twin studies in behavioral and health sciences

Organized by **Emma Otta,**
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Maria de Lourdes Brizot

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DEDICATION

To the fascinating world of twin studies and all those who have dedicated their lives to unraveling the intricate relationship between nature and nurture in human development. Twins are a window into human nature, says Nancy Segal, revealing insights into our humanity, who we are, and where we came from.

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ACKNOWLEDGMENTS

The organizers of the book TWIN STUDIES IN BEHAVIORAL AND HEALTH SCIENCES would like to express their gratitude to Professor Paulo Alberto Nussenzveig, Vice-President for Research and Innovation at the University of São Paulo, for his invaluable support of the event of the same name, which served as the foundation and starting point for this book. This event, made possible through the EDITAL DE APOIO A EVENTOS CIENTÍFICOS – 1/2024, provided a critical platform for the exchange of knowledge and ideas that ultimately contributed to the creation of this volume. We are also thankful to the University of São Paulo's Agency for Libraries and Digital Collections (ABCD), whose support through a call for funding the publication of original digital books resulting from scientific and academic studies allowed us to bring this book to fruition. In addition to institutional backing, we wish to highlight the significant financial support received from FAPESP (São Paulo Research Foundation), which played a pivotal role in the development of the research whose findings are presented in this book. Through the Thematic Research Grant, the organizers carried out the USP Twins Panel: Research on Behavior, Health, and Well-Being of Twins project (Process 22/02107-6). This grant not only provided the essential resources for conducting high-quality research but also nurtured a collaborative environment, facilitating national and international engagement in twin studies and their implications for behavioral and health sciences. Without the support of these institutions and agencies, this book would not have been possible. We are grateful for their commitment to fostering scientific knowledge and innovation in this important field of study. We are also especially grateful to the twins and their relatives who participated in the research projects and shared with our team the enthusiasm for the Painel USP de Gêmeos.

Emma Otta

Edgard Michel Crosato

Maria de Lourdes Brizot

FOREWORD

The unforgettable event which forms the basis for this book took place in Sao Paulo University, May 2024. It was located in a campus encased by staggering trees and vines and which at the same time, was hosting a festival of birds in all their resplendence. Leading twin experts from Brazil, Professor Emma Otta, Dr Edgard Michel Crosato and Dr Maria de Lourdes Brizot had brought together both national and international experts with a wealth of experience on the topic of twins.

My own fascination with twins started as a young child. I would marvel at individuals who looked identical, noting the visual thrill, fun, and challenges sometimes associated with that. Without realizing it, at the time, perhaps my true fascination stemmed from finding that some people are so very alike and others incredibly different; and the miracle of science and moreover nature and indeed genetics in leading to this.

All of the excellent presenters, turned authors of this book are united in having spent much of their careers focusing on twins. Like me, a sizeable proportion have studied huge samples of twins – both identical and non-identical in order to understand more about what makes people alike and different. As described so well in this book, different research methodologies are available – but as standard, comparing the similarity of identical twins and non-identical twins allows us to work out the extent to which genes and environmental influences explain differences between people. In this book, these differences include those in oral health, sleep and mental health. Why is it that some people are more prone to cavities than others, or more likely to get anxious or struggle to sleep when faced with the type of stressors we all experience from time-to-time?

The standard twin design makes a number of assumptions - one of which is that twins are similar to non-twins. When we use the twin design after all, we are not only trying to understand why twins are alike and different, but we are trying to extrapolate this information to other people (to the wider population of non-twins) as well. This is only possible if twins and non-twins are alike – and scientific research to date shows that they are in many ways. They

might look alike and have that unique status in life of being a twin, but when it comes to many of the variables we study – such as mental health, they don't seem to differ from nontwins much at all. This book, however, also highlights some of the differences between twins and singletons – such as the peculiarities of twin pregnancies and high-risk newborns (stemming, at least in part from the greater risk associated with twin than singleton pregnancies).

The notion of heritability is a central construct from twin research. This refers to the proportion of a variance of a trait that is due to genetic differences among individuals. A large group of people will differ greatly when it comes to anxiety levels for example, but to what extent do their genetic differences explain this? This concept is population specific, meaning that when we look at anxiety in a different population, genes might be more or less important in explaining differences in anxiety scores. This is particularly noteworthy given that a large proportion of twin studies are based in Northern Europe and North America. One of the exciting features of this book is therefore that some of the work presented is based on data from the University of Sao Paulo Twin Panel, the only twin registry that is currently active in South America. This work therefore provides a crucial addition to the wider field. Not only is heritability population specific but it is often misunderstood by the wider population. A common misunderstanding is that heritability refers to the likelihood of passing a trait onto one's children. In my own chapter, I talk about points of confusion and note that careful thought is needed when communicating with the general public in order to avoid misunderstandings, from which serious consequences can follow (such as perceptions about the acceptability of certain treatments).

In addition to twins teaching us about our differences and knowledge about unique features of being a twin, authors of this book have also examined twins to tell us about the development of other things, such as manual skills for example. Indeed, twins touch and push one another in the womb - and can show strong responses to these approaches from their goading sibling. When it comes to recognition skills, it is noteworthy that parents of identical twins can often tell them apart – which arguably makes them experts in facial recognition. Studying identical twins can also help us understand more about how the human brain processes visual information to recognize others.

Overall, the breadth of topics covered in this book is remarkable and one of its unique strengths. The varied methodologies and research topics covered here make this book invaluable for anyone wanting to stay abreast of the field or to learn about twins for the first time from both a research or practical perspective. While I wish that the readers of this book had been at the event on which this book is based – in a beautiful campus, enjoying the temperate climate of Sao Paulo in May, what we can give you, is a voyage into this event, providing a rich resource to enter or develop knowledge in this endlessly exciting field of research.

I hope you enjoy this journey as much as I have.

Alice Gregory

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1. INTRODUCTION TO TWIN STUDIES IN BEHAVIORAL AND HEALTH SCIENCES

Emma Otta, Edgard Michel Crosato, and Maria de Lourdes Brizot

The book TWIN STUDIES IN BEHAVIORAL AND HEALTH SCIENCES is a collaborative effort by professors of psychology, dentistry, and medicine, who are principal investigators of the FAPESP Thematic Project USP 'Twin Panel: Research on Behavior, Health, and Well-being of Twins'. Studies on behavior, health, and well-being from an ontogenetic perspective using twins as a model is an active area of research. The book connects readers with established and recent research findings. It fosters the interdisciplinary development of theoretical and practical knowledge and opportunities for multidisciplinary action projects. The increasing prevalence of twin births is an important public health issue given the risks to children and mothers. The book, which consists of 13 chapters authored by leading experts, is divided into three sections: early twin development, dentofacial development, and twin research concerning psychophysiological characteristics. The organizers are confident that the authors´ expertise and enthusiasm will inspire students and professionals to explore new avenues for professional practice and research.

Twin studies: A brief overview

Studies involving twins in the fields of development, behavior, and well-being began with Sir Francis Galton (1875), whose pioneering paper, 'The History of Twins as a Criterion of the Relative Contributions of Nature and Nurture,'

laid the conceptual and methodological groundwork for classical twin studies. Global registries have been created, supporting studies on the heritability of various traits throughout the life cycle. The oldest is the Danish Twin Registry, created in 1954, with over 85,000 registered pairs (Pedersen et al., 2019). The largest registry is in Sweden, with 216,258 registered pairs (Zagai et al., 2019). Denmark's population is 5,927,668, and Sweden's 10,647,920. The most recent update includes 75 registries worldwide. Every five years, *Twin Research and Human Genetics*, the journal of the International Society for Twin Studies, publishes a special issue with global updates on twin registries. Professor Yoon-Mi Hur, from Mokpo National University in South Korea, edited the 2019 special issue. The Painel USP de Gêmeos (USP Twin Panel) was the only Brazilian group among the 61 articles on twin registries from 25 countries, and the only active registry in South America (Otta et al., 2019).

Founded with the encouragement of Professor Fernando Leite Ribeiro, the University of São Paulo (USP) Twin Panel (Painel USP de Gêmeos) was established in 2017 at USP´s Institute of Psychology. This initiative aimed to create a comprehensive resource for studying twins, providing a unique platform for investigating the interplay between genetic and environmental factors in human behavior, health, and well-being. The panel was created by a dedicated team of faculty members under the leadership of Professor Emma Otta, whose expertise in psychoethology and behavioral studies was crucial in shaping its mission and scope. It recruits twins interested in research participation and accessing services provided by the university. Despite its recent establishment, the registry has already compiled a database of 6,126 participants and produced significant scientific output since its founding (e.g., Otta et al., 2016; Otta et al., 2019; Varella et al., 2019; de Oliveira Landenbergeret al., 2021; Ferreira et al., 2023; Varella et al., 2023; Fernandes & Otta, 2024). The USP Twin Panel is a valuable data repository and collaborative hub for interdisciplinary research, engaging experts in psychology, odontology, medicine, and other fields to advance the understanding of twin dynamics and their broader scientific implications. Since its inception, the USP Twin Panel has supported numerous studies, contributing significantly to research on genetics, behavior, and health, and serving as a vital tool for both academic inquiry and practical applications in diverse domains. It also fostered the establishment and strengthening of national and international collaborations with researchers focused on twin

studies related to behavior, health, and well-being. Many of these researchers contributed chapters to this book.

Classical twin studies estimate trait heritability by comparing the similarity between monozygotic (MZ) twins, who share 100% of their genes, and their dizygotic (DZ) counterparts, who share on average 50% of their genes and a common environment during their early development. Heritability, a statistical indicator expressed as h^2 and ranging from zero to one, indicates the proportion of population variability in a trait due to genetic differences among individuals. For example, in a study investigating telomere length in Swedish seniors aged 63 to 95, Pedersen et al. (2007) found higher intra-pair similarity in MZ twins ($r = 0.57$) than DZ twins ($r = 0.20$), resulting in estimated heritability of 56%. Mortality was three times higher in twins with shortened telomeres compared to their co-twins with longer telomeres.

Classic twin studies serve as the foundation for Polderman's comprehensive meta-analysis, which encompasses 50 years of research (Polderman et al., 2015). This meta-analysis synthesizes the findings of numerous twin studies to provide a robust understanding of the genetic and environmental influences on human traits and behaviors. By analyzing data collected over decades, it underscores the enduring value of twin studies in disentangling the complex interplay between heredity and environment, offering critical insights into areas such as mental health, physical traits, and developmental processes.

In addition to classic twin studies, researchers have developed a wide range of innovative designs to investigate individual differences, offering deeper insights into the complex interplay between genetic and environmental factors (Segal, 1990). These designs, summarized below, include: 1) Cotwin Control Studies: In this design, one twin serves as the "experimental" case and the other as the control. This method is particularly effective for studying the effects of specific environmental exposure or interventions; 2) Singleton Twins: These studies involve individuals born as twins but whose co-twin died either during or shortly after birth. These individuals, though raised as singletons, carry the genetic makeup of a twin; 3) Dizygotic Twin Designs: These studies were conducted with DZ twins, comparing, for example, same-sex and opposite-sex pairs; 4) Longitudinal Twin Studies: Tracking twins over extended periods, providing dynamic data on how genetic and environmental influences evolve across different developmental stages and life transitions; 5) Twin-Family De-

sign: This method incorporates data from extended family members, such as parents, siblings, and offspring, to disentangle genetic and environmental factors further and explore intergenerational transmission. 6) Twins as Couples: Twins are studied both individually and as a pair, under standardized conditions; 7) Twins and Nontwins: Investigators study the singleton siblings of twins, sibling pairs of similar age, or pairs of unrelated, age-matched singletons. 8) Partially Reared Apart Twins: Comparison of twins who have lived apart for a period with those who have always lived together. 9) Twins Reared Apart: A hallmark design in behavioral genetics, these studies involve twins who were separated at birth and raised in different environments, providing compelling evidence for the influence of genetics independent of shared upbringing. Subsequently, Segal et al. (2003) categorized existing psychological research on twins into four primary theoretical frameworks: 1) Behavioral Genetics: which investigates the genetic and environmental influences on behavior and traits, emphasizing quantitative genetic methods; 2) Social Genetics: a subdiscipline of behavioral genetics focusing on how genes influence social behaviors; 3) Evolutionary Psychology: explores how human psychology and behavior have evolved to provide adaptive advantages in specific environments. It examines the influence of natural selection on cognitive processes and behavioral traits over the course of human phylogenetic development. 4) Psychoanalytic/Psychodynamic Perspectives: Focuses on how early developmental experiences and twin-specific dynamics influence psychological outcomes, emphasizing the unique impact of shared development with an identical sibling. This diversity in research designs and theoretical frameworks highlights the versatility of twin studies in advancing our understanding of human behavior, development, and health.

In their article "Twin Studies Continue to Be Valuable in the Omics Era," van Dongen et al. (2012) conclude that the integration of classical twin studies with new technologies represents a powerful approach to investigating individual differences in complex traits. Hur (2019, p. 428) emphasized that "While the focus of twin studies has been on establishing the heritability of a wide variety of phenotypes, twin studies offer many other ways to gain insights into the mechanisms involved in individual differences. This includes but is not limited to the design of discordant MZ twins, which provides the best case-control match for genetic profile, pregnancy, age, sex, and childhood environment. These studies remain significant not only for obtaining phenotypic information

but also because they can shed light on the effects of (epi)genetic alterations that occur shortly after conception or throughout life."

To gain deeper insights into research on Brazilian twins in the international scientific community, a team from the Painel USP de Gêmeos conducted a scoping review. The findings were published in *Twins Research and Human Genetics*. Six databases (CAPES, BDTD, PEPSIC, PubMed, Google Scholar, and SciELO) contain studies in the fields of Medicine (n=73), Psychology (n=29), Dentistry (n=20), and Physical Education (n=11). Most of these were conducted in Brazil's Southeast (55%) and South (26%) regions. A majority were carried out with children (44%), a third with adults (35%), and 15% with adolescents. More than half (62%) focused only on monozygotic twins. In 80% of the studies, zygosity classification was based exclusively on the opinion of the twins/parents, without using validated classification instruments. Case studies predominated (52%), followed by those using self-report measures (36%), laboratory experiments (8%), and direct observation (4%). The most investigated topics in psychology were personality (16%), attachment (12%), cognitive assessment (12%), parental perceptions of development (8%), motherhood experience (8%), and parent-child relationships (8%). Based on the profile of existing studies, we suggest some prospects for the field, which could benefit from an increase in research including zygosity determination through genetic analysis and validated DNA-based questionnaires, comparison between monozygotic (MZ) and dizygotic (DZ) twins, larger sample sizes, expanded data collection designs, the use of appropriate statistical techniques for twin study designs, and enhanced sample representativeness. Initiatives such as the Painel USP de Gêmeos and collaborative research networks can play a significant role in promoting Brazilian studies on twin behavior.

Increasing twinning rates and challenges

The Painel USP de Gêmeos research team has been conducting population surveys focusing on twins' birth rates. The twin live birth rate in the city of São Paulo in 2003-2014 was investigated (Otta et al., 2016), based on data from the Sistema de Informações sobre Nascidos Vivos (SINASC) [Live Birth Information System of the São Paulo Health Department], encompassing 140 hospitals (56 public and 84 private) in the city. The average twin and

multiple birth rates, calculated from a total of 2,056,016 births, were 11.96‰ and 0.36‰, respectively. When considering the mother's residential address, a significant variation between 9.85‰ (Itaim Paulista) and 24.32‰ (Pinheiros) was observed across the 31 districts of São Paulo. The twin birth rate was higher in the central regions of the city.

A national survey (North, Northeast, Central-West, South, and Southeast regions) between 2002-2013 (Varella et al., 2019) showed that the more developed regions (Southeast: 10.34‰ and South: 10.06‰) exhibited higher twin birth rates than the developing regions (North: 7.32‰ and Northeast: 8.68‰), with the Central-West in an intermediate position (9.05‰). The underlying causes for these regional differences require further investigation. Several factors may have contributed to the higher twin rates reported in the developed Southeast and South regions, creating a regional discrepancy: more advanced maternal age due to a trend of women delaying childbearing to pursue a career, more access to assisted reproductive technologies, and greater nutritional diversity and abundance.

Advanced maternal age increases the likelihood of twin pregnancies, based on natural selection favoring double ovulation in response to declining fertility (Hazel et al. 2020). The incidence of twin pregnancies has been increasing in Brazil and worldwide in recent decades as a result of postponed childbearing and the use of assisted reproduction techniques (Russell et al., 2003; Varella et al., 2017, 2019).

Twins exhibit a higher rate of prematurity, with an average birth three weeks earlier and a birth weight approximately 1 kg less than their singleton counterparts (Luke et al., 2005). Assunção et al. (2010) analyzed the perinatal outcomes of 289 twin pregnancies delivered at the Hospital das Clínicas of USP´s School of Medicine (HCFMUSP) between 2003 and 2006. Only 11 (3.8%) pregnancies resulted from assisted reproduction, while most were spontaneously conceived. Of the 578 newborns (NB), 60.5% were from dichorionic pregnancies (DC), 30.8% monochorionic diamniotic (MCDA), and 6.6% monochorionic monoamniotic (MCMA). The average gestational age at delivery was 34.6 weeks (SD= 3.9) and the average birth weight was 2,031g (SD= 693). In DC pregnancies, the average gestational age was 35.4 weeks, MCDA 33.6 weeks, and MCMA 32.9 weeks. In singleton pregnancies, term newborns have a gestational age between 37 and 41 weeks (de Souza Pimenta, Calil &

Krebs, 2010). In NB resulting from twin pregnancies, the frequency of NB below the 10th percentile for twins was 15.7% for DC, 22.5% in MCDA, and 26.3% in MCMA. Malformations were identified in 21.3% of NB resulting from monochorionic pregnancies and 7.4% of dichorionic pregnancies. Hospitalization duration for MCMA NBs was longer than for other NBs (DC: 17.1; MCDA: 17.3 and MCMA: 23.3 days).

The FAPESP Thematic Project, "The USP Twin Panel: Research on Behavior, Health, and Well-Being of Twins," which organized this book, gathered interdisciplinary teams from various fields to examine socio-emotional, cognitive, and psychomotor indicators, as well as the developmental contexts of twin infants. These teams include researchers from the fields of:

• Psychology: Experts in human ethology, bioacoustics, cognitive psychology, and learning,

• Medicine: Specialists in fetal medicine, gynecology, obstetrics, neonatology, and clinical pediatrics.

• Dentistry: Featuring specialists in pediatric and forensic dentistry.

This collaborative approach enriches the study of twins by integrating diverse perspectives and areas of expertise. They share an ontogenetic perspective, with a focus on prenatal risk factors for the global development of children, and an interest in understanding how genetic, biological, environmental, and behavioral influences interact to promote education, health, and well-being. Monozygotic (MZ) and dizygotic (DZ) twins share 100% and 50% of their genetic makeup, respectively. The twin design (considering zygosity, gestation type, and average birth weight) is an important tool for advancing our understanding of how environmental and genetic factors combine to shape human characteristics and behaviors (see also D'onofrio et al., 2013; Donovan & Susser, 2011; Sahu & Prasuna, 2016).

The first part of the book focuses on the early development of twins, covering topics such as peculiarities of twin pregnancies (Chapter 2), high-risk newborns (Chapter 3), facial expressions in fetuses (Chapter 4), fetal origins of sensorimotor behavior (Chapter 5), building bonds from early interactions, with implications for the mental health of parents and developing twins (Chapter 6). The second part of the book focuses on the dentofacial development of twins, covering topics such as the influence of genetic and environmental factors on

oral health problems (Chapter 7), molar-incisor hypomineralization in mono- and dizygotic twins (Chapter 8), morphological variations of the face in twins (Chapter 9). The third part of the book explores psychophysiological characteristics in twins, covering topics such as bioacoustic analysis of newborns' crying (Chapter 10), genetic and environmental influences on sleep (Chapter 11), longitudinal associations between sleep problems and psychopathology (Chapter 12), the first systematic case study of Brazilian twins separated at birth and reunited in adulthood (Chapter 13). The final chapter presents the main conclusions derived from the preceding chapters and outlines potential directions for future research. It provides a summary of the key findings, discusses their broader implications, and identifies areas where further investigation could deepen understanding or address existing gaps in the field.

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PART I

TWINS EARLY DEVELOPMENT

The first section of this book comprises five chapters dedicated to exploring prenatal and perinatal risk and resilience factors that significantly influence twin development. These chapters address the early diagnosis of conditions that can profoundly impact twins' lives. Given the rising rates of twin births globally, including in Brazil, it is imperative to prioritize twins' health and well-being. To achieve optimal twin welfare, it is important for professionals and researchers across diverse health-related fields and specialties to collaborate and support caregivers to ensure twins' optimal welfare.

2. THE PECULIARITIES OF TWIN PREGNANCIES

Sckarlet Ernandes Biancolin Garavazzo

Abstract: This chapter examines the unique challenges associated with twin pregnancies, characterized by greater uterine expansion, increased weight gain, and significant metabolic changes, resulting in higher risks compared to singleton pregnancies. Premature birth, particularly before 27 weeks of gestation, significantly raises neonatal mortality and the likelihood of cerebral palsy. Early ultrasound assessment of chorionicity is essential for risk assessment and management. Dichorionic twins have separate placentas, and as a result experience fewer complications than their monochorionic counterparts, who share a placenta. Monochorionic pregnancies are particularly susceptible to severe complications, including Twin-to-Twin Transfusion Syndrome, Twin Anemia Polycythemia Sequence, and Twin Reversed Arterial Perfusion. Accurate early diagnosis and proactive monitoring are essential to mitigate risks and optimize outcomes.

