão Mendes that receive regular follow-up visits and a cohort of *young and adult twins* from the BHP study area.

Prospective Twin Cohort

Inclusion. Newborn twins from the whole Bissau area and surroundings are included daily at the main National Hospital Simão Mendes. The maternity ward has around 7,000 deliveries each year, including approximately 190 twin pairs. Furthermore, we also include twins born at the smaller Catholic Cumura Hospital outside the city. In total, more than a thousand twins have been included from 2009–2012, with the process described in detail elsewhere (Bjerregaard-Andersen et al., 2012). At the Simão Mendes Hospital, data regarding pregnancy and birth is collected. HIV testing of the mother is carried out, provided she consents. The newborn twins are examined clinically and anthropometric measures are recorded. Ballard score for maturity is assessed and filter paper blood is obtained for genetic analyses. After discharge the twins and mothers are driven home to enable follow-up. A cohort of randomly selected clinical controls (every fifth singleton delivery from the BHP study area) is included to facilitate comparisons between twins and singletons (see Figure 1).

Follow-up of twins and singleton controls. The first follow-up visit at home is conducted at 2 months of age and hereafter every 6 months (i.e., 6, 12, 18, 24 months, and so on). At follow-up, vital status is noted along with data on consultations, feeding practices, and anthropometric measurements. In the case of movement within the Bissau area, follow-up is attempted at the new address. Follow-up is not done outside Bissau city, that is, twins from the interior of the country are currently not followed.

The follow-up routine is expected to be ongoing, hence the twins are intended to be followed into adolescence and young adulthood. Additional assessments such as measurement of blood pressure and blood glucose levels are being planned.

Linkage with hospital data. All twins have a unique study number, which is also put on a sticker on the children's common vaccination cards. In Guinea-Bissau, whenever a child presents at hospital the mother will usually bring this vaccination card along. Hence the cohort can be directly linked to the clinical data collection at the pediatric ward at the National Hospital Simão Mendes, the main pediatric ward in the country (Biai et al., 2011; Veirum et al., 2007). The hospital database contains detailed clinical and laboratory information, including blood culture results, malaria slide, and hemoglobin and leukocyte count. Hospitalization outcome and discharge diagnoses are registered as well. This enables us to study which major infections and other diseases affect the twins.

Vaccines. Vaccine studies form a key component at the BHP (Aaby et al., 2012a, 2012b). Hence, information on

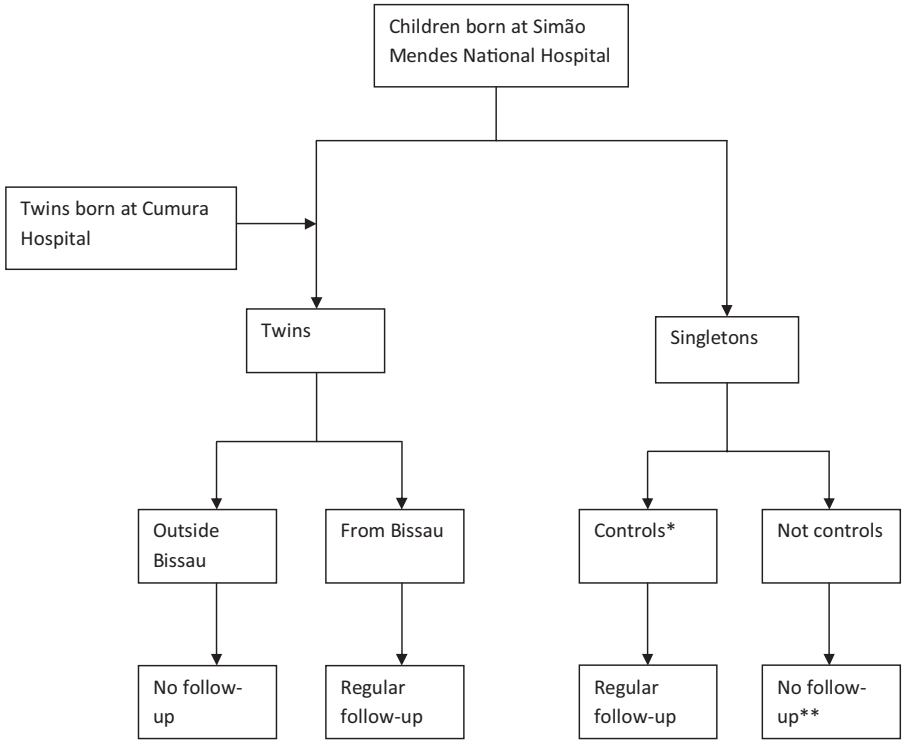

FIGURE 1

Prospective cohort of newborn twins.
Note: *Every fifth singleton birth from the BHP study area at the Simão Mendes National Hospital.
****If from the BHP study area follow-up will however happen through the HDSS.**

the vaccination cards is recorded meticulously at each contact with the children. The *prospective* cohort of newborn twins offers an opportunity of investigating how genetic and intrauterine factors affect vaccine effectiveness.

Young and Adult Twins From the BHP Study Area

Based on the HDSS database, almost 600 twins between 5–31 years in the study area have been identified. In a first study we screened this cohort for diabetes (DM) and metabolic syndrome (MS). For a subset of around 200 twins an oral glucose tolerance test has also been performed. The work includes an age-matched control cohort with the aim of comparing metabolic phenotypes for twins and singletons. This cohort is followed by the HDSS (see Figure 2).

Laboratory Analyses in Denmark

For both a subset of the *prospective* twin cohort and the cohort of *young and adult* twins, filter paper blood from live pairs has been analyzed for genetic zygosity in Denmark at Odense University Hospital, providing highly accurate zygosity status (Christiansen et al., 2003). Currently more than 100 same-sex pairs have been analyzed here, with monozygotic twins accounting for 19% of the total number of newborn twins (Bjerregaard-Andersen et al., 2012). In the cohort of *young and adult* twins, blood has been collected for metabolic measurements, which have also been carried out at Odense University Hospital.

Ethics

The twin studies described in this article have received ethical approval from the Ethical Committee in Guinea-Bissau. The establishment of the twin cohort has further received consultative approval from the Central Ethical Committee in Denmark. Written informed consent is obtained for all participants. Future additional studies (e.g., interventions) will require separate ethical approval.

Registry Management

The registry is a collaboration between the BHP (Guinea-Bissau), Odense University Hospital (Denmark), Danish Twin Registry (Denmark), and Research Center for Vitamins and Vaccines (CVIVA, Denmark). MB-A is the registry's principal investigator, with MS, HB-N, PAA, CB and AR as senior partners.

Perspectives

Twinning occurs commonly in Sub-Saharan Africa (Pison, 1992). This makes the region a good place to conduct twin studies (Pison, 1992). Yet, until now very few formal twin