Keywords: Twin pregnancies, Chorionicity, Dichorionic twins, Monochorionic twins, Twin-to-Twin Transfusion Syndrome, Twin Anemia Polycythemia Sequence, Twin Reversed Arterial Perfusion, Ultrasound assessment, Risk management

Women carrying twins undergo significant changes in their bodies. The notable peculiarities of twin pregnancies must be considered. Among maternal conditions that are more frequent in twin than singleton pregnancies are greater uterine expansion and higher weight gain associated with elevated levels of hormones such as human chorionic gonadotropin (hCG) and estrogens, as well as increased heart rate (HR) and blood volume. Metabolic alterations occur and often result in high glucose levels and insulin resistance. Preeclampsia, an obstetric complication characterized by persistent high blood pressure, also occurs more commonly in twin pregnancies.

Twin gestations are associated with an increased risk of postpartum hemorrhage due to uterine atony, a complication in which the uterus fails to contract properly during or after childbirth (Francois et al., 2005). Peripartum hysterectomy occurs more frequently in multiple than singleton gestations and is a major cause of maternal mortality in developing countries. Concerning fetal risks, twin pregnancies exhibit a significantly higher risk of premature birth compared to their singleton counterparts, with a noteworthy proportion born before 37 weeks of gestation (Alexander & Salihu, 2005; Assunção, 2008; Blencowe et al., 2012). Birth before 27 weeks of gestation is considered extreme prematurity. To date, there is no proven effective method to prevent preterm birth (Brizot et al., 2015; Martin, 2011). The lower the gestational age, the lower the birth weight. A higher number of fetuses is associated with increased neonatal mortality and a greater risk of cerebral palsy.

Chorionicity is the primary determinant of perinatal outcome, and two types can be differentiated by ultrasound: dichorionic and monochorionic (Figure 2.1). The chorion is the outermost fetal membrane, whereas the amnion is the innermost membrane that surrounds the fetus and contains the amniotic fluid. Monochorionic (MC) twins share one chorion and a single placenta. Dichorionic (DC) twins have separate chorions and individual placentas. The former has a 3-5 times higher risk of perinatal death than the latter.

In dichorionic-diamniotic (DCDA) pregnancies, each twin is enclosed within its chorion and amnion, meaning they have separate placentas and amniotic sacs. This is the most common arrangement in dizygotic (fraternal) twins since they originate from two separate fertilized eggs. However, DCDA pregnancies can also occur in monozygotic (identical) twins if the single fertilized egg divides within the first three days after fertilization. In monocho-

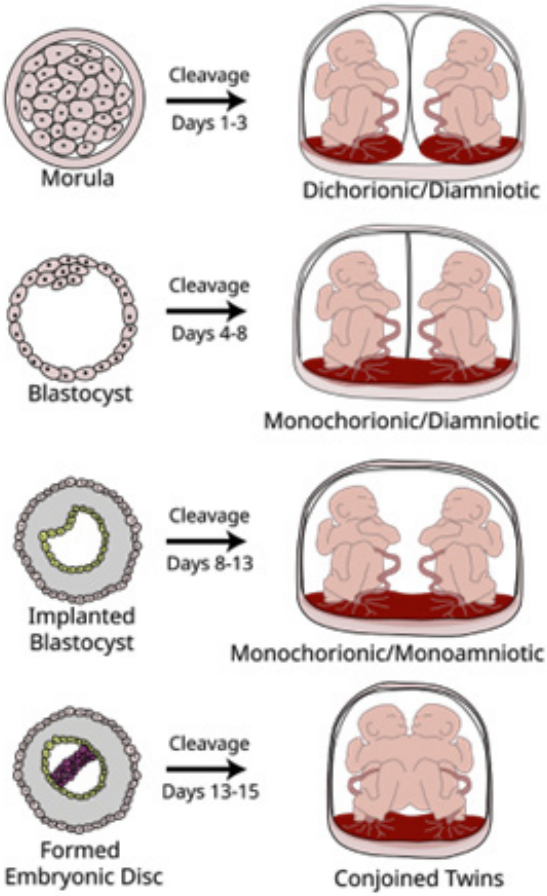

Figure 2.1 Types of chorionicity and amnionicity in twins (Source: Kevin Dufendach, CC BY 3.0 https://creativecommons.org/licenses/by/3.0>, via Wikimedia Commons)

rionic-diamniotic (MCDA) pregnancies, the twins share a single chorion and therefore a single placenta but possess separate amniotic sacs. This configuration occurs exclusively in monozygotic twins when the fertilized egg splits between 4 and 8 days post-fertilization. In monochorionic-monoamniotic (MCMA) pregnancies, the twins share both a single chorion (placenta) and single amniotic sac. This rare configuration occurs when a monozygotic embryo splits between 8 and 13 days after fertilization. MCMA pregnancies are associated with the highest risks among twin gestations. Conjoined twins are

an extremely rare outcome that occurs when a monozygotic embryo splits after day 13 of fertilization. Due to the late timing of the split, embryo separation is incomplete, resulting in the twins being physically connected. The extent and location of their connection vary, often depending on the time and nature of the incomplete division.

Early diagnosis is essential in twin pregnancies. Establishing chorionicity and amnionicity early, ideally during the first trimester, is critical for assessing and managing potential risks. This is typically accomplished through ultrasound, which evaluates key markers such as the number of placentas and thickness of the dividing membrane. Accurate early assessment facilitates timely intervention and appropriate monitoring, thereby reducing the likelihood of complications.

Monochorionic twin pregnancies are among the most complex and high-risk types of twin pregnancies due to their shared placenta, which can lead to several complications. Twin-to-Twin Transfusion Syndrome (TTTS) occurs when there is an unequal blood exchange between the twins through shared placental vessels. The donor twin transfers more blood than it receives, leading to restricted growth, anemia, and low amniotic fluid. The recipient twin receives excessive blood, causing overgrowth, hypertension, cardiac strain, and excessive amniotic fluid. Twin Anemia Polycythemia Sequence (TAPS) arises from an imbalance in the red blood cell transfer through tiny vascular connections in the shared placenta. The donor twin becomes anemic due to red blood cell deficiency, while the recipient twin develops polycythemia, characterized by an excessive concentration of red blood cells. Twin Reversed Arterial Perfusion (TRAP) is a rare condition affecting monochorionic twins where one twin (the acardiac twin) lacks a functioning heart and relies on the blood supply from the other twin (the pump twin) through abnormal placental connections. This places a significant burden on the cardiovascular system of the pump twin, substantially increasing the risk of heart failure.

Single intrauterine fetal death occurs in approximately 2-7% of twin pregnancies and poses significant risks to the surviving co-twin. The likelihood of co-twin demise is substantially higher in monochorionic compared to dichorionic pregnancies, with monochorionic twins facing a five-fold increased risk (Hillman, Morris, & Kilby, 2011).

For a comprehensive review of twin pregnancy complications, please refer to Biancolin (2017), Khalil and Thilaganathan (2019), Machado (2013), and Wang et al. (2020).

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3. HIGH-RISK TWIN NEWBORNS

Vera Lucia Jornada Krebs

Abstract This chapter highlights the substantial increase in twin pregnancies, which has contributed to a 76% increase in multiple births over recent decades and a corresponding rise in prematurity rates. Twin pregnancies pose heightened risks for maternal and neonatal health, including hypertension, intrauterine growth restriction, and prematurity. These complications often lead to low birth weight, respiratory distress, and neurodevelopmental delays. Monochorionic twins face additional challenges, such as twin-to-twin transfusion syndrome, anemia-polycythemia sequence, and growth restriction, with greater risks of cerebral injury and cardiovascular complications. Research identifies gestational age and birth weight as critical predictors of neonatal outcomes, with optimal ranges between 34–36 weeks and 1500–2499g. Long-term follow-up of twins with neonatal complications is essential to identify and manage late sequelae and mitigate the risks of chronic diseases.

Keywords: Twin pregnancies, Multiple births, Neonatal health risks, Low birth weight, Respiratory distress, Neurodevelopmental delays, Cardiovascular complications, Gestational age, Neonatal outcomes

The rising frequency of twin pregnancies has contributed to increasing the rate of prematurity in the world population. The rate of multiple births increased by 76% in the past three decades. These pregnancies pose a high risk to maternal and fetal health, and this risk increases according to the number of fetuses. Zygosity and chorionicity are important predictors of adverse outcomes.

In in vitro fertilization, the occurrence of monozygotic fetuses is higher (Ward & Caughey, 2022; Rissanen et al., 2022; Broughton & Jungheim, 2018).

In twin pregnancies, there is a greater frequency of complications such as hypertension, and restricted intrauterine growth with a higher risk of premature birth and consequent increase in morbidity and neonatal mortality. The main complications in preterm twins are: low birth weight, respiratory distress syndrome, admission to the Neonatal Intensive Care Unit, intraventricular hemorrhage, neurological disorders, sepsis, necrotizing enterocolitis, retinopathy of prematurity and bronchopulmonary dysplasia (Ishida et al., 2022).

A recent study comparing neonatal and infant morbidity and mortality in concordant and discordant twin newborns revealed significant differences in gestational age, birth weight, 5-minute Apgar scores below 7, neonatal intensive care unit admission, morbidity, intubation, and neurodevelopmental delays at 1 year of age (Kim et al., 2023).

A study in Japan in 2022 that analyzed neonatal and infant mortality with birth weight showed that the best scenario lies between 1500g and 2499g. At birth weight extremes, less than 1000g and greater than or equal to 4000g, mortality was higher at all ages, from 0 to 7 days, 8 to 28 days, and 29 days to 1 year of age. In the same study, the optimal gestational age was between 34 and 36 weeks. Similar to birth weight, gestational age extremes (22 to 27 weeks and greater than or equal to 40 weeks) had higher mortality (Ishida et al., 2022).

A study carried out at Hospital das Clínicas, in São Paulo, analyzed a cohort of 57 pairs of newborn twins, with an average gestational age of 36 weeks to describe the concentration of total and specific IgG antibodies (anti-Streptococcus B, anti-lipopolysaccharide of Klebsiella spp, and anti-lipopolysaccharide of Pseudomonas spp) in the umbilical cord and investigate the association between neonatal infection and antibody concentration in umbilical cord blood. In infants younger than 34 weeks there was less total IgG and anti-LPS Pseudomonas IgG transfer. The highest incidence of infection in the group with lower serum antibodies reinforces the importance of the mother´s antibody transfer (Yoshida et al., 2020).

In regard to the evolution of extremely preterm twin newborn pairs according to sex, a cohort study of 11 countries and 20,924 newborns showed an outcome disparity between the sexes in extremely preterm twins. Girls had

Figure 3.1 Preterm newborn in the Newborn Intensive Care Unit of Hospital das Clínicas, School of Medicine, University of São Paulo

lower risk than boys and opposite-sex pairs than same-sex pairs (Gagliardi et al., 2021).

In monochorionic twins, placental vascularization and the respective clinical presentation in the newborns were analyzed. The authors reported the following complications: twin-twin transfusion syndrome, twin anemia polycythemia sequence, selective fetal growth restriction, and other vascular disorders. It was found twin-twin transfusion syndrome survivors exhibited a higher propensity for cerebral injury. Recipient twins are at increased risk of cardiovascular complications and twin anemia polycythemia sequence donors of perinatal morbidity and mortality, and should be screened for hearing loss. The underlying mechanism for the increased risk of hearing loss and developmental delay in twin anemia polycythemia sequence donors remains unknown. A proportion of monochorionic twins are also monoamniotic, with additional risk for perinatal morbidity and mortality. Neonatal complications in monoamniotic twins are primarily driven by prematurity. Monochorionic twins with restricted intrauterine growth exhibit differences in morbidity. The larger and smaller twins have an increased risk of acute respiratory distress and chronic lung disease, respectively. In fetal programming, the smaller newborn with

restricted intrauterine growth is at greater risk of adult chronic diseases such as metabolic syndrome and cardiovascular disease (Groene et al., 2022; Marciniak et al., 2017).

We concluded gestational age in twin pregnancies continues to be one of the most important predictors of neonatal outcome. Long-term follow-up of all twin newborns with neonatal complications is essential to detect late sequelae and prevent chronic diseases.

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4. FACIAL EXPRESSIONS IN FETUSES

Mariana Azevedo Carvalho

Abstract This chapter delves into the study of facial expressions of emotions, starting with Darwin's groundbreaking work in 1872, which suggested a genetic basis for these expressions. Paul Ekman later confirmed the universality of facial expressions across different cultures, despite cultural variations. Fetal facial expressions, detectable in 4D ultrasound, develop prenatally, with distinct movements occurring as early as 24 weeks of gestation. Studies have demonstrated that fetuses respond to stimuli, including music and the mother's diet, with recognizable facial expressions. Research on fetal pain and facial expressions has led to the development of innovative tools such as Kurjak's Antenatal Neurodevelopmental Test, with promising potential for predicting neurodevelopmental outcomes.

Keywords: Facial expressions of emotions, Universality of emotions, Fetal facial expressions, 4D ultrasound, Fetal pain, Kurjak's Antenatal Neurodevelopmental Test, Fetal neurobehavioral assessment

Charles Darwin (1872) pioneered the study of facial expressions of emotions, investigating basic emotions such as fear, disgust, and anger (Figure 3.1). In the 19th century, he hypothesized a genetic origin or a specific area of the brain responsible for the manifestation of facial expressions. This has been confirmed because the limbic system and important cranial nerves that produce our facial expressions have been identified.

Figure 4.1 Weeping children from Darwin's Expression of Emotions (CC BY 4.0)

The psychologist Paul Ekman tested Charles Darwin's theory on the universality of emotions, given that many researchers questioned whether facial expressions were consistent across cultures. While cultural variations exist—such as the tendency of some Japanese individuals to suppress overt emotional expressions in social settings—Ekman demonstrated that certain facial expressions are universally recognized and associated with basic emotions. Ekman conducted research with an isolated community in New Guinea that had no prior contact with people from other cultures. With the help of a translator, he asked an elder to express facial emotions in four situations: (1) his friend had arrived, (2) his son had died, (3) he was about to fight, and (4) he stepped on a dead pig. Using a cross-cultural approach, he found compelling evidence of universal facial expressions because members of Western cultures could correctly interpret the emotions expressed by a member of a preliterate culture not in contact with Westerners.

Charles Darwin's work in 1872 sparked a renewed interest in the development of facial expressions of emotion over time. It is now known that facial expressions begin to develop in healthy fetuses prenatally and are discernable in the womb through ultrasound imaging. Four-dimensional sonography (4D-US) has been used for this purpose. Using 4D-US, Kurjak et al. (2003) observed frequent fetal eyelid and mouth movements between 30 and 33 weeks of gestation. Adapting Paul Ekman's adult Facial Action Coding System (FACS) for infants, Reissland et al. (2011) studied facial movement in two fetuses. At 24 weeks, they found 50% of single-action unit facial movements. With an increase in gestational age, there was a corresponding decrease in single action units and an increase in the co-occurrence of several action units. Increasing brain maturation was associated with quadruple or quintuple action unit patterns. In the third trimester, two configurations were observed (''cry-face-gestalt'' and ''laughter gestalt''). In a study conducted with 15 fetuses in the second and third trimesters of pregnancy, Reissland et al. (2013) found a developmental progression toward an increasing complex "pain/distress gestalt" as they matured, mirroring the facial expression observed after birth.

López-Teijón et al. (2015) conducted a study to compare fetal reactions to different types of stimulation, including flute monody and vibratory stimulation via intravaginal application of stimuli, ranging from 14 to 40 weeks of gestation. The study found that fetuses between 16 and 39 weeks of gestation exhibited repetitive mouthing and tongue ejection movements to intravaginally applied music, but not to abdominal music or intravaginal vibration. These facial action-based results suggest that the fetus perceives musical stimuli from an early age and that intravaginal application may be more effective in transmitting musical stimulation due to less interference.

Fetuses´ facial expression reactions to the mother's diet transmitted to the amniotic fluid. They exhibit a laughter-face gestalt when the mother eats carrots and of cry-face gestalt when she eats kale (Ustun et al., 2022).

Researchers have been using facial expressions to investigate fetal pain. Fetal pain is a controversial topic because many researchers argue that consciousness is required to feel pain. According to the cortical necessity hypothesis, cortical structures that develop after 24-28 weeks of gestation are necessary for conscious pain perception. Conversely, the subcortical modulation hypothesis posits that subcortical structures can mediate fetal pain perception before 24

weeks of gestation (Thill, 2023). The Fetal Pain Study Group examined fetal facial expressions in response to nociceptive stimuli (Bernardes et al., 2021). They analyzed 5 fetuses with diaphragmatic hernia requiring intrauterine surgery and 8 healthy fetuses. Facial expressions were recorded under three conditions: (1) during anesthetic injection into the thigh for intrauterine surgery, (2) during scheduled ultrasound examinations for the control group, and (3) in response to an acoustic stimulus. Using the Neonatal Facial Coding System, researchers found that changes in fetal facial expressions can be consistently identified and quantified, mirroring assessments in newborns undergoing medical procedures.

The study of fetal facial expressions is a new field of research with numerous potential applications, such as fetal neurobehavioral assessment tests designed to predict neurodevelopmental outcomes, such as Kurjak's Antenatal Neurodevelopmental Test (Kurjak et al., 2021). New methodological approaches are being investigated to analyze changes in fetal facial expressions using artificial intelligence to quantify and study them (Miyagi et al., 2023).

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5. THE FETAL ORIGIN OF MANUAL SKILLS

Jacqueline Fagard

Abstract This chapter explores the fetal origin of manual skills, emphasizing how fetuses develop sensorimotor behaviors before birth. From the third week of gestation, the nervous system begins forming, leading to early spontaneous movements. By the 14th to 15th week, targeted arm movements emerge, indicating intentionality. Fetuses explore their environment, demonstrating curiosity and proprioception. Around 18 to 22 weeks, coordinated movements become more controlled, with evidence of cortical control. The chapter also highlights the importance of fetal playfulness, seen in movements like the "balloon play." These early motor experiences significantly shape motor skills and cognitive abilities, challenging misconceptions about learning beginning at birth.

Keywords: Sensorimotor behaviors, Fetal intentionality, Proprioception, Fetal playfulness, Motor skills development, Prenatal exploration, Cognitive development, Early spontaneous movements, Neural development, Fetal environment interaction, Prenatal learning

This chapter delves into the fascinating fetal origin of manual skills. When admiring a masterpiece painting, we often overlook the countless hours of dedicated practice behind it. Even the greatest artists are relentless in their quest for perfection, devoting themselves to improving their technique, often right up to their final days. The first manual skill we witness in infants is grasping. Despite their initial lack of grasping skill, they swiftly learn to touch and grasp objects. These grasping abilities undergo significant changes in the first few

months of life (Fagard, 1998; Fagard, 2016). Intrigued by the development of infant grasping, I studied the onset of manual skill acquisition (Fig. 5.1). I questioned the extent of manual skills present in the womb and pondered how movements initiate and progress during this critical period. I am currently preparing a book titled "The Fetus' Hidden Life: Fetal Origin of Movements," which delves deeply into the fetal origin of sensorimotor behavior.

Figure 5.1. View of a fetus in the womb (drawing by Leonardo da Vinci 1510, public domain)

In the third week of gestation, the embryo develops a crucial structure called the neural plate, which forms the foundation for the nervous system. As development progresses, the neural plate undergoes folding to create the neural tube. This process is essential for the formation of the nervous system. Neural cell differentiation starts in the spinal cord. During this phase, neural cells migrate from the ventricular layer to the mantle layer of the spinal cord, first forming motor columns and, a few days later, sensory columns. Limb development begins as small buds around the fourth week of gestation and progresses to full formation by the eighth week. Muscle development occurs between the fifth and sixth weeks, aligning with the growing complexity of the limbs.

Nerve cells (motor neurons) have spontaneous (endogenous) electrical activity, as do muscle cells. When the number of spontaneously activating neurons reaches a critical mass, synchronized activity emerges. This activity is first synchronized for small ensembles of neurons, then additional neurons connect. When the number of fibers that have synchronized their electrical activity reaches a critical mass, information begins to flow and nerve circuits are formed. When enough nerve cells are connected to enough muscle cells, the motor command is transmitted to the muscle: around the sixth week, the first neuromuscular junction occurs. This process likely marks the emergence of what we refer to as the first central pattern generators. It is precisely at this time that the first barely perceptible movements, known as vermicular movements and startles, can be observed. These initial fetal movements occur prior to the development of sensory systems and are therefore not a result of sensory stimulation. Following these initial movements, sensory connections develop, leading to the emergence of the first reflexes around the eighth week. As the skin and proprioceptive sensory receptors mature, these movements subsequently induce sensory stimulation. General movements gain in complexity as descending brainstem fibers reach the spinal cord and movements are controlled by brainstem CPGs (central pattern generators). Figures 5.2 and 5.3 show summaries of the process involved.

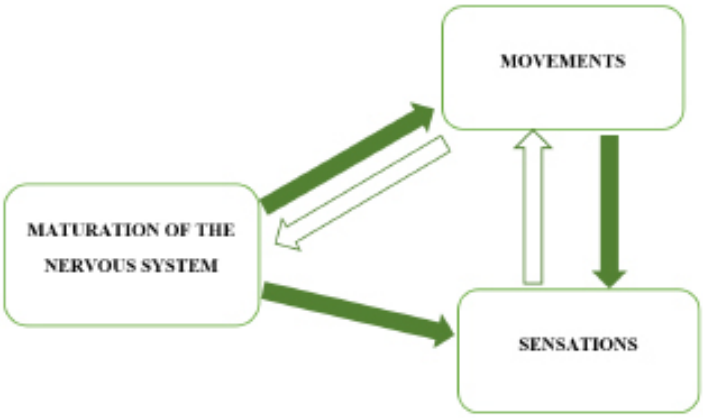

Figure 5.2 Synthetic scheme outlining the relationship between the maturation of the nervous system, fetal movements, and sensations during development: (1) maturation of the motor system induces endogenous movements; (2) movement is necessary for the normal development of sensory receptors; (3) once the maturation of sensory receptors has begun, movement produces sensory feedback.

Embryo	Fetus				
	11th–13th wk	14th wk		20th wk	21th wk
Maturation of NS	Motivation to repeat	Intention (search of sensations)	Curiosity	Coordination	Contingencies detection?
- vermicular - startles - GM	-random spontaneous GM	-targeted isolated arm movements	-exploratory movements (motor babbling)	-anticipatory movements	-controlled movements
				maturation of the cerebral cortex	

Figure 5.3 Overview of prenatal motor development (NS=nervous system, GM=general movement)

Spontaneous general movements are observed around the 11th to 13th week, which may be a passive consequence of startles. By the 14th to 15th week, targeted isolated arm movements appear, indicating the beginning of intentional movements in the search for sensations. Exploratory movements emerge as fetuses begin to explore the dynamic boundaries between well-innervated and less-innervated areas, demonstrating curiosity and a growing sense of proprioception. Through exploratory movements, the fetuses actively interact with their environment, gaining insight into their developing bodies. Coordinated anticipatory movements, such as opening the mouth before the hand reaches it, further demonstrate coordination and anticipation (Myowa-Yamakoshi & Takeshita, 2006). Research by Zoia et al. (2007) indicates different movement patterns towards the eyes and mouth, suggesting a level of control and contingency detection. Zoia et al. performed a kinematic analysis of hand movements and found that movements towards the eyes and mouth differ significantly. Touching the eyes could be painful, while touching the mouth is not, suggesting a degree of control and the ability to detect contingencies. Thus, around 18 to 22 weeks of gestational age, reaching movements become more coordinated and better controlled. This could reflect the maturation of the cortical plate and the fact that descending and ascending pathways start to connect the cortex to the spinal cord. This possibility of cortical control over movements at that age is confirmed by the fact that anencephalic fetuses exhibit different motor activity than normally developing fetuses starting around the 20th week of pregnancy.

To gain insight into fetal playfulness, watch this informative video by François Farges (2007) captured after an amniocentesis procedure. [https://www.dailymotion.com/video/x8kg1gy] Amniocentesis is an ultrasound-guided puncture procedure commonly performed in the second trimester of pregnancy. It involves removing amniotic fluid from the uterus for testing using a thin needle. In one out of 300 procedures, a blood clot was formed, leading to observations by the doctor performing the amniocentesis that are relevant to the issues discussed in this chapter. The clot gave rise to a "balloon play" recorded on video. After 45 minutes of "learning", the fetus moves her hands to palpate the 'balloon', placed in front of the face, then put on the knees and pushed by the legs. The video repeatedly features this 'balloon play'. The medical team was concerned about the bleeding during the examination, but the pregnancy progressed normally. The mother gave birth to a healthy baby girl, who is still doing well today.

In the video by Farges (2007), compelling evidence is presented showing that a fetus can exhibit playful movements in the womb, demonstrating control and coordination. This challenges the misconception that learning begins at birth, as the fetus already displays motivation, intention, curiosity, and anticipation. It is crucial to acknowledge the months of sensorimotor experiences during gestation, as they significantly contribute to the development of motor skills and other more cognitive skills. While the movements of newborns may appear much less coordinated compared to those of fetuses, it is important to consider the adjustment to increased gravity, increased visual stimuli, and feeding requirements after birth, which can be much attention-demanding. This perspective suggests that birth marks a period of adaptation, including some apparent regression of sensorimanual coordinations, rather than a sudden onset of learning.

In conclusion, fetuses have many months in the womb to learn about themselves and their environment, including people. In particular, twins typically exhibit strong reactions to being touched or pushed by the co-twin between 11 and 13 weeks, with earlier responses in monochorionic twins (Piontelli, 2010). Oriented hand movements towards the co-twin occur earlier and are more frequent than towards their own body (Zoia et al., 2012). But even in singletons, social cognition can emerge before birth, as the fetus has to adapt to the other individual (the pregnant mother) within which she spends several months.

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6. FROM AN INTEREST IN EARLY SENSUAL DEVELOPMENT BY TOUCH AND SOUND TO A GENERAL LONG-TERM CONCEPT FOR THE MENTAL HEALTH OF PARENTS-TO-BE AND THEIR OFFSPRING

Birgit Arabin

Abstract This chapter explores the development of prenatal sensory abilities and their impact on overall well-being. It emphasizes the gradual emergence of fetal sensory and social capacities beginning in the first trimester. Research reveals how fetal movements are integrated with sensory awareness, resulting in coordinated behaviors and responses to external stimuli. Notably, fetal interactions, especially among twins, provide valuable insights into social cognition and environmental adaptation. The chapter also highlights the importance of maternal care, discussing interventions like Kangaroo Mother Care and music programs that promote maternal and fetal/newborn well-being. Ongoing studies aim to assess the long-term effects of these interventions on maternal stress and fetal development.

Keywords: Twin fetal interactions, Kangaroo Mother Care, Music programs in pregnancy, Maternal well-being, Fetal well-being, Newborn well-being, Maternal stress reduction, Fetal development interventions, Long-term effects of prenatal interventions

Aristotle already anticipated that sensory development is a gradual process beginning prenatally and that the origin of the soul is rooted in sensory capabilities. However, until the 19th century, there was widespread skepticism regarding fetal sensory and social capacities (Arabin, 2002). The introduction of ultrasound and fetal heart rate (FHR) monitoring has enabled the investigation of fetal sensory organs and behavioral responses from the first trimester onward. This has revealed how self-generated movements integrate with somatosensory awareness, leading to coordinated fetal movements (FMs). Consequently, it highlights the continuity of behavioral patterns and responses from the prenatal to neonatal stages, including reactions to both maternal and external stimuli. More recently, it was described that the fetal brain is highly attuned to social interactions and that this may predict other individuals' mental states. Fetuses also develop a preliminary concept of others earlier than establishing a solid concept of oneself, which represents a biological adaptation to later social life (Faraji and Metz, 2023). The developmental origins of fetal senses thus have biological, medical, and social implications.

My early research was focused on the crucial role of early fetal sensory development through touch and sound (Arabin et al., 1999; Arabin, 2009). Thereby long-term ultrasound observations played a crucial role. This chapter adopts the WHO's definition: "Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity". Professionals, researchers, and philosophers have touched upon this concept, emphasizing the significance of nurturing sensory systems for overall well-being. Heinz Prechtl (1984) is regarded as the pioneer in describing the continuity of prenatal and postnatal sensory and motor development and "behavior". He created the General Movements Assessment (GMA) based on the "Gestalt Perception" of videoed movements in young infants, which indicates how the neurological system is developing.

Attending the first Congress of Fetal Origin of Adult Diseases in 2000 in Mumbai was enlightening. Michael Meaney's research on maternal care and its influence on gene expression in rats provided profound insights. Rat offspring that received ample maternal licking exhibited improved social behavior and heightened grooming behavior toward their offspring. His studies on oxytocin receptors, and the enduring impact of maternal care on grooming behaviors in subsequent generations, emphasize the vital role of balanced nurturing in

ensuring the overall well-being of offspring (Meaney, 2001). How a mother cares for her offspring can directly impact the expression of genes responsible for stress reactivity and cognitive function in her offspring. Early studies with animals (preferably rats and mice) have shown the importance of the concept of "environmental enrichment" and its beneficial influence on cognitive and intellectual capabilities (e.g., Hebb, 1947; Rosenzweig & Bennett, 1996).

Over the years, I have been fascinated by studying twins and have conducted behavioral studies on them. Ultrasound videos have revealed the first reactions of twins towards each other starting at 10 weeks, demonstrating their sensory capabilities (Arabin et al., 1996). Simple movements gradually transition into complex movements, with twins embracing each other in utero by 99 postmenstrual days. The reactions of twins towards each other depend on gender and chorionicity. Monochorionic twins start interacting earlier and show reactions to each other earlier than dichorionic twins, likely due to a thinner or non-existent membrane between them. The first contacts with response were observed at 65 days in monochorionic monoamniotic twins, 72 days in monochorionic diamniotic twins, and 81 days in dichorionic diamniotic twins. The gender composition of twin pairs and variations in testosterone levels may lead to significant differences. Male-male fetal pairs showed quicker initiatives and responses compared to female-female and opposite-sex pairs. Female-female pairs displayed complex body contacts (>5 sec) with repetitive actions and reactions earlier than male-male pairs.

There is a gradual increase in cutaneous sensory receptors in human fetuses across gestational weeks: perioral area (7 weeks), face, hands, and feet (11 weeks), trunk, arms, and legs (15 weeks), and all cutaneous and mucous surfaces (20 weeks). Synapses develop in the dorsal horn of the spinal cord (6 weeks), perioral area (7 weeks), neocortex/thalamus (8 weeks), sensory nerves grow into the spinal cord (14 weeks), and synapses in the neocortex (24 weeks).

Beneficial effects have been claimed for interventions like haptonomy, which aims to stimulate prenatal emotional contact between parents and fetuses through touch, although there is no solid scientific evidence of its effectiveness (Arabin & Metz, 2020). In contrast, the beneficial effects of Kangaroo Mother Care (KMC), depicted in Figure 6.1, have been well-demonstrated for both preterm infants and parents (Arabin et al., 2021; Charpak & Montealegre-Pomar, 2023). Rey (1983) developed KMC for low-birth-weight pre-

mature infants at the Instituto Materno Infantil in Bogotá, Colombia, as an alternative to the lack of newborn incubator care. Touch has improved outcomes in premature babies, including reduced infection and mortality rates, enhanced development as measured by head circumference, improved immunology, and increased breastfeeding rates. KMC became a worldwide practice that influenced the Western world, humanizing neonatal care in affluent countries.

Figure 6.1 Two twin boys receiving 'kangaroo care,' strapped to their grandmother's chest in a hospital in Malawi. (Licensed under the Creative Commons Attribution 2.0)

Music during pregnancy is another intervention that enhances maternal stress resilience for the benefit of the offspring (Maul et al., 2024). The study of the fetal sound environment in a comparative perspective is an active area of research. Experiments on sound attenuation during sheep gestation have provided insights into the human case (Armitage, Baldwin & Vince, 1980). By using hydrophones implanted into the amniotic sac of sheep fetuses, researchers were able to confirm that external sounds are attenuated by approximately 16 to 37 decibels (dB). External sounds at levels corresponding to those of a normal conversation were picked up without masking by maternal cardiovascular sounds. They could be heard by the sheep fetus since there is evidence that its auditory system is functional at 100 days of gestation. In the realm of hearing physiology, much of our understanding comes from experiments conducted on sheep. Technical details of the importance of sound amplitude, sound pressure energies, and the transmission of music and voices through the maternal abdomen have been discussed elsewhere (Arabin 2002). It is worth noting that lower-pitched sounds, such as male voices or deep musical notes from instruments

like the double bass or cello, traverse the maternal abdomen more easily. In contrast, higher-frequency sounds are more readily absorbed but can be better discriminated by the fetal ear (Arabin, 2002). However, higher-pitched sounds require more energy to penetrate the maternal tissues effectively.

A music program, titled "Creativity Program during Pregnancy", has been developed by us in cooperation with the Foundation Berliner Philharmoniker. Singing workshops aim to promote well-being experiences in groups of pregnant women, enhancing body awareness and connection with their developing children. The concert music is selected by musicians from the Philharmonic orchestra, workshops are designed by the Department of Education of the Berlin Philharmonics by Katja Frei. The women also receive a CD with Mozart's music to relax at home. The lyrics of John Miles sum up this program.

Music was my first love
and it will be my last.
Music of the future
and music of the past.

To live without my music
would be impossible to do.
In this world of troubles,
my music pulls me through.

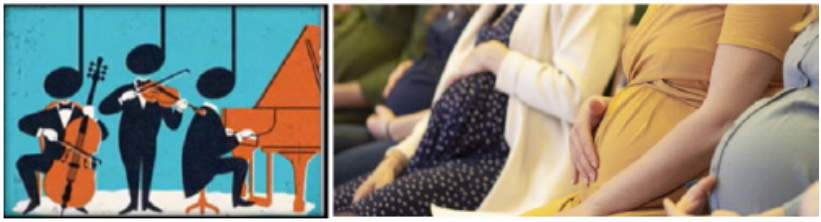

Figure 6.2 Creativity in pregnancy: a project combining music and research (Picture: Annette. Hauschild), see https://clara-angela.info and https://www.berliner-philharmoniker.de/

Before and after each intervention, participants in the Creativity in Pregnancy Program are monitored both physiologically and psychologically. Physiological monitoring includes measuring cortisol levels from saliva and assessing maternal heart rate variability (a stress marker). Psychological evaluation is conducted using the PANAS test, a validated questionnaire designed to measure affect. All test results were significantly improved after the variety of interventions. To assess long-term effects, we utilized validated tests for anxiety, stress, and depression through mail-based follow-ups, along with measuring cortisol levels in maternal hair at the end of pregnancy and collecting standard obstetric data. To evaluate the impact on the fetus, umbilical cord blood and placental tissue were sampled immediately after birth to analyze telomere length and fetal exposure to maternal cortisol. These results are compared with a control cohort that did not undergo the interventions.

During the initial study intervals (2021–2022 and 2022–2023), we observed that singing was associated with slightly greater immediate improvements, as reflected both in objective measures and in subjective evaluations of maternal feedback. Women especially appreciated the music selection in the concerts and the empathy of the team of artists and scientists. The project is ongoing and we plan to start a controlled trial in January 2025, whereby pregnant women either take part in life concerts and courses within the Philharmonic building as before or have the chance to participate just by digital media. If the effects between the cohorts are small it is justified to involve larger groups of potential candidates for stress reduction and maternal-fetal well-being, mainly for risk groups in areas of poor socioeconomic level or even global crises or conflicts.

We aim for participation in the "Creativity in Pregnancy Program" to enhance maternal self-awareness and strengthen parental bonding. Furthermore, we anticipate that it may have transgenerational physiological benefits, as evidenced by studies on telomere length measurements (Verner et al., 2021). Creative empathic interventions induce maternal stress resilience and lower harm for the offspring due to reduced fetal exposure to maternal cortisol or pro-inflammatory markers. In the current world of unnecessary global conflicts, this may interrupt vicious circles of trans-generational programming, because music programs may pass any frontier and even re-unify pregnant mothers of hostile environments in our hope for humanity's dream: a peaceful future.

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PEER COMMENTARY

Mario Henrique Burlacchini de Carvalho, Claudia Monteiro Peixoto, and Maria de Lourdes Brizot

As coordinators of the discussion section in the first part of this book, we collected and compiled the insightful questions posed to the authors during the 'Twin Studies in Behavioral and Health Sciences' event, which inspired this publication. This event, which brought together experts in the field, provided an invaluable platform for in-depth discussions and the exchange of ideas on the latest developments, challenges, and future directions in twin studies related to behavioral and health sciences. The questions from the event reflect the diverse perspectives and critical inquiries essential to advancing research in this area. By including these questions into this section, we aim to further enhance the reader's understanding of the topics discussed, providing a deeper exploration of the key themes and issues raised during the event.

Question 1: Dr. Sckarlet was asked about the definition and meaning of preeclampsia (Audience Question).

Answer: *Preeclampsia is characterized by high blood pressure combined with proteinuria, the presence of excess protein in the urine. Both of these conditions are necessary for a diagnosis. It is a serious and potentially life-threatening form of hypertension that can occur during pregnancy. (Sckarlet Ernandes Biancolin Garavazzo)*

Question 2: Dr. Mariana and Dr. Jaqueline were asked the following question: "I am curious about the current understanding of fetal pain percep-

tion before the emergence of facial expressions typically associated with pain. Is there evidence to suggest that fetuses can experience pain before displaying these expressions? If so, are anesthetic interventions currently used in such situations?" (Patricia F. Monticelli)

Answer 1: *We have observed that when a painful stimulus is applied, fetuses often respond with specific movements, such as flexing their necks or bringing their hands toward their mouths. However, it is widely considered that fetuses are unlikely to experience pain before 20 to 22 weeks of gestation, as the spinothalamic connections required for pain perception are not fully developed by that stage. While nociceptors are present in the skin, the neural pathways essential for processing pain remain immature. Research in this area has advanced significantly over the past decade. Recently, we conducted a validation study on a fetal pain scoring system in collaboration with the Grupo de Estudo da Dor Fetal (Fetal Pain Study Group) at USP. This study included five fetuses with diaphragmatic abnormalities and led to the validation of the Fetal-7 scale (Bernardes et al., 2021; Bernardes et al., 2024). Our findings indicated that these fetuses exhibited frequent movements in response to stimuli, suggesting potential early sensory responses that warrant further investigation. (Mariana Azevedo Carvalho)*

Answer 2: *Research indicates that while the first pain receptors in fetuses begin to develop around the seventh week of gestation, the neural pathways required for experiencing pain are not fully formed until after the 11th week. Early evidence of fetal sensitivity is observed during medical procedures, where accidental needle contact with the fetus often triggers a rapid withdrawal response, suggesting an unpleasant sensation. Further indications of sensory perception are seen in early ultrasounds, typically performed between 11 and 14 weeks, which reveal fetal responses to external stimuli. For example, gentle tapping on the mother's abdomen can prompt the fetus to change position. These observations suggest that even in the early stages of development, the fetus exhibits some level of sensory awareness. (Jacqueline Fagard)*

Question 3: Dr. Jacqueline was asked the following question: "I have read that atypical movement patterns in neonates may be indicative of autism spectrum disorders. I am curious to know if any evidence or research is suggesting that unusual fetal movements might also be associated with these conditions." (Camila, fetal medicine doctor in the twins' group at Hospital das Clínicas)

Answer: *That is a very interesting question. I know that some studies have examined movement patterns in fetuses, particularly in cases of anencephaly, where motor activity differs from that of typically developing fetuses. However, I am not currently aware of any research directly linking fetal movements to the later development of autism spectrum disorders. This is certainly a fascinating area of inquiry, and I would be happy to explore it further and share any relevant articles I come across. (Jacqueline Fagard)*

Question 4: My question for Dr. Mariana is about twin studies. You have discussed the differences between twins and non-twins, as well as the distinctions between monozygotic and dizygotic twins. These variations are central to understanding genetic and environmental influences. Given the latest findings and advancements in our knowledge of twins, how might these developments refine or reshape the twin study design and its applications in research? (Alice Gregory)

Answer: *From the first two presentations, we learned the importance of defining key factors such as gestational age at delivery, birth weight, and prenatal conditions. Without understanding these variables, it is difficult to draw reliable conclusions about environmental influences. It is essential to consider all of these factors, particularly in cases involving monoamniotic twins or those with discordant birth weights, as they can have a significant impact on neurodevelopment and other aspects of the twins' growth. (Mariana Azevedo Carvalho)*

Question 5: The fifth question was directed to Professor Arabin, asking whether she believes it is important for a mother to talk to her belly during pregnancy and if such interaction could strengthen the bond between mother and baby. (Audience Question)

Answer: *I don't believe that talking to the belly is as effective as music. I think a positive mood, combined with singing, has a stronger impact. When a mother sings, and the baby hears the same melody repeatedly, the baby begins to recognize both the melody and the maternal voice. This has been demonstrated in research—not by me, but by other studies. Babies exhibit more positive behavior when exposed to music and maternal songs, as opposed to just hearing the voice. In this case, music proves to be more beneficial than talking. (Birgit Arabin)*

Question 6: The sixth question was directed to Professor Arabin, focusing on the benefits of music and other interventions. Do you think music should be prescribed to pregnant women, similar to how we prescribe vitamins or aspirin to prevent preeclampsia? In countries like Brazil, where there are many stressful situations and high anxiety levels in cities like São Paulo, do you believe music should be recommended for all pregnant women?