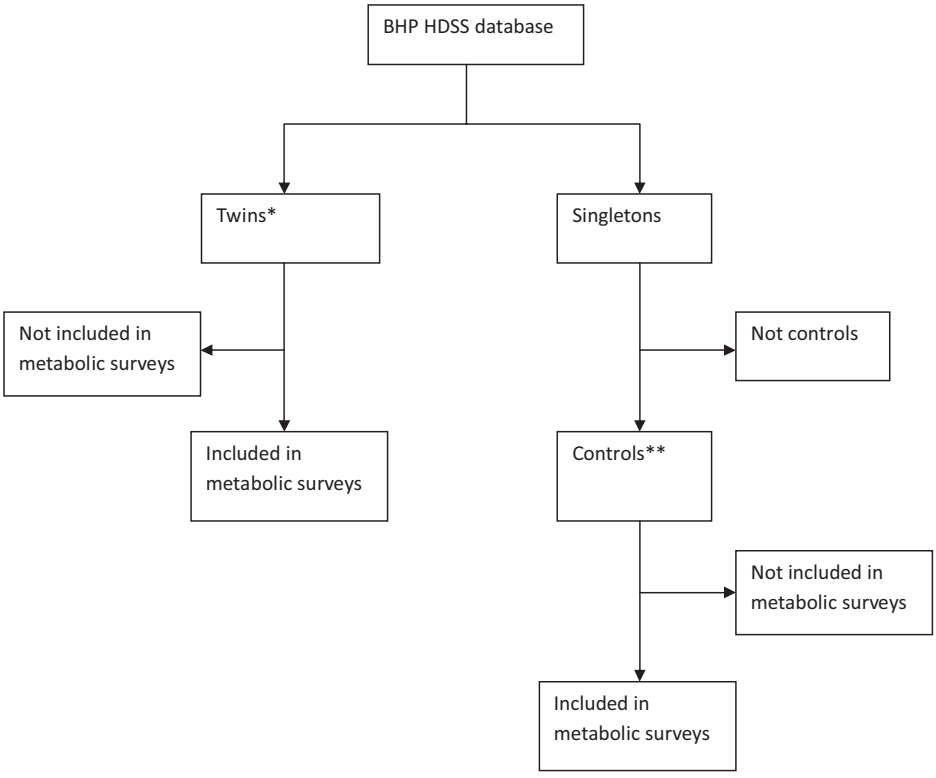

FIGURE 2

Cohort of young and adult twins.
Note: *Between 5–31 years.
****Matched on date of birth, i.e., each twin has a specific singleton control.**

registries have been established here, making comprehensive evaluations of twin health difficult.

Along with the Nigerian registry (Hur et al., in press), the twin registry established at the BHP in Guinea-Bissau is the first in Sub-Saharan Africa. We have several aims with this registry. First, we intend to do a detailed description of twin mortality, morbidity, and associated risk factors. Such data is necessary in order to design evidence-based interventions. Our first study found a 22% perinatal twin mortality making such initiatives imperative (Bjerregaard-Andersen et al., 2012). Second, by sampling a large number of twins, our registry can be used to differentiate between genetic, intrauterine, and environmental risk factors for disease where the relative importance might be different from high-income settings. Monozygotic twins here represent a particularly strong tool as analyses within monozygotic pairs control for both common environmental and genetic factors (Johansson et al., 2008). Until now our interest has been metabolic conditions such as diabetes, metabolic syndrome, and impaired glucose tolerance, but it could be used to study infections such as HIV, tuberculosis, and malaria as well. Recent data demonstrate that monozygotic twins can be strikingly different in terms of adult disease phenotypes, thereby supporting the evidence of substantial epigenetic influences (Fraga et al., 2005; Petronis, 2006).

The majority of twins are born with LBW (Bjerregaard-Andersen et al., 2012). Indeed, twins account for 20–23% of all LBW children in our setting (Aaby et al., 2011). LBW — as a marker of the fetal environment — has previously been associated with conditions such as diabetes, metabolic syndrome, and cardiovascular disease (Barker, 1990; Christensen et al., 1995; Hales & Barker, 2001; Vaag & Poulsen, 2007). Yet the etiology of LBW is not the same in a high mortality country as Guinea-Bissau. Maternal HIV infection (Habib et al., 2008) and malaria (Guyatt & Snow, 2004) are risk factors for LBW, while socio-economic factors, nutrition, and access to health services likely also play a role. Hence, more studies are needed to explore the association between LBW and various disease phenotypes in Sub-Saharan Africa. Our twin registry here represents a good opportunity to conduct such studies.

Finally, twin studies could answer the question whether there are genetic advantages to be being a twin. Prior to modern healthcare, fewer children survived from a similar number of twin and singleton deliveries (Pison, 1992) and twin deliveries were associated with 2–3 times higher maternal mortality (Hoj et al., 2003). Hence twinning, which

is strongly hereditary in Africa, should have disappeared. As it has not and the twinning rate is particularly high in Africa, there could be important health benefits associated with the genes that control twinning (Sirugo et al., 2012).

Our overall intention is that the twin registry in Guinea-Bissau will provide a future platform not only to improve twin healthcare in the region, but also help clarify the pathogenesis and underlying factors of various diseases, including how epigenetic processes modulate disease risk in low-income countries. The registry therefore has exciting prospects, but ideally more African twin registries are needed both for data comparisons and combined analyses.

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