Answer: *This is indeed a very important question, and there is research supporting this idea. For example, in Canada during the ice storm, many pregnant women experienced stress due to being cut off from basic resources, and the IQ of their babies deteriorated significantly over time. There are also studies by Professor Olson and others that demonstrate while interventions like creative writing are beneficial, music and singing have an even greater positive impact. Creative interventions, including music, can help prevent cognitive delays and metabolic diseases in future generations. In some places, like Brazil, these interventions are not yet covered by insurance or government programs, which is unfortunate. However, I believe attitudes will shift once we demonstrate, in a few years, that music improves both metabolic profiles and IQ. These interventions are affordable, non-invasive, and have a positive impact on both mothers and babies. I am confident in their potential and am actively working to publish these findings. Furthermore, I fully agree that music holds particular significance for pregnant women in high-stress environments, such as those found in São Paulo. Even in places like Venezuela, where music programs have proven successful, we have seen positive effects. It is crucial to reassure women that they can significantly benefit their babies by singing just three times a week. This is a simple and cost-effective intervention, but it is essential to ensure that women feel supported and reassured. They can sing alone or in groups, with group singing being especially beneficial. During the pandemic, many women found comfort in singing together. Although this intervention may not yet be accessible to everyone, I firmly believe it has great potential for positive change.*

Question 7: The seventh and final question was also directed to Professor Arabin. What is the role of community-based interventions in supporting the mental health of babies and families? Parents today are often overworked and stressed. (Audience Question)

Answer: *Yes, I completely agree. There is a study from Spain that examined anti-stress programs involving Mediterranean diets and other interventions.*

Although these programs were not focused on music, they showed a significant reduction in intrauterine growth retardation. This suggests that community-based interventions, such as music or creative activities, can help reduce stress and improve outcomes for both mothers and babies. I do not believe social media alone can provide the emotional support mothers need. Small group settings, where women can connect, are much more beneficial. In our music intervention program, for example, we organized small groups of up to 10 women who participated in workshops where they sang, engaged in creative writing, and took part in other activities. The results were overwhelmingly positive, with reductions in stress and improvements in both fetal and maternal health. Singing, in particular, proved to be the most effective activity. Singing is also beneficial for the fetus, as the vibrations travel directly through the mother's diaphragm. For this reason, I always encourage singing—it doesn't require instruments, just a good teacher and the right support. It is a simple, low-cost intervention with a significant impact.

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PART II

DENTOFACIAL DEVELOPMENT OF TWINS

The book's second section explores the fascinating world of twins' dentofacial development. These in-depth chapters use twins as an experimental model to explore crucial factors impacting oral health, such as genetics, prenatal and perinatal conditions, and environmental influences. Chapter 7 discusses oral health problems and their impact on children's quality of life, while Chapter 8 provides insights into molar-incisor hypomineralization in monozygotic and dizygotic twins. Finally, Chapter 9 elucidates the morphological facial variations in twins. This section contains valuable and potentially groundbreaking insights that will benefit both the scientific and dental communities.

7. INFLUENCE OF GENETIC AND ENVIRONMENTAL FACTORS ON ORAL HEALTH PROBLEMS

Julia Gomes Freitas

Abstract This chapter highlights the critical role of twin studies in understanding the complex interplay between genetic and environmental factors in oral health. Studies provide valuable insights into conditions such as dental caries, periodontal disease, malocclusion, and other oral health issues. Research has demonstrated that genetic factors contribute to caries susceptibility while environmental factors influence microbial acid production. Our research group, CARDEC Trials (CARies DEtection and management in Children), has been conducting studies involving twin children investigating dental caries, dental fear, anxiety, and the quality of life of twins. Regarding our preliminary findings, no significant anxiety differences were observed between monozygotic (MZ) and dizygotic (DZ) twins. However, the MZ twins exhibited stronger correlations in caries indicators, underscoring the genetic influence on dental health. In terms of oral health-related quality of life, we also found a stronger correlation among monozygotic twins.

Keywords: Twin studies, Monozygotic twins, Dizygotic twins, Dental Caries, Dental fear, Dental anxiety, Oral health indicators, Oral Health-related Quality of life

Twin studies are essential to advancing our understanding of oral health since they focus on the intricate interplay between genetic and environmental factors in the development and progression of various conditions. Twin studies in dentistry play a crucial role in providing valuable insights into oral hygiene, dental caries, periodontal disease, malocclusion, and dental developmental defects. These studies significantly contribute to predicting and preventing diseases, improving treatment approaches, and enhancing patient behavior management.

Monozygotic (MZ) and dizygotic (DZ) twins share 100% and 50% of their genes, respectively. If there are genetic influences, the correlation between MZ twins will exceed that of their DZ counterparts. Twin studies in dentistry show the effective use of the twin research design. Evidence suggests that genetic factors influence caries susceptibility, while environmental factors modulate microbial acid production (Bretz et al., 2005). Research on oral care habits has found no significant differences between MZ and DZ twins (Liptak et al., 2024). However, there is weak evidence indicating a higher concordance in MZ compared to DZ twins in terms of the hypomineralization of second primary molars (Silva et al., 2019). It has also been revealed that environmental factors, such as maternal smoking during pregnancy, significantly contribute to the development of this dental condition. Furthermore, twin research has demonstrated that traits related to intra-arch tooth positioning are generally more heritable than those related to inter-arch positioning after craniofacial growth completion (Lin, Hughes & Meade, 2023). This finding has significant implications from a clinical perspective, suggesting that occlusal traits influenced more by genetic factors may pose greater challenges for orthodontic interventions compared to those primarily influenced by environmental factors.

Our research group CARDEC Trials (CARies DEtection and management in Children) from the School of Dentistry of the University of São Paulo, Brazil has been conducting studies on twins (https://sites.usp.br/cardec/pesquisas_de_diagnostico/cardec-twins/). This chapter presents the findings of our research on the influence of zygosity on dental caries, dental fear and anxiety related to dental treatment, and children's oral health-related quality of life (OHRQOL). It is important to note that 10% to 20% of children experience some degree of dental anxiety (Cianetti et al., 2017). In Brazil, dental fear af-

fects 21% of children (Costa et al., 2017). Figure 7.1 illustrates a continuous cycle of dental fear.

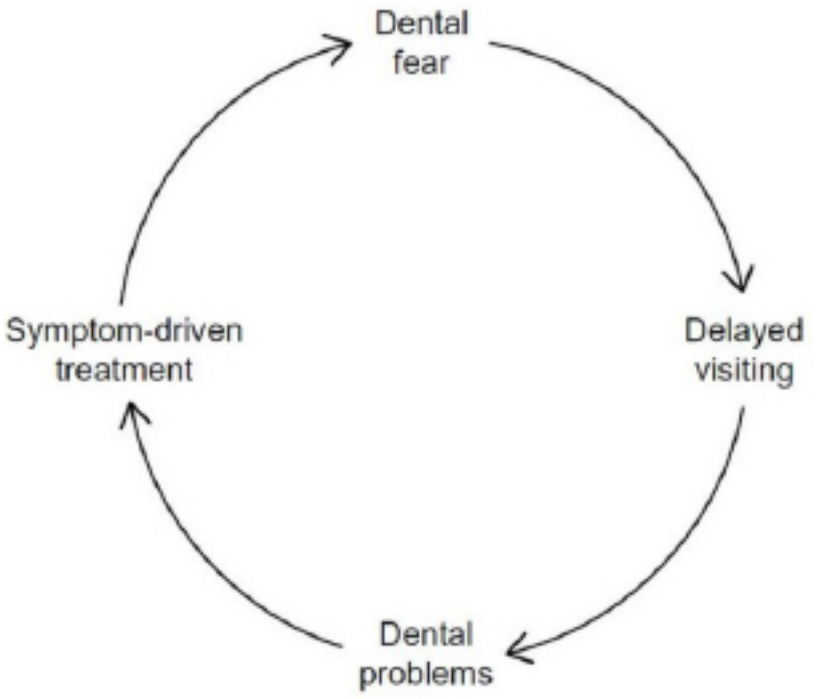

Figure 7.1 Vicious cycle of dental fear (4.0 CC-BY-4.0)

In our twin cohort study, we recruited twins born between 2007 and 2017 at Hospital das Clínicas, São Paulo, Brazil, and parents were asked about their interest in dental treatment for their children and their willingness to authorize research participation. The children also consented to participate. Our sample comprised 144 twin children. Zygosity was classified using the Brazilian version of the Zygosity Questionnaire by Christiansen et al. (2003), which was completed by the twins' guardians (Otta et al., 2019). We collected information on sex, age, anxiety, and oral health conditions. The children responded to the Modified Child Dental Anxiety Scale (MCDAS), which originally consisted of 8 questions, although the last 2 may not apply depending on the local context. In this study, we applied only the first 6 questions, which concerned different situations that they might encounter in our dental office (Howard & Freeman.,

2007; Freeman et al., 2020; Barbosa et al., 2022), such as having their teeth examined, scaled and polished, or extracted (Fig. 7.2). Each question was rated on a 5-point Likert scale, 1 being relaxed/not worried, 2 very slightly worried, 3 fairly worried, 4 significantly worried, and 5 very worried (Howard & Freeman, 2007). The final scores are calculated by summing the six questions, with a maximum and minimum of 30 and 6, respectively, with lower scores indicating less dental anxiety (Freeman et al., 2020). The questionnaire was administered before the start of the treatment session in the dentist´s office. After the children's health conditions were assessed, a clinical examination was performed, and an individual treatment plan was devised based on each participant´s dental needs. Statistical analyses were conducted using multilevel linear regression. Intraclass correlation coefficient (ICC) and Kappa analyses were performed and stratified by zygosity.

How do you feel about ....

• going to the dentist generally?	1	2	3	4	5
• having your teeth looked at?	1	2	3	4	5
• having your teeth scraped and polished?	1	2	3	4	5
• having an injection in the gum?	1	2	3	4	5
• having a filling?	1	2	3	4	5
• having a tooth taken out?	1	2	3	4	5

Figure 7.2 Twin children evaluated their dental anxiety through faces (MCDAS-f)

The study found no significant differences in dental fear and anxiety scores between Monozygotic (MZ) and Dizygotic (DZ) twin pairs. This contrasts with other research suggesting that genetic influences play a significant role in the development of dental fear and anxiety (e.g., Randall, Shaffer, McNeil, et al., 2017). Concerning gender, the study found that girls reported higher MCDAS-f scores, indicating greater dental fear and anxiety toward dental treatment than boys. This is consistent with previous research (Kothari & Gurunathan, 2019) and may be attributed to cultural norms that allow girls to express

their feelings more openly, while boys are expected to suppress them. No statistically significant association between MCDAS-f scores and age was found.

The present study also examines the influence of zygosity on the agreement of caries indicators in children. After the oral clinical examination, we calculated the decayed, missed, and filled primary and permanent teeth index, considering all lesions (including initial-stage decay, moderate decay, and cavitated lesions) (dmft-D1/DMFT-D1) and only cavitated lesions (dmft-D3/DMFT-D3). We calculated the agreement in caries parameters between monozygotic and dizygotic twins using the Intraclass Correlation Coefficient (ICC) with 95% confidence intervals (95% CI). Heritability (h2) was assessed using Falconer's method, as detailed in Table 7.1. The agreement between the pairs was consistently higher for MZ, with the ICC (95% CI) varying from 0.601 (0.336 to 0.778) to 0.842 (0.707 to 0.918) for dmft-D3 and dmft-D1, respectively. For DZ twins, the higher ICC value was for DMFT-D1 (0.695; 0.484 to 0.829). The h2 values were higher than 0.700 in most cases. In conclusion, the agreement observed between monozygotic twins underscores a genetic influence on dental caries occurrence.

Table 7.1. Agreement and heritability (h2) in the caries indicators in MZ and DZ twins

Quantitative variables	Monozygotic	Dizygotic	h^2
	ICC (95% CI)	ICC (95% CI)	
dmft-D1	0.842 (0.707 to 0.918)	0.388 (0.095 to 0.623	0.908
dmft-D3	0.601 (0.336 to 0.778)	0.236 (-0.094 to 0.516)	0.730
DMFT-D1	0.740 (0.539 to 0.861)	0.695 (0.484 to 0.829)	0.090
DMFT-D3	0.748 (0.553 to 0.866)	0.371 (0.067 to 0.613)	0.754
dmft-D1 + DMFT-D1	0.698 (0.473 to 0.837)	0.226 (-0.094 to 0.504)	0.944
dmft-D3 + DMFT-D3	0.635 (0.386 to 0.799)	0.267 (-0.051 to 0.536)	0.736

Notes: ICC = Intraclass Correlation Coefficient (ICC) and 95% Confidence Intervals (95% CI), h2 = Heritability calculated using Falconer's method, dmft = decayed, missed or filled primary teeth; DMFT = decayed, missed or filled permanent teeth, D1 = All lesions, D3 = cavitated lesions

In the current study, we also evaluated the influence of zygosity on the response patterns of twins to the short form of the Brazilian version of the Child Perceptions Questionnaire (CPQ11-14) before receiving dental treatment. This questionnaire is designed to measure oral health-related quality of life (Torres

et al., 2009). Intraclass Correlation Coefficient (ICC) analyses with 95% confidence intervals (95% CI) included 28 pairs of MZ twins and 28 of DZ twins. The findings revealed a stronger agreement in the response patterns among MZ, ICC = 0.622 (95% CI = 0.163 to 0.865), compared to DZ twin pairs, ICC = 0.443 (95% CI = -0.098 to 0.774).

In conclusion, an in-depth understanding of these interrelations enables oral health professionals to create more impactful prevention and treatment strategies aimed at enhancing patients' oral health and overall well-being. Our research group's primary objective is to conduct comprehensive studies on pediatric dentistry using twins as models and an interdisciplinary framework. By doing so, we aim to significantly contribute to the advancement of children's oral health by integrating behavioral and social science. Our studies have been made possible through the generous participation of over 100 pairs of twins and their parents, who have willingly dedicated their time to furthering our understanding of how genetic and environmental factors influence the development of oral health, as well as shedding light on the intricacies of the twinning process.

Statement: The authors declare that the data presented here are preliminary results of our studies, which have also been presented at Brazilian and international conferences.

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8. MOLAR-INCISOR HYPOMINERALIZATION IN MONO AND DIZYGOTIC TWINS

Francisca Aline da Silva Matias, Lúcia de Fátima Almeida de Deus Moura, and Marina de Deus Moura de Lima

Abstract This chapter focuses on enamel, the hardest tissue in the human body, which forms in two stages. Defects in this process can lead to conditions such as Molar-Incisor Hypomineralization (MIH), characterized by weakened and discolored teeth. MIH has both genetic and environmental origins. Our research on twins revealed a strong genetic influence, with 75.5% concordance in monozygotic twins compared to only 10% in their dizygotic counterparts. This underscores the fundamental role of genetics in the development of MIH. Understanding the genetic factors involved can improve early diagnosis and pave the way for more effective treatment strategies.

Keywords: Twin studies, Genetics of dental diseases, Early diagnosis, Tooth development, Amelogenesis, Enamel defects, Molar-Incisor Hypomineralization

Teeth are composed of the following mineralized tissues: cementum, dentin, and enamel. Enamel, the outermost layer of the tooth crown, has the highest mineral content in the human body, protecting the underlying dental pulp from physical, thermal, and chemical insults (Elliott et al., 1997; Lacruz et al., 2017).

Enamel formation is regulated by cells called ameloblasts, in a process known as amelogenesis. This process occurs in two main phases: secretion and maturation (Lacruz et al., 2017). The former takes place when ameloblasts secrete proteins that form the organic matrix of the tissue. The latter is the mineral deposition phase, whereby the matrix loses water and proteins, and the ameloblasts regulate and transport ions to the tissue (Zhang et al., 2023).

Thus, amelogenesis is a highly regulated process, and any disruption can result in defective enamel. When disorders occur during the secretion phase, the organic matrix is not deposited in specific dental crown regions, leading to areas devoid of enamel, known as quantitative defects or hypoplasias (FDI, 1992). However, disruptions during the maturation phase can lead to defects in the mineral content of the enamel. These defects were characterized by Weerheijm et al. (2001) as "abnormalities in the translucency (opacities) of the enamel".

Molar-Incisor Hypomineralization (MIH) is an enamel defect that has raised concerns among the dental community worldwide. This condition occurs when defects affect one to four first permanent molars, with or without incisor involvement (Weerheijm et al., 2001; Lygidakis et al., 2022). The global prevalence is 13.5% and is characterized by demarcated opacities such as white, yellow, and/or brown (Ghanim et al., 2018; Lopes et al., 2021).

The defective enamel tends to be porous, with lower calcium and phosphorus mineral content and higher carbon content (Jälevik et al., 2001). Thus, molars and incisors severely impacted by this condition may exhibit posteruptive enamel breakdown (Weerheijm et al., 2001; Ghanim et al., 2018), dentin hypersensitivity (Castro et al., 2021; Linner et al., 2021), and rapidly progressing carious lesions (Lygidakis et al., 2022; Mazur et al., 2023). Furthermore, studies show that MIH has a negative impact on the quality of life of children and adolescents (Dantas-Neta et al., 2016; Amrollahi et al., 2023; Kisacik et al., 2024).

The multifactorial etiology of MIH needs further explanation. Studies suggest that it may be related to pre-, peri- and postnatal environmental factors (Lygidakis et al., 2022; Franco et al., 2023), which occur between the end of pregnancy and the beginning of the child's fourth year of life (Alaluusua, 2010). Genetic and epigenetic factors also play a role (Zhang et al., 2022; Teixeira et al., 2023; Figueira et al., 2023).

In a cross-sectional study, Elzein et al. (2022) analyzed salivary DNA in a sample of 659 Lebanese children aged 7 to 9 years diagnosed with MIH. They observed that 37 SNPs were associated with the occurrence of MIH in the following genes: AMTN, MMP-20, STIM1, STIM2, ORAI1, SLC34A2, SLC34A3, VDR, PVALB, HSP90B1, TRPM7, SLC24A4, CA6, SLC4A2, TNFRSF11A, IL10RB, ARNT, ESR1, and CYP1B1.

A systematic review conducted by our research group (Figueira et al., 2023) showed that single nucleotide polymorphisms (SNPs) in genes related to amelogenesis, immune response, xenobiotic detoxification, ion transport, aquaporin function, and vitamin D receptors may result in MIH. However, we also concluded that the certainty of evidence from available studies remains low or very low, requiring improved designs, diagnostic standards, and long-term follow-up.

Studies with monozygotic (MZ) and dizygotic twins (DZ) are crucial for understanding disease etiologies. Comparing the concordance of traits between MZ and DZ co-twins, who share 100% and 50% of their genetic material, respectively, provides estimates of genetic and environmental contributions to the traits under investigation (Hagenbeek et al., 2023). The higher correlation observed between MZ than DZ twins demonstrates the genetic influence on the trait, whereas similar correlation values in MZ and DZ twins indicate a more significant environmental influence.

Research involving twins is particularly valuable in MIH, where both genetic and environmental factors are believed to play significant roles. Twin studies can help determine heritability and pinpoint specific environmental factors that contribute to its development (Vassend et al., 2022). Recent studies have shown that using both MZ and DZ twins can reveal complex mechanisms and identify potential targets for preventive and therapeutic interventions (Vassend et al., 2022; Hagenbeek et al., 2023; Oz & Kırzıoglu, 2023).

In this respect, our research group (Buccæ Geminæ) was the first to evaluate the occurrence of MIH in twins. We analyzed a census sample composed of twins aged 8 to 15 years from the city of Teresina, Piauí state, Brazil, totaling 167 pairs, with 94 monozygotic (MZ) and 73 dizygotic (DZ) pairs. The concordance of MIH in both individuals of MZ and DZ pairs was 65.5% and 34.5%, respectively. This statistically significant difference in MIH concor-

dance between MZ and DZ pairs (p=0.021) suggests a genetic influence on MIH occurrence (Teixeira et al., 2017).

Using our data, Vieira (2019) calculated MIH heritability to be 20%. This reinforces a genetic component in MIH development of MIH. However, further studies are needed to broaden our understanding of the genetic and environmental factors involved in this condition.

Moreover, twin research can significantly enhance our understanding of gene-environment interactions, which is crucial for numerous health conditions. By elucidating how environmental factors modulate genetic expression, we can develop more effective strategies to prevent and treat various diseases.

Understanding the genetic basis of MIH can provide valuable insights for early diagnosis, prevention, and treatment strategies. Additionally, investigating gene-environment interactions in MIH can clarify its complex etiology. This dual approach may lead to more targeted interventions and a better understanding of how both genetic predispositions and environmental exposures contribute to the development of MIH.

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9. EXPLORING FACIAL MORPHOLOGY IN TWINS: CHALLENGES AND EMERGING RESEARCH APPLICATIONS

Edgard Michel Crosato, Emma Otta, Fausto Medeiros Mendes, Laura Regina, Antunes Pontes, Tania Kiehl Lucci, Mariana Novaes, Maria Gabriela Haye Biazevic

Abstract This chapter explores the complexities of distinguishing monozygotic (MZ) twins, who share near-identical genetic material. It highlights challenges in face recognition, providing historical examples such as the Mauch twins and studies on self-recognition and parental recognition of twin faces. The chapter reviews the biological differences between MZ and dizygotic (DZ) twins and explores advances in artificial intelligence (AI) for facial identification. A novel AI-based system analyzed the facial features of twins using advanced algorithms, achieving high accuracy in distinguishing even subtle differences. These findings demonstrate AI's potential for applications in forensics, healthcare, and security, enhancing identification reliability and addressing twin-specific challenges.

Keywords: Monozygotic twins, Dizygotic twins, Face recognition, Twin identification, Artificial intelligence (AI), Facial identification algorithms, Forensic applications, Facial feature analysis, Twin-specific challenges

This chapter focuses on twins, specifically exploring variations in their facial features and their practical implications. In everyday life, distinguishing

monozygotic (MZ) twins is frequently challenging due to their near-identical appearances. A striking example is the case of the Mauch twins, William John (Billy) and Robert Joseph (Bobby), famous child actors in the 1930s (Figure 9.1). Their remarkable resemblance often led to misidentification, even by their mother. In an interview with Time Magazine, she revealed her strategy for distinguishing between them: Bobby wore a ring with a stone, while Billy wore a simple band, allowing her to easily recognize her sons. Their portrayal of the Prince of Wales and a pauper in the 1937 film *The Prince and the Pauper*, made them ideal for the roles, since the plot revolves around their exchange of identities and misidentification. The comedic misunderstandings central to the plot allowed each boy to navigate the other's life with both challenges and revelations. The use of identical twins allowed the film to depict the mistaken identity that drives the narrative convincingly, enhancing the realism and emotional depth of the story.

Figure 9.1 Monozygotic twins can be remarkably hard to tell apart, as exemplified by the striking similarities between Billy and Bobby Mauch, child actors in the 1930s.

Despite being raised apart from infancy and only reunited as adults, MZ twins remain, as the saying goes, "like two peas in a pod," with one mistaken for the other (Figure 9.2; Chapter 14, this book). Nancy Segal recounts several instances where their reunion was facilitated by identity confusion (Aron, 2024; Segal, 2012). In these cases, individuals familiar with one twin often mistook the other for their sibling, leading to unexpected encounters and surprising revelations.

Figure 9.2 Raised apart since birth and only reunited as adults, one twin was mistaken for the other (Photo: Tomaz Maranhão)

MZ share identical genetic material but are not completely identical

Monozygotic (MZ) and dizygotic (DZ) twins originate from different biological processes during conception and early embryogenesis. The former are

formed when a single fertilized egg divides into two separate embryos. This division occurs early in development, typically within the first two weeks after fertilization. The exact cause is not fully understood, but it is believed to be a random event. The timing of the division affects the type of placental and amniotic structures the twins share. If it occurs early, between days 1 and 3, the twins will have separate placentas and amniotic sacs. If it occurs later, between days 4 and 8, they will share a placenta but have separate amniotic sacs. If the division occurs even later, between days 8 and 13, the twins will share both a placenta and an amniotic sac. DZ twins occur when two separate eggs are fertilized by two distinct spermatozoa during the same ovulation cycle. This process involves hyperovulation, where the mother releases two or more eggs that are then fertilized. Each embryo develops independently, with its own placenta and amniotic sac.

Table 9.1 Key differences between monozygotic and dizygotic twins

Feature	Monozygotic Twins	Dizygotic Twins
Number of Eggs	1	2
Number of Spermatozoa	1	2
Genetic Similarity	100%	~50%
Cause	Random zygote division	Hyperovulation and dual fertilization
Placental Sharing	May or may not share	Always separate

DZ twins share on average 50% of their DNA, similar to non-twin regular siblings. This means they can differ in sex, appearance, and various other traits. By contrast, MZ twins share 100% of their DNA. Originating from a single zygote, MZ twins are similar because of their shared genetic makeup, but they are not identical. Minor physical and non-physical differences can arise due to a number of factors, making each twin unique. A variety of intrauterine factors and genetic mechanisms can lead to phenotypic, genotypic, and epigenetic differences (Gringras & Chen, 2001; Silva et al., 2011). Advances in molecular techniques are making these differences increasingly easier to identify and understand.

Are MZ twins and their parents experts in face recognition?

Humans are extremely skillful in recognizing and interpreting faces compared to other objects. This ability is deeply ingrained in the cognitive systems, facilitating rapid and accurate distinction between individuals, even among large groups. This expertise stems from specialized neural mechanisms, such as the fusiform face area in the brain, which is specifically attuned to facial perception (Chen et al., 2023). Studying twins and their parents enhances our understanding of how the human brain processes and differentiates faces, even under challenging conditions. Insights from these individuals can guide the development of advanced facial recognition algorithms, enhancing their robustness in addressing high-similarity challenges, such as distinguishing between identical twins. While twin studies and face recognition studies have both been growing significantly, surprisingly little research has been conducted into the ability of adult MZ twins to differentiate their faces from those of their co-twins.

An individual's face is a crucial element of their bodily identity, and people generally exhibit a remarkable ability to recognize their own face, exceeding their recognition of other familiar faces. In a groundbreaking study, Martini et al. (2015) used monozygotic twins—who share nearly identical facial features—as a model to investigate self-face processing. The research, using rapid stimuli presentation, assessed how well these twins could differentiate their own face from that of their co-twin and a highly familiar individual. The participants were recruited from the National Twins' Register of Istituto Superiore di Sanità of Rome. The results revealed that the MZ twins failed to discriminate the self-face from their co-twin's face, suggesting that the perceived physical similarity between twins overrides the potential advantage of self-recognition.

Sæther and Laeng (2008) conducted a comparative study with parents of monozygotic twins and those of non-twin siblings (the control group). The remarkable findings showed that when exposed to familiar and unfamiliar twin faces, the parents of twins recognized their own children faster than the control parents. However, they were slower in distinguishing unfamiliar twin faces, indicating that their recognition abilities are more attuned to their own offspring.

Facial recognition and facial identification in psychology and technology

Facial recognition and identification are related but distinct processes often used in psychology and technology. Facial recognition involves detecting and analyzing a face in an image or video to determine if it matches any faces stored in a database. This process is commonly used to verify identity. For instance, a security system identifies authorized personnel by recognizing their faces. It works by detecting the presence of a face in an image or video, extracting features such as interocular distance, nose shape, and jawline, and comparing these features to a database to find potential matches. On the other hand, facial identification is a more specific process that confirms an individual´s identity based on unique facial characteristics. This method is used to establish who a person is, often more reliably than facial recognition. For example, law enforcement might identify a suspect by comparing a photograph to a database of mugshots. This process focuses on analyzing unique facial traits and matching them against a known identity, often for legal or forensic purposes. In summary, facial recognition is a general process that detects and identifies a face from many possibilities (e.g., "This is a face I recognize"), while facial identification accurately determines an individual´s identity (e.g., "This face belongs to John Doe"). Both processes play essential roles in applications such as biometric security, forensic analysis, and the study of social and psychological aspects of human interaction.

Consider the following analogy to understand better how a facial recognition system processes and identifies faces. Imagine that a facial recognition system is a librarian in a large library. When you enter, your face is like the cover of a book—it is the first thing the librarian notices to identify you. The librarian has access to an extensive catalog of book covers (photos of faces) and their corresponding details (names, IDs, etc.). This catalog represents the system's database. Rather than memorizing every detail of the book cover, the librarian focuses on key features, such as the title's font, colors, or design elements. Similarly, a facial recognition system extracts unique facial characteristics, such as interocular distance, the curve of your lips, or the shape of your jawline. When comparing your face, the librarian searches through the catalog for a match. Similarly, the system analyzes your facial features and compares them against its database. If the librarian finds a match, your identity is confirmed. The

system also identifies you by verifying if your face corresponds to an entry in its database. However, errors can occur. The librarian might mistake one book cover for another if they appear too similar or fail to identify a book that is not listed in the catalog. Likewise, facial recognition systems may misidentify individuals (false positives) or fail to recognize them (false negatives). By addressing these challenges, this study provides insights into how facial recognition technologies can evolve to better distinguish between monozygotic twins, leveraging subtle differences in their facial features to enhance identification accuracy.

New approaches to facial recognition and identification in twins using artificial intelligence (AI)

Advancements in Artificial Intelligence (AI) have paved the way for innovative research in facial recognition and identification, especially among twins. A study led by the first author explored novel methods and technologies to distinguish between MZ twins using AI algorithms (Crosato et al., 2024). The research employed a streamlined automation process to facilitate the comparative identification of the faces of MZ twins registered in the Painel USP de Gêmeos (USP Twin Panel). This process combined image segmentation, feature extraction, and machine learning techniques. The study analyzed images of 50 pairs of MZ twins, using Python 3.10 and the Google Colab platform (version 3.7.13), along with libraries such as NumPy, Matplotlib, and OpenCV (CV2). During the initial image preparation phase, all images were standardized to grayscale, with brightness and contrast adjusted to optimize quality for analysis.

AI-based recognition was achieved by visualizing color-coded lines, which highlighted specific similarities and differences between images, even with variations in the twins' facial expressions (Figure 9.3). These lines represent key facial aspects and structural patterns, such as jawline shape, interocular spacing, and nasal and labial contours, which are often difficult to discern with the naked eye. By analyzing these intricate details, the AI system demonstrated its ability to go beyond superficial recognition, revealing the subtle, unique facial features that differentiate identical twins.

The proposed AI program presents the primary image alongside comparative images, highlighting the degree of similarity between them. Photographs are displayed side by side, with matching colorations indicating shared facial

Figure 9.3 AI-powered recognition relies on identifying and analyzing patterns of colored lines in images, enabling it to recognize similarities even when facial expressions differ.

features. Using a dataset of 50 pairs of twin images, the program demonstrated an average similarity of 90.3% when comparing images of the same individual taken at different times. By contrast, the image similarity of different individuals averaged only 10.8%.

This marked differentiation underscores the program's effectiveness in distinguishing between individuals, even in the challenging context of identical twins. The use of color-coded visual cues not only enhances the interpretability of the results but also provides a practical tool for accurately identifying subtle and unique features.

This approach highlights the ability of AI to address one of the most challenging tasks in facial recognition technology—distinguishing between individuals with near-identical facial features. By employing advanced techniques such as feature mapping and machine learning, the system can process and analyze high-dimensional data far more efficiently and accurately than traditional methods.

Conclusion

The findings presented in this chapter emphasize the significant potential of AI-based facial recognition methodologies for practical applications across

diverse fields. In forensics, where precise identification is paramount, these advancements offer a powerful tool for distinguishing between individuals, even in the case of monozygotic (MZ) twins. Similarly, in healthcare, facial biometrics could enhance personalized diagnostics by identifying subtle and unique traits relevant to patient care. Furthermore, this technology is promising for refining biometric systems used in security and access control, providing improved reliability and minimizing false positives. By addressing the challenges inherent in complex identification scenarios, this innovative methodology establishes a robust framework for the continued advancement of AI-driven tools.

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PEER COMMENTARY

Mariana Minatel Braga Fraga

As the coordinator of the discussion section for Part II: 'Dentofacial Development of Twins' in this book, I organized and compiled the insightful questions posed to the authors during the Twin Studies in Behavioral and Health Sciences event. This event facilitated a rich exchange of ideas among leading experts. By incorporating these questions into this section, we aim to enhance the reader's understanding of the topics addressed. These questions provide valuable context and encourage deeper engagement with the chapters. They illuminate the nuances of research, highlight methodological complexities, and explore the broader implications of the findings. By showcasing key themes and issues raised during the event, this section seeks to stimulate reflection, critical thinking, and ongoing dialogue within the evolving field of twin studies related to dentofacial development.

Question 1: How was zygosity measured? The first question was addressed to Dr. Julia and Professor Marina, with additional insights contributed by Professor Emma for this section. (Audience Question)

Answer: *Information regarding zygosity was initially obtained from the Hospital das Clínicas database. Additionally, we utilized a questionnaire to gather parents' and children's perceptions of similarity. The classification of zygosity was based on a combination of data from the hospital records and responses from the questionnaire, ensuring a more comprehensive assessment. (Julia Gomes Freitas) Answer: This is indeed a very important question and a common challenge in twin*

studies. In our study, we did not perform DNA analysis to determine zygosity. Instead, we relied on a parent-reported questionnaire. While we acknowledge that this is not the ideal method, we are currently using a validated questionnaire developed by Dr. Emma, which consists of four questions related to the children's appearance. This method has proven effective for zygosity determination in similar studies (Marina de Deus Moura de Lima).

Answer: *Odontological treatment is being provided to school-aged twins born at the Hospital das Clínicas of the Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), whose mothers received antenatal care at the Twin Obstetric Clinic. Information from the medical records of the newborns, particularly on chorionicity and amnionicity, provided valuable clues about zygosity. Chorionicity refers to the number of placentas present in a twin pregnancy, while amnionicity pertains to the number of amniotic sacs surrounding the twins. These factors are key to understanding zygosity and are classified into the following combinations: two placentas and two amniotic sacs (DCDA = Dichorionic-Diamniotic), one placenta and two amniotic sacs (MCDA = Monochorionic-Diamniotic), one placenta and one amniotic sac (MCMA = Monochorionic-Monoamniotic). MCDA twins, resulting from embryo splitting between days 4–8 post-fertilization, and MCMA twins, arising from splitting between days 8–13, are always monozygotic. DCDA twins can be either monozygotic if the embryo splits within the first 3 days after fertilization, or dizygotic. Notably, while all dizygotic twins are DCDA, not all DCDA twins are dizygotic. Dr. Julia also mentioned the Brazilian version of a validated 4-item questionnaire, originally designed for adults and compared against DNA-based zygosity classification, that was adapted for use with parents of twin children and the children themselves. In adults, the questionnaire demonstrates 96.7% agreement with DNA analysis, highlighting its reliability (see Varella et al., 2023). Currently, DNA analysis from buccal swabs is being completed for twin children at the Laboratório de Genética e Biologia Molecular (Laboratory of Genetics and Molecular Biology), LIM40-HCFMUSP, under the coordination of Professor Cintia Fridman. This will enable Dr. Julia and her team of pediatric dentists to compare zygosity classifications across various data sources. (Emma Otta)*

Question 2: It was Dr. Julia's turn to answer the next question. Thanks for the fascinating talks. Did I understand correctly that you found a large shared

environmental influence on children's dental anxiety? Is that right?(Audience Question)

Answer: *Indeed, that is correct. In our analysis of the anxiety data, we considered both genetic and environmental influences. However, our findings indicated a stronger impact from environmental factors. As the project is still ongoing, we have not yet fully explored or discussed these findings in depth. Nonetheless, we hypothesize that the environment plays a more significant role, particularly due to the contexts in which children are raised. (Julia Gomes Freitas)*

Question 3: Dr. Julia was also asked the following question. Did you have access to the reasons why children feared going to the dentist? (Audience Question)

Answer: *No, we did not have access to the underlying reasons for children's dental fear and anxiety before their appointments. The questionnaire, however, provided insights into specific situations that triggered fear or anxiety during the appointment itself, such as tooth extractions. Unfortunately, we lack data on the historical or root causes of their fears. (Julia Gomes Freitas)*

Question 4: The following question about facial recognition was addressed to Dr. Laura, a representative of Professor Edgar's team. Could you explain how the system captures and analyzes different facial expressions? For example, does it compare 50 images of the same individual displaying various expressions, such as smiling or looking serious, with their twin's expressions? (Audience Question)

Answer: *Yes, the system compares multiple images—about 50—from both individuals. These images are used to analyze and measure how facial features change across different expressions. The algorithm for this analysis was developed by Professor Edgar. (Laura Regina Antunes Pontes)*

Question 5: Continuing the discussion with Dr. Laura, the following question was posed. Does the system account for conditions like facial palsy, where certain parts of the face may have limited movement? (Audience Question)

Answer: *That is a great point. While the system is robust, conditions like facial palsy might require additional considerations. The algorithm primarily focuses on dynamic changes in facial expressions. For such cases, further adjustments might be necessary. (Laura Regina Antunes Pontes)*

Question 6: It was Professor Marina's turn to answer another question. Is there any way to prevent MIH? (Audience Question)

Answer: *That's an excellent and complex question. Currently, there is no known way to prevent Molar Incisor Hypomineralization (MIH). Early diagnosis is critical for minimizing tooth damage. Most studies on MIH are cross-sectional, making it challenging to identify specific environmental factors. Current research points to potential contributors from pre-birth, birth, and post-birth factors, but the exact causes remain unclear. Addressing MIH is a significant challenge for dentists, and further research is essential to better understand and manage this condition. (Marina de Deus Moura de Lima)*

Question 7: Another question was directed to Professor Marina. Have you come across any animal studies investigating MIH? For example, could genetic therapies, potentially involving the use of viruses to introduce new genes, be explored? (Audience Question)

Answer: *MIH was first described in 2008, so research in this area is still relatively new. Some animal studies, primarily using rats, have investigated the effects of bisphenol A (BPA), but the results have been inconsistent. Currently, no studies have explored genetic therapies as a potential treatment for MIH.*

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PART III

RESEARCH WITH TWINS ON PSYCHOPHYSIOLOGICAL CHARACTERISTICS

Part III explores twin research from a psychobiological standpoint, underscoring the unique insights twins provide into human development and individual differences. This section showcases studies on psychophysiological traits, such as the bioacoustic analysis of newborn crying and the links between sleep and mental health. It highlights diverse methodologies, including longitudinal research on sleep and psychopathology, which sheds light on phenotypic variability. The section concludes with a fascinating case study of Brazilian twins reared apart from birth, illustrating the dynamic interplay of genetic and environmental influences.

10. BIOACOUSTIC ANALYSIS OF NEWBORN CRYING

Patrícia Ferreira Monticelli, Adriana Sicuto de Oliveira

Abstract This chapter explores crying as a universal behavior in vertebrates, including humans, which signals discomfort, fear, or separation to elicit caregiving. Nonhuman infant vocalizations, often termed distress or isolation calls, exhibit conserved behavior across species, including sound structure and neural processing. Mammalian infant cries typically exhibit specific frequency modulation patterns—chevron, flat, or descending—reflecting their ancestral origins. In humans, cries initially signal needs such as hunger, pain, or fear that evolve with age as vocal control matures. Despite sharing similarities with other species, human cries are distinctive in their developmental trajectory and integration with speech precursors. Research on crying provides valuable insights into its ecological, psychological, and healthcare implications.

Keywords: Crying behavior, Human cry development, Psychological insights into crying, Comparative bioacoustics, Infant communication evolution, Nonhuman and human cries

One might think of a cry as a human infant's vocalization when hungry, tired, scared, or seeking maternal attention. While that is accurate, it is incomplete: nonhuman infants also cry (Newman, 2004), even without shedding tears. Vocalizing when separated from the mother (the primary source of attachment and nourishment in mammals) or when facing danger is an ancestral

behavior. Although named "distress calls" or "isolation calls" (ICs) in other species depending on the context of emission, caregiver sound structure and elicited responses are very similar, indicating that the production and processing of crying are highly conserved in vertebrates (Newman, 2004; Lingle et al., 2012). For instance, precocial birds, capable of independent movement and foraging shortly after hatching, also emit these calls in this situation to successfully attract the attention of their caretaker (Newman, 2004; Lingle & Riede, 2012). Lingle et al. argued that the differences between isolation or distress calls across vertebrate species are more influenced by arousal differences than discrete functions.

In mammals, Newman (2007) described an integrated mammalian crying system, also citing preserved elements of production, perception, and neural processing. Starting with sound structure, infants from 20 primate species, such as prosimians, Old World and New World monkeys, great apes, and humans, produce ICs that consist of voiced notes with little or no noise, have a rich harmonic structure, and are generally uttered in series corresponding to expiratory phases (Newman, 1992; Newman, 2004). Figure 10.1 shows visual similarities in spectrograms of different mammalian species.

In most of the infant distress vocalizations analyzed by Lingle et al. (2012),

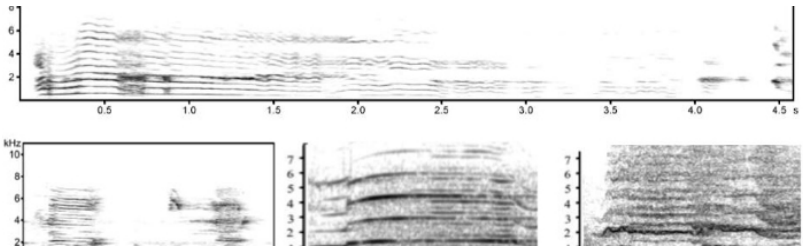

Figure 10.1 Spectrograms of mammalian isolation or distress calls. Human baby cry (above), goat infant recorded on the internet (below left), and capybara (middle) and otter adults recorded at zoos. The images were produced in Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany) and Raven Pro 1.6.5 (Cornell Lab of Ornithology, USA).

the authors identified three frequency modulation patterns (the melody) that are also described in human infant cries, appearing individually or together in a note: chevron (rising then falling), flat (frequency change less than 10% of the mean fundamental frequency, F0), or descending shape. Várallyay et al. (2007) found that in human infants, the cry melody (the F0 contour) typically

starts with a rising or flat unit and ends with a falling or flat unit. They suggest this pattern is due to the tension of the vocal cords before (transitioning from a static condition) and after sound production, which may be similar in other mammalian infants.

In addition to the sound structure, evidence from mammals supports the hypothesis of the ancestral origin of crying in communicative function and elicited responses (behavioral, neural, and neurochemical) (Newman, 2004; Lingle et al., 2012). Susan Lingle demonstrated that wild deer in Canada respond to the distress calls of various mammalian species, such as marmots, fur seals, and humans, and by rushing protectively toward the calling infant (Lingle & Riede, 2014). Lingle and Riede (2014) proposed the commonality of vertebrate infant vocalizations that attract the attention of caregivers or other family members in different contexts.

The Human Infant Cry

The human infant cry has been extensively investigated by scientists across various fields, such as medicine, linguistics, and psychology. While displaying numerous similarities with other species, some features are unique to the human infant cry. During the first year of life, crying is initially triggered by hunger, pain, micturition (which may cause discomfort), and noise (which is believed to frighten infants) (Lockhart-Bouron Anikin et al., 2023; Soltis, 2004; Cornec et al., 2024), as well as by parental absence. After the newborn phase, crying can also be prompted by boredom, fatigue, mechanical provocation, or fear (Rothgänger, 2003). Whether cries uttered in each context are consistent is still debated in the literature. Most researchers distinguish between cries of hunger and pain; some also identify cries of pleasure, while others recognize only pain and non-pain cries or classify all cries after the newborn period as discomfort cries (Rothgänger, 2003). Green et al. (1998) described motivational changes in hunger cry notes over the course of a crying bout, comparing the structure of five cries from the beginning and five cries two minutes later. The later cries exhibited lower mean and maximum peak frequencies, less energy in the higher frequency ranges, and were consistently shorter.

Cry structure evolves with age but never develops into articulated sounds such as cooing, babbling, or words (Oller et al., 2013, 2019). During the first

two months, babies primarily cry and produce other 'protophones' functionally flexible, non-cry vocal precursors to speech. As described by Oller et al. (2019), these protopones are the earliest vocal precursors to speech, emitted even by premature newborns in neonatal intensive care, and are more prevalent than cries (Oller et al., 2013, 2019). These sounds include vocants, squeals, and growls (Oller et al., 2019).

Crying has a typical fundamental frequency (F0) that remains consistent between 404 and 464 Hz during the first 18 months of life (Benyo et al., 2007). However, the duration of cries varies over these 18 months: 95% of cries last between 0 and 2 seconds, with the average increasing from approximately 0.8 to 1.0 seconds after the first two months of life. During this period, infants cry more spontaneously, and the melody is shaped by the source part of the vocal tract (larynx). As the supralaryngeal mechanisms mature, cries become more controlled, and consonant- and vowel-like elements emerge and appear (Wermke et al., 2021). At between 3 and 5 months of age, a baby's cries exhibit the effects of laryngeal constrictions, a regular phenomenon in healthy, normally developing infants that also occurs during crying, cooing, and marginal babbling. This may represent infants' early vocal exploration (Robb et al., 2020).

Cry F0 contour variability increases primarily after the first two months of life. Várallyay et al. (2007) identified the twenty most frequent types of cry melodies from birth to 18 months. They analyzed 2,640 cry melodies, assigning numerical values to three basic units: falling (-1), flat (0), and rising (1). They found 77 different sequence patterns, with 95% occurring in only 20 combinations. A cry typically begins with a rising or flat unit and ends with a

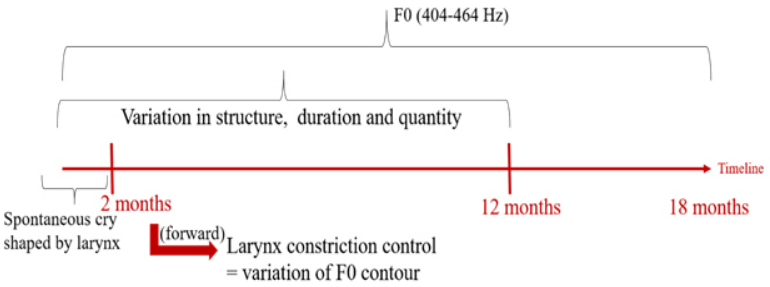

Figure 10.2 Summary of key cry features observed in human infants over the first 18 months of life.

falling or flat unit, which the authors attribute to vocal cord tension in static conditions. Figure 10.2 illustrates and summarizes some cry features across the first 18 months of human infants.

Conclusion

In summary, vocalizations that elicit the attention of caregivers and other family members, emitted over a range of discomfort feelings such as hunger, separation, fear, pain, or even threat, may be categorized differently across different species and contexts. However, cries, distress calls, and isolation calls are similar in sound structure, neural processing, elicited responses, and communicative function across multiple vertebrate species. This commonality, which is even more pronounced in mammals, reinforces the shared origin of infant vocalizations and challenges the premise that crying is an exclusively human vocal behavior. Based on bioacoustics studies on unique and shared human cry features, scientists are generating and disseminating knowledge pertinent to health, ecology, and psychology.

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11. TWIN STUDIES ON SLEEP

Stephania Ruth Basilio Silva Gomes, Mario Andre Leocadio Miguel, and John Fontenelle Araújo

Abstract This chapter explores heritability, which refers to the proportion of phenotypic variation attributable to genetic factors. Comparing the similarities between monozygotic and dizygotic twins provides valuable insights into the relative contributions of genetics and environment. Sleep traits, such as chronotype, duration, and quality, are moderately heritable, underscoring the substantial influence of genetic factors. For example, chronotype heritability estimates range from 0.32 to 0.55, and sleep duration between 0.31 and 0.46. Anxiety and depression also have genetic components, with heritability estimates of 0.48 and 0.31, respectively. Notably, poor sleep quality is associated with increased symptoms of anxiety and depression, emphasizing the intricate interplay between genetics, sleep, and psychological well-being.

Keywords: Heritability, Genetics and environment, Sleep traits, Chronotype, Sleep duration, Sleep quality, Anxiety, Depression, Sleep and psychological health, Heritability of sleep traits

Understanding heritability and the importance of twin studies

Heritability quantifies the proportion of variation in a trait or characteristic in a population that can be attributed to genetic differences between individuals (Falconer, 1989). Broad-sense heritability (H^2) refers to the total proportion of variation in a trait or characteristic in a population that is attrib-

utable to genetic differences between individuals, including both the additive genetic effects and genetic interaction. On the other hand, narrow-sense heritability (h^2) refers to the proportion of variation in a trait that is due solely to additive genetic differences between individuals (Visscher et al., 2008; Furlotte et al., 2014).

Comparing the similarity between monozygotic (MZ) and dizygotic (DZ) twins in classic twin studies provides an initial estimate for the relative contribution of genetic and environmental factors to a specific trait (Verweij et al., 2012; Sahu & Prasuna, 2016). MZ twins exhibiting greater concordance than their DZ counterparts for a specific trait indicates greater genetic contribution. This difference in agreement between the two twin types is used to calculate phenotypic heritability (h^2) (Boomsma et al., 2002). Thus, the use of this analysis led to important changes in the understanding of disease determinants, highlighting the importance of the interaction between genetics and environment in determining the phenotype and providing valuable insights for disease prevention, diagnosis, and treatment.

Heritability of Sleep Traits

In recent decades, studies have contributed to the understanding of genetic and environmental factors that influence various sleep characteristics. In this sense, studies on chronotype, a person's individual preference for specific activity times and sleep throughout the day, have played a crucial role. Population-based studies, such as that carried out in Baependi, Brazil, show the significant influence of genes on chronotype expression, with a mean MEQ questionnaire score of 63.5 and heritability, adjusted for sex and age, of 0.48 (Von Schantz et al., 2015). More recent research, such as the study conducted by Leocadio-Miguel et al. (2021), identified heritability values of 0.37 for MEQ, 0.32 for MCTQ, and 0.28 for a single-question chronotype evaluation, reinforcing the genetic basis of this behavioral trait. Furthermore, large-scale genomic analyses, involving almost 700,000 participants, identified 351 loci associated with morning preference, further highlighting the influence of genes (Jones et al., 2018).

Research on the heritability of sleep duration and quality reveals a significant influence of genetic and environmental factors. Madrid-Valero et al.

(2020) found an average heritability of 0.31 for subjective sleep quality and 0.38 for sleep duration. Klei et al. (2005) highlight the statistical significance of heritability for subjective sleep measures, including time, duration, and quality, ranging from 12.4% to 29.4%. Furthermore, Kocevska et al. (2021) reported that heritability estimates for sleep duration ranged from 42% to 45% in adulthood. Finally, SNP-based heritability for short and long sleep was estimated at 11.9% and 7.8%, respectively, as observed by Austin-Zimmerman et al. (2023), showing a modest but significant genetic influence on sleep duration.

Heritability of anxiety and depression symptoms

Heritability estimates of major depression and anxiety are approximately 67% and 49%, respectively, with a tendency to be higher among women (Feigon et al., 2001; Kendler et al., 2006; Ask et al., 2014; Guffanti et al., 2016). Furthermore, a study of a mixed-race Brazilian population showed a 66% overlap in genetic variation associated with the symptoms of these disorders (Taporoski et al., 2015). These figures reflect the importance of genetic factors in the predisposition to these psychological disorders. Twin studies reveal that genetic and environmental correlation varies between depression and different types of anxiety disorders, showing varying proportions of shared genetic factors than shared and nonshared environmental factors (Kendler et al., 1992; Kendler et al., 1993; Mosing et al., 2009). These findings highlight the complexity of the interaction between genetic and environmental factors in the manifestation of anxiety and depression symptoms.

Association between sleep and depressive and anxious symptoms

In contemporary society, inadequate sleep habits are prevalent, disrupting both the quantity and regularity of sleep, significantly influencing the development of depression and anxiety (Salgado-Delgado et al., 2011; Coles et al., 2015). Individuals who exhibit the evening chronotype, that is, those who naturally prefer later sleep and wake times, often struggle to maintain an adequate sleep routine due to conflicts with established social standards (Roepke & Duffy, 2010; Kabrita et al., 2014). As a result, there is often a mismatch between

sleeping and waking times, especially between working and non-working days, leading to a chronic discrepancy between the individual's biological clock and social demands, a phenomenon known as Social Jetlag (SJL) (Roenneberg et al., 2019). One of the consequences of this maladjustment is reduced weekday sleep duration due to early awakening, which, in the long term, can result in chronic sleep deprivation (Taillard et al., 2021).

Adults with an evening chronotype tend to experience more severe depressive and anxiety symptoms compared to those with a morning or intermediate chronotype (Walsh et al., 2022). On the other hand, Social Jetlag (SJL) is associated with depressed mood regardless of sleep deficit, representing a risk factor for the development of depressive symptoms in people experiencing SJL for more than 2 hours (Feng et al., 2023; Im et al., 2023). With respect to sleep duration, both short and long periods are linked to a greater risk of depression and anxiety, compared to the recommended range of 7 to 9 hours of sleep (Zhai et al., 2015; Zhou et al., 2020; Chunnan et al., 2022). Furthermore, both short and long sleep duration are associated with poor sleep quality in people with depression, whereas in anxious individuals, worse sleep quality is more related to short periods of sleep (Müller et al., 2017; Shim & Kang, 2017; Chan et al., 2022).

Connect Twins Project

The Connect Twins Project is a core initiative in a doctoral study conducted by the Postgraduate Program in Psychobiology at the Federal University of Rio Grande do Norte (UFRN), in collaboration with the Twins Panel at the University of São Paulo (Painel USP de Gêmeos). Its main objective is to investigate the lifestyle habits associated with symptoms of anxiety and depression among Brazilian twins, thereby expanding knowledge in these areas.

Objectives

Given the association between inadequate sleep habits and the emergence of psychological disorders, this chapter aims to describe the heritability of sleep-related variables (chronotype, social jetlag, sleep duration, and quality) and symptoms of anxiety and depression. Furthermore, it evaluates how these

characteristics are associated with one another and investigates whether the studied sleep traits differ in terms of the presence or absence of anxiety and depression symptoms in a sample of Brazilian twins, using data from the Connect Twins Project.

Methodology

Participants and recruitment

Participants registered in the Twins Panel at the University of São Paulo (USP) were recruited online and invited to complete a series of questionnaires using Google Forms. Twins not registered in the USP Twin Panel were recruited through social media using a similar procedure to expand our sample nationally. Eligibility criteria included being at least 18 years old, and both siblings completing the questionnaires individually.

Instruments

To assess symptoms of depression and anxiety, we used the Hospital Anxiety and Depression Scale (HADS), developed by Zigmond and Snaith (1983). For both subscales (anxiety: HADS-A; depression: HADS-D), scores less than or equal to 7 represent the absence of symptoms, and 8-21 the occurrence of mild to severe cases (Stern, 2014). Chronotype was determined using the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2004), and we also applied the Morningness-Eveningness Questionnaire (MEQ) (Horne & Ostberg, 1976). Social Jetlag (SJL), sleep duration during working (Sd-wd), and non-working days (Sd-fd) were obtained from MCTQ questions. To assess sleep quality, we used the Pittsburgh Sleep Quality Index (PSQI) (Bertolazi et al., 2011). Twin pairs were classified as MZ and DZ using the questionnaire-based assessment of twin zygosity, validated by Christiansen et al. (2003).

Data analysis

The data were submitted to descriptive statistical analysis, evaluating measures of central tendency and standard deviation for quantitative variables.

Data normality was assessed using the Shapiro-Wilk test. We also applied the Spearman and Pearson correlation test to assess the associations between the variables of interest. Finally, the independent t-test was used to analyze possible differences in the mean MEC, MCTQ, Sd-wd, Sd-fd, HADS-A, and HADS-D scores concerning the groups with and without anxiety symptoms, as well as between the groups with and without depressive symptoms. Mann-Whitney U tests were also performed to verify possible differences in the SJL and PSQI scores between these groups. The heritability of depression and anxiety symptoms, sleep quality, sleep duration, chronotype, and social jetlag was assessed using the maximum likelihood estimation method, with an approach based on variance components, using the SOLAR Eclipse software version 8.4.2 (Almasy & Blangero, 1998), which uses sequential oligogenic linkage analysis routines. The model was adjusted for each trait to include age and sex as fixed effects.

Results

Participants

Sixty-one twins participated in the research, comprising 29 pairs and one trio, distributed across the five Brazilian geographic regions, with a predominance in the Northeast (58.3%) (Fig. 11.1). Among these 61 participants, 80.32% and 19.67% were monozygotic (MZ) and dizygotic (DZ) twins, re-

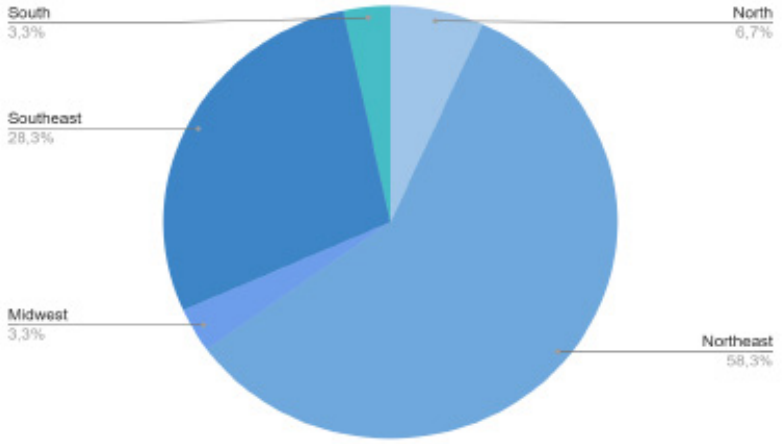

Figure 11.1 Distribution of participants by region of Brazil (%)

spectively. The majority of participants were female (72.12%), with ages ranging from 18 to 66 years (M = 30.5, SD = 11).

The participants´ MEC scores varied from 25 to 72 (M = 54.1, SD = 10.7). MCTQ scores ranged from 53.6 to 409 (M = 227, SD = 77.9), and SJL scores in minutes from 0 to 315 (M = 92.3, SD = 61.5). PSQI scores ranged from 4 to 17 (M = 8.69, SD = 3.05), HADS scores from 2 to 19 for anxiety (M = 9.08, SD = 4.07), and 0 to 15 for depression (M = 6.48, SD = 3.91). Sleep duration on working days ranged from 270 to 720 minutes (M = 459, equivalent to 7 hours and 39 minutes; SD = 94.6), while on non-working days, it ranged from 315 to 690 minutes (M = 526, corresponding to 8 hours and 46 minutes; SD = 82.1).

A significant heritability was identified in the chronotype measures obtained from the MCTQ score (h^2=0.55), but not for the MEC score (h^2=0.21). Heritability was significant in both sleep duration on non-working days (h^2=0.59) and in anxiety (h^2=0.48) and depressive symptoms (h^2=0.31) (Table 11.1). Additionally, MEC, SJL, and HANDS-D showed significant effects of both sex and age, while MCTQ exhibited a significant effect of sex.

Table 11.1 Narrow-sense heritability (h2) estimates for the trait explored in this study

Trait	$h^2 \pm SE$	*p-value*	*p* Value of Covariates	Variance Explained by Final Covariates
MEQ score	0.21 ± 0.16	0.0939	Age (0.0678) Sex (0.07)	0.1292
MCTQ score	0.55 ± 0.12	0.0008	Sex (0.0088)	0.1260
SJL	0.03 ± 0.23	0.4329	Age (0.0818) Sex (0.0177)	0.1588
PSQI score	0.28 ± 0.17	0.0639		
Sd-wd	0.16 ± 0.20	0.2185		
Sd-fd	0.59 ± 0.13	0.0003		
HADS-A score	0.48 ± 0.15	0.0048		
HADS-D score	0.31 ± 0.16	0.0421	Age (0.0151) Age*Sex (0.0130)	0.0979

Abbreviations: SE=standard error; MEQ=Morningness-Eveningness Questionnaire; MCTQ=Munich Chronotype Questionnaire; SJL=Social Jetlag; PSQI=Pittsburgh Sleep Quality Index; Sd_wd=Sleep duration on working days; Sd_fd=Sleep duration on non-working days; HADS-A=Anxiety score on the Hospital Anxiety and Depression Scale; HADS-D=Depression score on the Hospital Anxiety and Depression Scale.

A significant positive correlation was found between the HADS-A and HADS-D scores (r=0.542; p= < 0.001), PSQI and HADS-A (r=0.483; p=< 0.001), and PSQI and HADS-D (r=0.494; p=< 0.001). A significant positive correlation was also observed between sleep duration on non-working and working days (r=0.376; p=0.003), and a negative correlation between MEQ and MCTQ scores (r=-0.575; p= < 0.001).

Significant differences were found in HADS-A scores between groups with and without depressive symptoms (t(59)=–3.91,p<.001), with the former exhibiting a higher mean (M = 11.28, SD = 3.85) compared to the latter (M = 7.55, SD = 3.50). Similarly, statistically significant differences were found in HADS-D scores between groups with and without anxiety symptoms (t(59)=–4.21,p<.001), with the former exhibiting a higher mean score (M = 8.03, SD = 8.00) compared to the latter (M = 4.24, SD = 3.00).

Furthermore, significant differences in PSQI scores were observed between groups with and without depressive symptoms (U=185, p<.001) and between those with and without anxiety symptoms (U=262, p=.005). Participants with depressive symptoms exhibited higher PSQI scores (M = 10.48, SD = 2.78), as did those with anxiety symptoms (M = 9.60, SD = 9.50).

We also found significant differences in average working-day sleep duration (Sd-wd) between groups with and without depressive symptoms (t(59)=–2.35, p=.02). Participants with depressive symptoms exhibited a longer sleep duration (M = 492 minutes, SD = 100.7) compared to those without (M = 436 minutes, SD = 83.9).

Discussion

Previous studies reported higher heritability for the MEQ score than that observed in the present study, ranging between 0.37 and 0.52 (Barclay et al., 2010; von Schantz et al., 2015; Toomey et al., 2015; Leocadio-Miguel et al., 2021). The heritability of the MCTQ score was higher than that found in a prior study that used this instrument to evaluate chronotype ($h^2 = 0.32$), although both studies exhibited significant genetic variance (Leocadio-Miguel et al., 2021). These discrepancies may be attributed to our relatively small sample size.

No studies are currently investigating sleep duration heritability on non-working days. However, research conducted in different countries has fo-

cused on self-reported sleep duration during a typical week and on a single night. These studies showed that overall sleep duration heritability is estimated at 46%, based on a meta-analysis conducted by Kocevska et al. (2021). In the present study, it was found that non-working day sleep duration (Sd-fd) exhibited high and significant heritability, suggesting less influence of the shared environmental component in Sd-fd, such as social pressures on sleeping and waking times during a working day.

The estimated heritability for anxiety (0.48) and depression (0.31) in this study is consistent with previous findings. For example, a family cohort study found heritability estimates of 0.30 and 0.32 for depressive and anxiety symptoms, respectively, in sex and age-adjusted models (Taparoski et al., 2015). Moreover, other research revealed a higher heritability of 0.67 for depression, 0.49 for anxiety, and 0.53 for comorbid depression and anxiety (Guffani et al., 2016). These findings suggest that both anxiety and depression are significantly influenced by genetic and environmental factors.

This study found a strong correlation between anxiety and depressive symptoms (r=0.54). It was observed that individuals with anxiety symptoms tended to obtain higher scores on the HADS-D, while those with depressive symptoms exhibited higher scores on the HADS-A, suggesting that symptoms worsen under these conditions. Other studies corroborate these findings, showing a significant association between anxiety and depression and their coexistence (Haung et al., 2004; Knowles & Olatunji, 2020). Additionally, Jacobson and Newman (2017) conducted a meta-analysis, and found that anxiety symptoms can predict the development of future depressive symptoms, and vice versa.

A positive and significant association was also found between sleep quality and both anxiety (r=0.48) and depression (r=0.49). Furthermore, higher PSQI scores were observed in individuals with depressive and anxious symptoms compared to their symptoms-free counterparts, demonstrating poorer sleep quality in the presence of these disorders. These findings are corroborated by Gregory et al. (2011), who found moderate associations between sleep quality and anxiety (r=0.39) and depressive (r=0.50) symptoms in young adults. Another study revealed that participants with poor sleep quality were more likely to develop anxiety and depression compared to those who had good quality sleep (Lee et al., 2021). This is reinforced by Teker and Luleci (2018), suggesting

that deterioration in sleep quality was linked to an increase in anxiety, and vice versa.

Longer sleep duration was observed on working days in individuals with depressive symptoms compared to those without these symptoms. No studies have specifically investigated the association between sleep duration on working or non-working days and depressive symptoms. However, the literature reports that both short and long sleep duration are associated with an increased risk of depression (Zhai et al., 2015; Furihata et al., 2015; Dong et al., 2022). A cross-sectional study carried out in rural populations explored the relationship between sleep duration and depressive symptoms, showing that short sleep duration is associated with an increased likelihood of high depressive symptoms (Chang et al., 2011).

Conclusion

The study indicates a strong genetic influence on chronotype, as measured by the MCTQ score, but not the MEQ score. Furthermore, a notable genetic influence was observed for non-working day sleep duration and depressive and anxiety symptoms. A positive correlation was observed between anxiety symptoms, depressive symptoms, and sleep quality, suggesting that individuals with more pronounced anxiety and depressive symptoms tend to experience poorer sleep quality. It was also found that participants with longer non-working day sleep duration also tend to exhibit longer working day sleep patterns. Moreover, individuals with depressive symptoms reported, on average, longer working day sleep duration compared to those without these symptoms. Additionally, those who suffer from both anxiety and depressive symptoms tend to have higher HADS-A and HADS-D scores, indicating greater symptom severity. Finally, individuals with depressive and anxiety symptoms also obtained higher PSQI scores, suggesting poorer sleep quality in thesegroups.

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12. LONGITUDINAL ASSOCIATIONS BETWEEN SLEEP AND PSYCHOPATHOLOGY

Alice M. Gregory

Abstract This chapter delves into the intricate relationship between sleep and mental health, emphasizing sleep's essential role in physical and cognitive development. Drawing on longitudinal, epidemiological, and genetically sensitive data, it highlights how sleep disturbances predict future mental health issues, including anxiety and depression. Twin studies reveal significant genetic influences on sleep traits, such as duration and quality. Misunderstandings about heritability persist, complicating public comprehension of genetics. The chapter stresses the importance of clear communication in behavioral genetics research to bridge gaps in understanding and advocates for improved education on genetics and its role in human behavior.

Keywords: Sleep quality, Sleep and mental health, Mental health predictors, Sleep disturbances, Genetically sensitive research, Twin studies, Heritability misconceptions, Behavioral genetics, Public understanding of genetics, Genetics Education

This chapter explores the relationship between sleep and mental health, a central focus of my research for decades. I have studied sleep disturbances and related traits using longitudinal designs, epidemiological samples, and genetically sensitive data. This chapter examines the role of sleep in child devel-

opment, delves into twin studies and their intriguing findings, discusses the connection between sleep and developmental psychopathology, and addresses the public communication of science. Sleep is vital for the growth and repair of our body and brain, as well as for information processing and memory. It facilitates the formation of new brain circuits and the pruning of unnecessary connections. A lack of sleep can impair emotional regulation. The pioneering researcher Allan Rechtschaffen (1971) once said, " If sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made."

However, sleep can also go wrong. According to the International Classification of Sleep Disorders (ICSD-3) of the American Academy of Sleep Medicine (2014), there are six main categories of sleep disorders: insomnia, sleep-related breathing disorders, hypersomnolence, circadian rhythm disorders, parasomnias, and sleep-related movement disorders (Table 12.1). Each category has several subtypes. My research has primarily focused on insomnia and general sleep difficulties, often reported by parents regarding their children or self-reported by adults experiencing poor sleep quality (e.g., Madrid-Valero et al., 2020; Madrid-Valero, Barclay & Gregory, 2024).

Table 12.1 – Main categories of sleep disorders (AASM, 2014)

Category	Brief Description
Insomnia	Persistent sleep difficulty
Sleep Related Breathing Disorder	Abnormal respiration during sleep
Central Disorders of Hypersomnolence	Excessive sleepiness
Circadian Rhythm Sleep-Wake Disorders	Misalignment of sleep-wake propensity and environment
Parasomnias	Physical events/experiences related to sleep
Sleep Related Movement Disorder	Movements that prevent/disrupt sleep

The intricate relationship between sleep and mental health continues to unfold. A decade ago, in collaboration with the late Professor Avi Sadeh, Director of the Laboratory for Children's Sleep-Wake Disorders at Tel Aviv University, we explored this connection in the literature (Gregory & Sadeh, 2016). Our findings revealed a compelling pattern: nearly all mental health conditions are associated with atypical sleep patterns. While the specifics may vary across studies—encompassing aspects such as poor sleep quality, insomnia, or restless leg syndrome—the overarching conclusion remains consistent: most mental

health disorders are intertwined with sleep challenges. Sleep problems may serve as a 'red flag' for the development of future disorders, but they are often overlooked by parents and clinicians, making them an 'invisible risk.'

During my PhD, I explored whether sleep problems could serve as an early indicator of later disorders. In 2005, I analyzed data from 1,037 children who were part of the Dunedin Multidisciplinary Health and Development Study, focusing on participants aged 5, 7, and 9, to investigate the relationship between their sleep problems and anxiety and depression in adulthood at ages 21 and 26 (Gregory et al., 2005). The main finding was that persistent sleep problems in childhood were significant predictors of anxiety disorders, but not depressive disorders, in adulthood (Figure 12.1). Regarding the etiology of the significant association, similar environmental risk factors, such as difficult peer relations and stressful life events, could influence both sleep problems and anxiety. Genes involved in the serotonergic system might also play a role as a potential mechanism underlying the observed association.

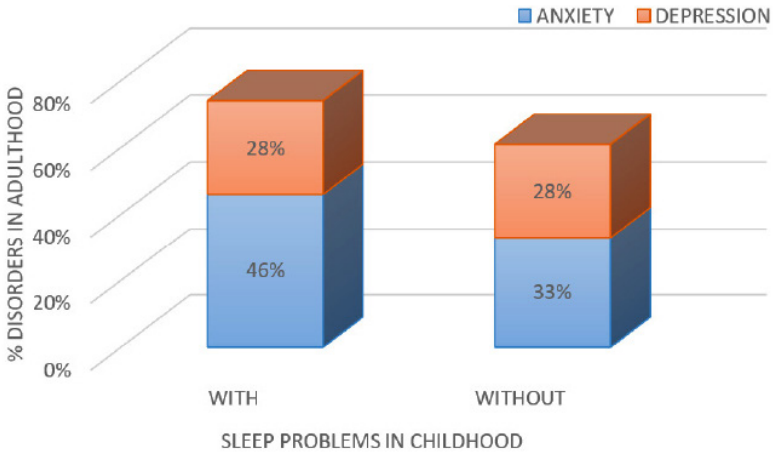

Figure 12.1 Proportion of individuals who developed anxiety and depression problems in adulthood as a function of sleep problems in childhood

A systematic comprehensive review has explored the intricate relationship between sleep disturbances, anxiety, and depression, revealing compelling find-

ings (Alvaro, Roberts, & Harris, 2013). It was discovered that childhood sleep problems are strong predictors of higher levels of depression and a combined depression/anxiety variable, with no evidence of the reverse relationship. Furthermore, the review highlighted that sleep difficulties can forecast future mental health challenges such as anxiety, depression, and even alcohol abuse. The review underscores the need for additional longitudinal studies to deepen our understanding of these complex dynamics.

Over the last decade, there has been an increase in twin studies focusing on sleep characteristics, aiming to unravel the influence of genetic and environmental factors on individual variability. Twin studies involve comparing the similarities between pairs of monozygotic (MZ) twins, who are genetically identical, and dizygotic (DZ) twins, who share, on average, 50% of their segregating genes. The assumption is that environmental influences are similar for MZ and DZ twins. Any greater similarity between MZ twins compared to DZ twins is attributed to genetic factors, which can help estimate additive genetic influences. These studies typically use models to estimate three components: additive genetic influences (A), shared environmental influences (C), and nonshared environmental influences (E). A review of these studies has revealed high heritability estimates for various sleep parameters, including sleep duration and quality, diurnal preference, and insomnia (Madrid-Valero & Gregory, 2023). These findings underscore the significant genetic influence on sleep patterns and challenge us to delve deeper into understanding the genetic factors contributing to our sleep behaviors. However, it is important to interpret the results with caution since most studies have been conducted in Northern Europe and North America. Heritability is defined as the extent to which differences in a trait within a population can be attributed to genetic factors (Plomin, DeFries, McClearn & McGuffin, 2008). A heritability estimate is unique to the population from which it originated and thus varies based on the characteristics of that sample. Further research in diverse populations is needed to fully understand the genetic factors influencing sleep behaviors.

Despite the valuable insights gained from twin studies regarding the impact of genetic and environmental factors on sleep indicators, the concept of heritability remains widely misunderstood, even within the scientific community. Table 12.2 presents common misconceptions about the concept of heritability, highlighting the most prevalent misunderstandings. Studies of heritabil-

ity examine the extent to which genetic factors influence differences in physical, physiological, or behavioral traits (from height to people's sleep parameters and even the size of corn stalks) between individuals within a population (Figure 12.2). This is not the same as asking to what extent genetic factors influence attributes in any one individual. The distinction between population-level heritability and individual traits is essential.

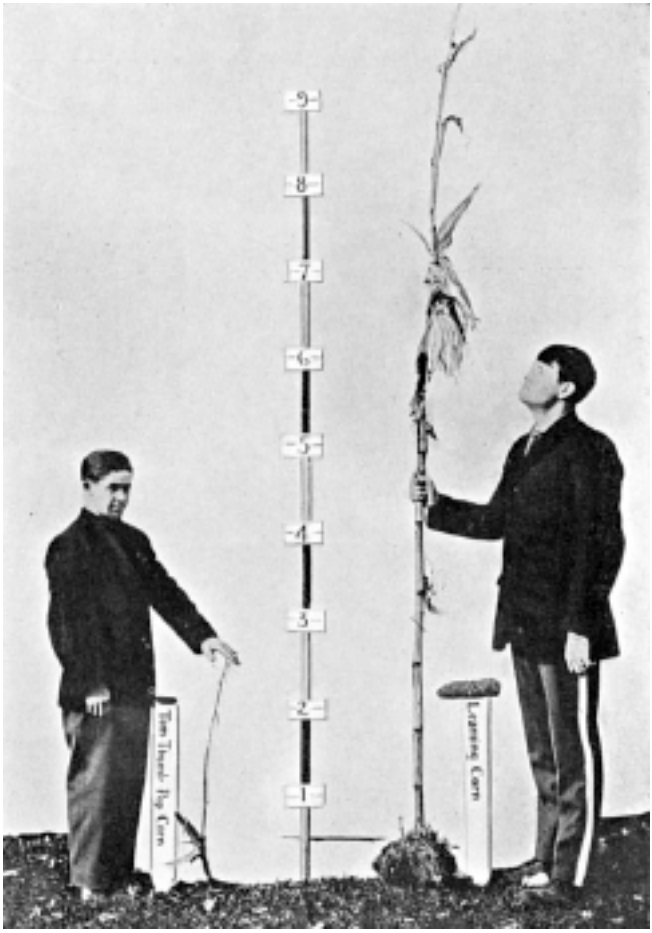

Figure 12.2 Two boys, one short and one tall, each holding a stalk of corn — one short, the other tall (Creative Commons CC0 License).

Table 11.2 Common misconceptions about the concept of heritability (Source: Visscher,

Misconception	Why the concept is incorrect
• Heritability is the proportion of a phenotype that is passed on to the next generation	• Genes are passed on, not phenotypes.
• High heritability means genetic determination	• Even with a strong genetic influence, environmental factors continue to significantly shape an individual's traits.
• Low heritability implies no additive genetic variance	• A low heritability implies that only a small portion of the observed variation is caused by differences in genotypes, but it doesn't necessarily mean that the additive genetic variance is small.
• Heritability is informative about the nature of between-group differences	• Heritability should not be used to predict changes in a population or differences between groups because it is specific to a particular population and environment.
• A large heritability implies genes of a large effect	• High heritability estimates are often due to a polygenic effect, where multiple genes each contribute a small amount to the overall variance.

Hill & Wray, 2008).

A multidisciplinary team comprising psychologists, geneticists, lawyers, educators, and philosophers collaboratively created the International Genetic Literacy and Attitudes Survey (iGLAS) to comprehensively evaluate public understanding and attitudes toward genetics (Chapman et al., 2017). Madrid-Valero et al. (2021) added items to iGLAS to investigate what people know about the heritability of sleep. Participants (N=3,658) answered the online survey and rated, on a scale of 0-100, how important genetic differences were in explaining individual differences in sleep quality, sleep length, and insomnia. They were also asked to interpret the statement 'insomnia is approximately 30% heritable' by choosing from the following alternatives: (a) If someone has insomnia, this is approximately 30% due to their genes; (b) Approximately 30% of people will experience insomnia at some point in their lives; (c) Genetic influences account for approximately 30% of the differences between people in insomnia (the correct alternative); (d) There is an approximately 30% chance that someone will pass insomnia onto their children. Additionally, participants were asked to evaluate the efficacy of various interventions (i.e., medication, talking therapies, gene therapy, and lifestyle changes) using five options ranging from 1 (not effective at all) to 5 (extremely effective). Two scenarios were presented: one focuses on environmental etiology ("Robert is suffering from insomnia. He thinks it is probably because his job is so stressful, and he has a lot else going

on in his life. Robert is keen to seek help for his disturbed sleep") and the other focuses on genetic etiology ("Peter is suffering from insomnia. He thinks it is probably because of his genes—after all, multiple family members suffer terribly from sleep too. Peter is keen to seek help for his disturbed sleep"). Less than a quarter of the participants correctly identified the heritability of insomnia, with almost half of the sample mistakenly believing that heritability refers to the likelihood of passing a disorder on to their children. It is noteworthy that there is a prevalent misunderstanding about the concept of heritability, even among individuals with otherwise strong knowledge of genetics. Additionally, the study found that participants provided varying estimates of treatment effectiveness based on their presumed understanding of the disorder's cause.

Final Comments

This chapter showed that sleep is important for mental health both in the short term and over time. Twin research has provided valuable insights into various aspects of sleep, including its heritability, developmental aspects, and sleep theories. Additionally, this research has deepened our understanding of the intricate connections between sleep and mental health, encompassing both immediate and long-term impacts. Despite the advances made, the concept of heritability is still misunderstood, and caution is recommended about communication and interpretation of the findings from these studies. From my point of view, researchers must carefully consider how they communicate and share behavioral genetics research findings. It is important to work closely with journalists when presenting results to the general public and to address any misunderstandings that may arise. Additionally, efforts should be made to enhance the public's understanding of genetics in general and to emphasize training in behavioral genetics sic science education, particularly in disciplines that study human behavior.

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13. BRAZILIAN TWINS REARED APART SINCE NEWBORNS: A CASE STUDY

Eloísa de Souza Fernandes, Jennifer Leão Correia, Tania Kiehl Lucci , FrauleinVidigal de Paula, Hellen Vivianni Veloso Corrêa, Mauro Dias Silva Júnior,Nancy L. Segal, Emma Otta

Abstract This chapter presents a case study of Brazilian monozygotic twins separated at birth and reunited in adulthood. We explore their psychological and behavioral similarities and differences. Although both were raised in Brazil, they grew up over two thousand kilometers apart—one in the Northeast and the other in the Southeast—exposed to extremely different cultural environments. Remarkable similarities were found in their personalities and shared interests, particularly their professional and artistic dedication to photography. However, significant differences, such as divergent sexual orientations, were also identified. We interpret these findings through the lens of genetic and cultural influences, shedding light on the complex interplay between nature and nurture in shaping individual development.

Keywords: Brazilian twins, adoption, genetic-cultural effects, psychological characteristics, behavior, reared-apart twins

Twins offer a unique perspective on human nature (Segal, 1993, 2010), providing a powerful tool for understanding the complex interaction between genetic and environmental factors in shaping behavioral traits (Segal, 1990; Segal et al., 2015). They also present a valuable opportunity to explore evolutionary-based hypotheses related to social affiliation (Segal et al., 2020). Twins

separated at birth and raised in different environments, but later reunited as adults, serve as a natural experiment for testing these hypotheses (Segal, 2011).

Examining case studies of reunited twins proves insightful, since they allow for real-life investigations following formal research methods. This approach not only improves and complements statistical hypothesis-testing research with twins (Ridder, 2020; Segal, 2017; Segal and Hur, 2022) but also provides the opportunity for in-depth qualitative analysis in real-world contexts.

A common critique of twin studies, including those involving reared-apart twins, is the possible underestimation of environmental influence. This criticism stems from the examination of siblings reared in similar environments, cultures, and family conditions, limiting the range of gene-environment interactions and, consequently, the diversity of potential behavioral outcomes (Segal, 2012; Segal et al., 2015).

Studies on twins raised apart from birth, with distinct life histories, reveal marked similarities in personality, habits, interests, and a number of mental health and psychological measures. These resemblances are largely attributed to genetic factors that influence temperament, occupational and leisure interests, job satisfaction, social attitudes, and medical history (Segal & Hur, 2022).

Despite growing up in different family and cultural contexts, monozygotic twins often exhibit shared personal characteristics, such as interests, talents, and personality traits, which may predispose them to seek out similar environments. This phenomenon aligns with the concept of active niche construction, as proposed by Scarr and McCartney (1983), suggesting that individuals actively shape their environments in ways that reflect their innate predispositions.

Brazil, a vast and culturally heterogeneous country, is underrepresented in twin studies, as evidenced by a meta-analysis on the heritability of human traits spanning fifty years of research (Polderman et al., 2015). The country's population is a highly heterogeneous genetic makeup, with contributions from European, African, and Native American ancestries, making Brazil one of the most genetically diverse nations (Souza et al., 2019).

Brazilian television reported a case involving twins who were separated shortly after birth and reunited two decades later. The twins were adopted over 2,000 kilometers apart, each experiencing distinct developmental trajectories. One twin was raised in one city by a traditional Christian nuclear family,

while the other grew up in several cities in a multi-parent household, following Afro-Brazilian cultural traditions (Figure 13.1). This provided a unique opportunity to conduct a systematic case study (Ventura, 2007) to determine how their different life histories influenced their behavioral and psychological development.

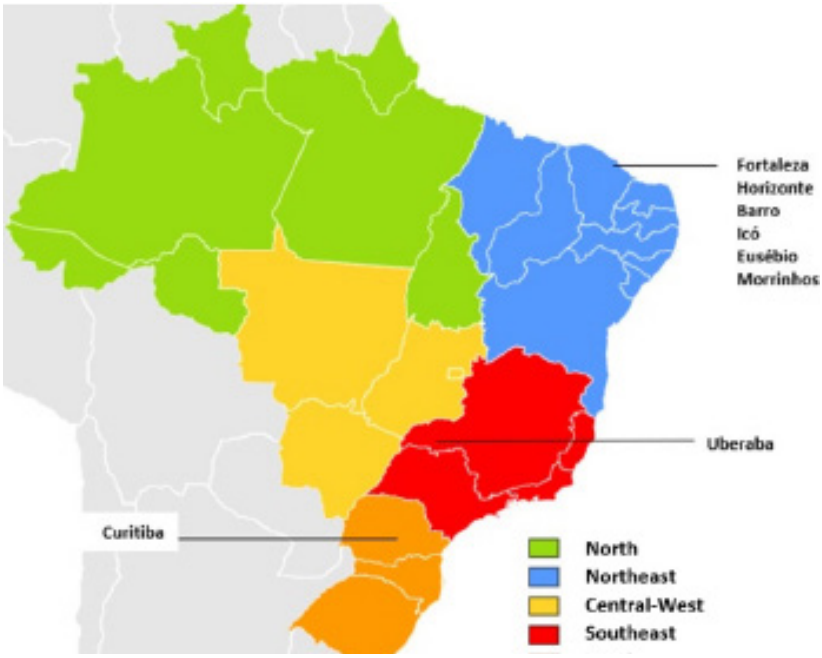

Figure 13.1. Geographic locations of reared apart twins: TM in six different cities of Ceará state, in the Northeast region, and GF, in only one city in the Southeast region. Following marriage, GF relocated to Curitiba, in the South region. (CC BY-SA 3.0)

This report presents the first systematic case study of Brazilian monozygotic twins separated at birth and reunited in adulthood. Inspired by the landmark Minnesota Study of Twins Reared Apart (MISTRA), conducted from 1979 to 1999 (Segal, 2012), with ongoing individual assessments at the Twin Studies Center at California State University, Fullerton (CSUF), our study arose from a research collaboration between the Painel USP de Gêmeos (Otta et al., 2019) and CSUF's Twin Study Center (Segal, 2012; Segal & Niculae, 2019).

The investigation aimed to explore the twins' behavior, interests, and psychological characteristics, drawing on insights from prior research. We hypothesized that the twins would share similarities in physical features, personality traits, and interests, which might lead them to seek similar developmental niches. However, we also anticipated personal differences to emerge, shaped by the dynamic interactions with their unique life contexts.

Methods and Materials

The twins TM and GF were born on June 25, 1997, on the outskirts of Fortaleza, a coastal city in Northeastern Brazil. Faced with adverse living conditions within their biological family, they were placed for adoption and separated at 15 days of age. They were reunited at the age of 23, having re-established contact on the internet approximately two months before the onset of our study.

Both twins provided saliva samples for DNA analysis, which was conducted by Laboratório Gene in Minas Gerais state. They were interviewed separately using the Brazilian version of the MISTRA Life History (Segal, 2012) and completed battery of psychological assessment tools. These included: the Perceived Vulnerability to Disease scale (Duncan et al., 2009); Social Relatedness measures (Segal, Hershberger, & Arad, 2003; Segal, 2005; Aron, Aron, & Smollan, 1992; Vázquez et al., 2017); Holland's Occupational Interests (Rigoni, 2018); the Minnesota Job Satisfaction Questionnaire (Weiss et al., 1967); the Wechsler Abbreviated Scale of Intelligence (WASI-III; Wechsler, 2014); the List of Possible Similarities (Curry & Dunbar, 2013); Big Five Personality Traits (Andrade, 2008; Laros et al., 2018); the Highly Sensitive Person Scale (Aron & Aron, 1997); and the Klein Sexual Orientation Grid (Klein et al., 1985; Klein, 2014). The twins also provided photographs, thereby enriching the study (Sheridan & Chamberlain, 2011).

The first author (ESF) interviewed one twin, and the second (JLC) the co-twin between August and October 2020. Five 60-to 120-minute video sessions were held via Google Meet. Questionnaires were administered online through the Google Forms platform. Both twins provided informed consent. The research protocol and consent form were approved by the Human Research Ethics Committee of the Instituto de Psicologia at Universidade de São Paulo

(CAAE: 48609515.6.1001.5561), following National Council for Ethics in Research (CONEP) guidelines. Furthermore, both twins reviewed the manuscript and approved the text and the photographs used for illustration.

The twins´ biographical data, preferences, and behavioral tendencies were qualitatively compared to identify both similarities and differences. A triangulation method, incorporating interviews, observations, and questionnaires, was used to gain a comprehensive understanding of their life trajectories (Denzin & Lincoln, 2011). Within-pair comparisons were made for psychological and behavioral measures. This multi-dimensional approach aimed to present a holistic portrayal of the twins' lives, psychological characteristics, and behaviors, ensuring a thorough investigation of the factors shaping their development.

Results

The results are presented below, categorized by assessment domains.

Zygosity Diagnosis

DNA testing confirmed the twins' monozygosity, revealing identical genetic profiles.

Medical and Physical Measures

The twins exhibited a remarkable physical resemblance, with dark brown skin, brown eyes, black hair, and similar facial features, such as an oblong face with a high forehead, pronounced chin, and full lips. Both had endomorphic body types. TM was 1.74m in height and weighed 84 kg, while GF was 1.70m tall and weighed 92 kg. Their similarities extended to their clothing choices, hairstyles (side-swept crew cuts), and round black eyeglass frames. Describing themselves as "like two peas in a pod," they recalled an acquaintance once mistaking one for the other in Uberaba.

TM suffered from severe asthma attacks during infancy and childhood, although his health improved with age. His Perceived Vulnerability to Disease scores were slightly above the midpoint: 4.42 for Perceived Infectability and

4.62 for Germ Aversion, on a 7-point scale. GF, who was adopted with severe anemia and recurrent fevers, also exhibited health improvements. In early adulthood, GF sustained wrist and knee fractures from a motorcycle accident. His questionnaire scores were at the lower end: 1.85 for Perceived Infectability and 3.5 for Germ Aversion, both lower than TM's scores.

Life History Interview

TM was raised in the Northeast region of Brazil and adopted by an unmarried, childless woman. His early childhood socialization occurred in a rural Quilombola community, which had a collectivist social structure. From middle childhood to pre-adolescence, he relocated between small towns in Ceará due to the occupation of his adoptive mother's partner, a truck driver, eventually settling in the state capital. TM identified as an Umbandista, reflecting the religious beliefs of his mother, SM. In contrast to the Umbandist nuclear family, extended family members adhered to the Igreja Universal do Reino de Deus (Universal Church of the Kingdom of God, UCJK) and the Igreja Apostólica Ministério 100% Trigo (Apostolic Church 100% Wheat Ministry).

GF's upbringing differed significantly from TM's. He was adopted by a devout married couple with three grown biological children, and his family followed the Christian Congregation (CC) religious tradition. Unlike TM, GF remained in Uberaba throughout his childhood. A traumatic event occurred when GF was 12: he was misinformed about his co-twin's supposed death, only to later discover that his biological father had been tragically murdered.

At 16, TM's biological mother sent him a photo of GF with incomplete names and "Uberaba" inscribed on the back, igniting TM's fervent desire to find his co-twin. After initiating a social media search and, seven years later, with police assistance, TM was able to reach out to GF's adoptive parents and connect with his co-twin. TM was astounded by the striking resemblance in their Facebook photos. Similarly, GF and his adoptive family also recognized the nearly identical appearance between the two young men.

Twin Relationship

The twins began communicating on June 1, 2020, through messages and video calls. To surprise GF on their shared birthday (June 25th, 2020),

TM, GF's wife, and a close friend arranged a freelance photography job at the airport. GF was astonished to find his co-twin there to meet him. TM spent about a month with GF in Uberaba before returning to Fortaleza. After the data for this study was collected, GF visited Fortaleza for a vacation with his co-twin.

In response to MISTRA's relationship questions, TM initially expected that they would be "closer than best friends," while GF anticipated they would be "as close as best friends." One month later, they both perceived their bond as "closer than best friends." On the Inclusion of Other in the Self Scale, both twins selected the pair of circles that reflected the greatest overlap, indicating a deep sense of closeness.

Perceived family stress and support

TM rated his perceived family stress and instability as 8 on a scale from 0 to 10, reflecting substantial challenges during his childhood and adolescence. He and his adoptive mother experienced domestic abuse by her partner, which was exacerbated by his excessive alcohol consumption. Conflicts about his sexual orientation also arose on the Christian side of his family, resulting in pressure for religious conversion therapy to "change" his gay identity.

By contrast, GF obtained a score of 5. He reported a generally positive relationship with his adoptive parents but experienced considerable difficulties with his middle adoptive brother. The adoptive father occasionally used physical punishment with his biological children, which fostered resentment in them, since they perceived that the adoptive child was treated more affectionately and leniently. Despite their divorce in 2005, the adoptive parents maintained an amicable relationship.

Occupational Interests, Education, and Employment

Using Holland's Interest Inventory, TM's profile is Investigative, Social, and Enterprising (ISE) with scores of 12, 12, and 12, respectively. By contrast, GF's profile is Investigative, Realistic, and Social (IRS) with scores of 13, 12, and 11.

TM attended public schools throughout most of his education. Despite frequent relocations and a delayed start to formal schooling, he remained a conscientious and committed student, consistently ranking among the top performers in his class. Beginning at age 13, TM worked part-time to help support his adoptive mother. At 20, he enrolled in a four-month photography course, which led to a position at the Museu de Fotografia de Fortaleza [The Fortaleza Photography Museum].

GF, also educated in the public schooling system, regarded himself as an average student, experiencing a slight decline in academic performance around age 12. However, he effectively balanced part-time work with his academic responsibilities, taking advantage of opportunities beyond the classroom. At 17, he began working part-time as an apprentice in a pharmacy, eventually transitioning to a full-time employee a year later. His interest in photography, initially a hobby, evolved into a freelance career in 2018.

Cognitive Assessment

In the vocabulary subtest of the Wechsler Abbreviated Scale of Intelligence (WASI), the twins obtained similar T-scores (TM: 51, GF: 54). However, a more pronounced difference was observed in the matrix reasoning subtest (TM: 45, GF: 53). When transforming the scale scores into composite scores and comparing them with Brazilian normative data, TM obtained a composite score of 96 (95% CI 89-103), corresponding to the 39th percentile. By contrast, GF achieved a composite score of 106 (95% CI 99-112), placing him in the 66th percentile.

Perceived Overall Interest Similarity

An Overall Similarity Score, ranging from 0 to 14, was calculated by summing shared items, resulting in scores of 6 for TM and 7 for GF. Both twins agreed on perceived similarities in "personality," "sense of humor," "socioeconomic status," "income," and "level of education/intelligence." However, they acknowledged differences in "religious beliefs," "occupation," and "sexual orientation."

Personality

The twins exhibited similar scores across the Big Five personality traits (Fig. 13.2), with TM scoring slightly higher than GF in extraversion, neuroticism, agreeableness, and openness to experience. Notably, GF scored higher than TM in conscientiousness. When compared to a Brazilian sample of 138 pairs of MZ twins raised together (Fernandes, 2021), the MZ twins in our study, raised apart, demonstrated score differences that closely approximated the average differences observed among MZ twins raised together. Their differences were: extraversion = 0.33 SD below, neuroticism = average, conscientiousness = 0.5 SD above, agreeableness = 0.5 SD below, and openness to experience = 0.16 SD below.

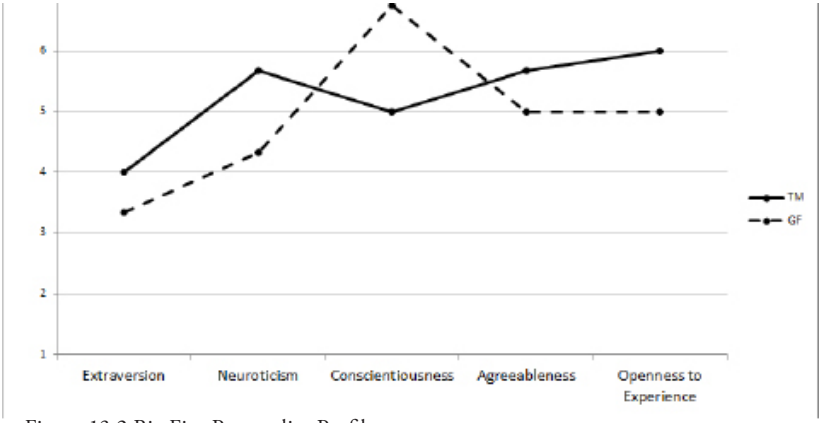

Figure 13.2 Big Five Personality Profiles

Sensory-Processing Sensitivity

Distinct differences were observed in the twins' sensitivity and responsiveness to the environment, with TM exhibiting heightened sensitivity and reactivity than GF (Fig. 13.3). This divergence was evident across all three dimensions of the Highly Sensitive Person Scale (HSPS), particularly in aesthetic sensitivity. The differences were most pronounced in the following domains: Low Sensory Threshold (TM = 4.6, GF = 2.3), Ease of Excitation (TM = 5.1, GF = 3.9), and Aesthetic Sensitivity (TM = 5.3, GF = 1.5).

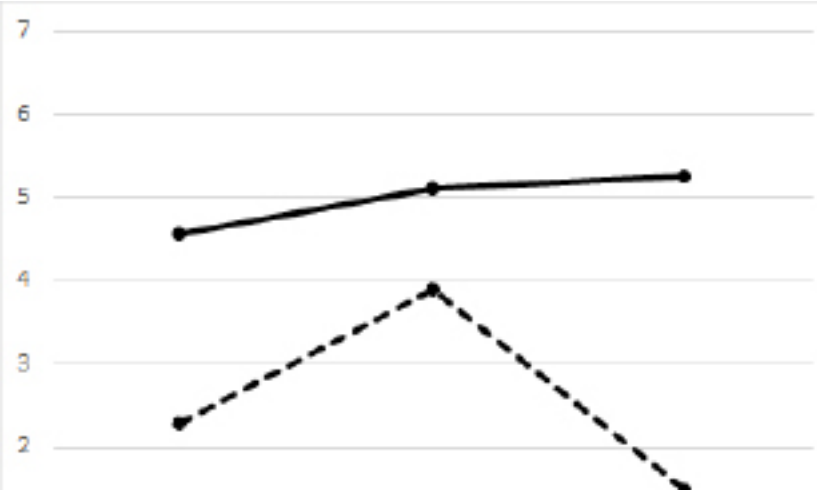

Figure 13.3 Sensory-Processing Sensitivity Profiles

Sexual Orientation

TM's sexual orientation, as assessed by Klein's Sexual Orientation Grid (KSOG), indicated exclusive attraction to individuals of the same gender across past, present, and ideal scenarios. His sexual identification consistently reflected a homosexual orientation in all these time frames.

By contrast, GF's responses showed that his sexual attraction, behavior, fantasies, and emotional preferences were exclusively directed toward the opposite sex in the past, present, and ideal scenarios. His sexual identity remained consistently heterosexual throughout these periods. At the time of the study, GF had been married for three years to a 22-year-old psychology undergraduate who worked as a human resources professional.

Photography: A Shared Passion

Both twins shared a passion for photography, using it as a form of creative expression, albeit focusing on distinct thematic areas (Fig. 13.4). TM's work was abstract, conceptual, and emotionally intense, often conveying social critique. One of his photographs, for example, depicts him in a white button-up shirt holding a Holy Bible, symbolizing a member of a neo-Pentecostal church.

Red fluid resembling blood seeps from the Bible, staining one of the shirt's cuffs, evoking a powerful statement on religious themes.

By contrast, GF's photography emphasized realistic depictions of the world, capturing nature, historical buildings, and people in meaningful, candid moments. Judges evaluated the photographs based on valence and arousal. TM's photos (M = 3.61, SD = 1.64, N = 14) received lower mean pleasantness ratings than GF's (M = 7.10, SD = 1.02, N = 10). They also elicited higher mean arousal ratings (M = 4.71, SD = 2.42, N = 14) compared to GF's (M = 2.95, SD = 2.13, N = 10).

Figure 13.4 Examples of the twins' different photography styles: A peaceful religious image depicting the Museum of Sacred Art of Uberaba contrasting with violent religious harassment (Source: GF's and TM's archives)

Discussion

Distinct Cultural Upbringings and Familial Challenges

In the present study, the twins were raised separately in diverse cultural and family environments in Brazil. TM grew up in the Northeast, in a Quilombola community practicing the Umbanda religious tradition. GF was raised in the Southeast in a Christian community. Born into poverty and exposed to the detrimental effects of their biological parents' unhealthy lifestyles, the twins faced considerable social vulnerability during infancy, which tragically escalated with the murder of their biological father.

The twins were adopted separately into working-class families with financial struggles. Both adoptive mothers displayed remarkable resilience and a strong commitment to providing nurturing homes, supported by their re-

spective communities. TM's family was part of a Quilombola community of Umbanda adherents, while GF's family belonged to a Christian Congregation community. Despite the contrasting environments, both twins exhibited adaptive developmental trajectories characterized by prosocial behavior, empathy, and a sense of responsibility. This underscores the critical importance of a "good enough" family environment in fostering positive developmental outcomes, transcending specific family characteristics (Ridley & Pierpoint, 2003).

Breaking the cycle of poverty, the siblings found opportunities to thrive within their adoptive families. Their relationships with their adoptive mothers were characterized by warmth and affection. However, TM faced family violence during preadolescence after his adoptive mother entered a new relationship. Living with a stepparent, as in TM's case, corroborates predictions of heightened risk for child abuse and neglect, as suggested by Daly and Wilson (1998). This finding echoes the inclusive fitness theory, which posits that parents are more inclined to invest in their genetic offspring.

Macro-level cultural factors often influence micro-level interpersonal interactions, as evidenced by TM's experiences of strained family dynamics and psychological distress. This was particularly evident in his exposure to conversion therapy. While Umbanda practitioners are generally inclusive toward homosexuals and provide social protections (Ogland & Verona, 2014), documented instances of growing religious intolerance against Afro-Brazilian religions complicate the picture. Organizations such as UCJK have played significant roles in perpetuating this intolerance (Neace, 2016). This hostility extends to "pastoral practices" aimed at altering the sexuality or gender identity of homosexuals, as reported by Dias (2019).

Nature via Nurture

In the present study, when the twins first met, they immediately experienced a profound social connection. This aligns with findings from previous research (Segal, 2005) and supports predictions derived from kin selection theory (Hamilton, 1964). Segal (2022) introduced the term genetically related intimacy (GRI) to describe the unique bond of love shared between reunited twins, distinct from any form of sexual attraction.

In our study, we anticipated behavioral similarities mediated by personality traits, despite notable differences in the twins' upbringings. This was cor-

roborated by evidence highlighting the genetic influence on personality, as demonstrated by studies by Jarnecke and South (2015). Twin studies estimate that approximately 40–50% of the variation in Big Five personality traits can be attributed to genetic factors (Vukasovic & Bratko, 2015). Consistent with this, the twins in our study displayed highly similar personality scores across the Big Five traits, with the exception of conscientiousness. GF's notably higher conscientiousness score may reflect the influence of his structured and rigorous religious upbringing. This finding corroborates Saroglou's (2002) meta-analysis, which identified conscientiousness as the personality trait most strongly associated with religiosity.

Building on the framework of Scarr and McCartney (1983), we suggest that the shared traits in the Five Factor Model (FFM) of personality and the vocational personality profiles of TM and GF shaped their responses to environmental opportunities and influenced the choices they made in selecting their environments. Adopted into working-class families, both were predisposed toward embracing the world of work. Early experiences in the workplace emphasized the development of skills, a sense of responsibility, and engagement with meaningful objectives. As noted by Gomes (1994), the focus on avoiding idleness and disorder is particularly significant. For individuals from economically disadvantaged backgrounds, the primary goal of such work-oriented "education" is often the cultivation of a strong "work ethic", instilling discipline and a commitment to productivity.

TM's vocational personality profile highlights humanistic traits, a strong propensity to help others, an enterprising mindset, and a preference for avoiding routine tasks. He also demonstrates analytical and observant qualities, with a clear focus on creative problem-solving. His roles in the Tutoring Programme and at the Museu de Fotografia de Fortaleza [The Fortaleza Photography Museum] align closely with these characteristics. On the MISTRA scale of occupational success (ranging from 0 to 10), TM achieved a commendable score of 8.

By contrast, GF's vocational profile reveals a preference for creative problem-solving, analytical reasoning, and attention to detail, combined with a desire for hands-on autonomy in tasks involving the repair or assembly of objects and operating machinery. He thrives in practical, task-oriented learning experiences that produce tangible results. His idealistic tendencies reflect a genuine concern for the welfare of others and a sincere enjoyment in helping people.

However, his role as a pharmacy clerk did not align well with his vocational profile. This mismatch is reflected in his MISTRA score of 5 on the occupational success scale.

After data collection, GF and his wife relocated to Curitiba, a city in Southern Brazil known for its high Human Development Index (HDI). GF transitioned into a systems analyst role, which better aligned with his vocational profile. While living in Uberaba, GF dedicated himself to learning computer programming. Within six months, he secured a remote junior programmer position, marking a significant step in his career. This allowed him to save and eventually move to Curitiba in March 2022.

GF found Curitiba more conducive to his aspirations, offering opportunities for personal and professional growth that contrasted with the perceived stagnation and conformist attitudes in Uberaba. The relocation resulted in several positive changes, including the development of new friendships, improved dietary habits, and a return to regular physical activity. Financially, GF invested in real estate, which he will receive within a year, and fulfilled a childhood dream of purchasing his desired car.

Photography as a Cathartic Outlet

Consistent with findings on twins reared apart (Segal, 2012), TM and GF exhibited striking shared interests, most notably a passion for photography. Despite the cost, it independently evolved for both into a source of income. TM secured a position at the Museu de Fotografia de Fortaleza [The Fortaleza Photography Museum], while GF pursued freelance photography to supplement his income. We propose that both twins independently discovered photography as a means of emotional expression and processing, underscoring its cathartic potential.

In response to the question, "Is photography a cathartic process for you?" posed by Cocking (2016), Mexican photographer Moreno emphatically affirmed, "Totally. Photography is a stabilizing force in my life." He described the medium as transformative, offering a deeper understanding of the human condition while providing an escape from an increasingly hostile world. Others share similar sentiments. Photographer Ruddy, for instance, views each image as an exploration of self-identity, reflecting on the questions, "Who am I,

and what is my role here on this earth?" Meanwhile, Morton underscores the importance of documenting everyday life, emphasizing its value in helping us understand our times and affording future generations a lens through which to reflect on their journeys (Laurent, 2017).

Closing Remarks: Limitations and Future Directions

This case study highlights resilience — the capacity to adapt effectively to challenging life experiences through cognitive, affective, and behavioral flexibility in response to both external and internal demands (APA Dictionary of Psychology). Drawing on the metaphor introduced by Lionetti et al. (2018) regarding individual differences in environmental sensitivity, both TM and GF exhibit traits reminiscent of "dandelion" personalities. Like these robust flowers that thrive in a variety of environments and rebound after adversity, both individuals display inherent resilience, protecting them from significant environmental threats and mirroring the tenacity of dandelions.

The case study underscores the interplay of genetic and rearing factors in shaping behavior. However, it does not allow for definitive conclusions about the relative contributions of these influences in elucidating individual developmental differences. Nevertheless, the findings contribute to the growing body of research on twins raised apart in non-WEIRD (Western, Educated, Industrialized, Rich, and Democratic) cultures, providing a foundation for generating hypotheses to be tested with larger, more heterogeneous samples.

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PEER COMMENTARY

Fraulein Vidigal de Paula, Cintia Fridman, Claudio Possani, and Natália Bezerra Dutra

As coordinators of the discussion section for Part III: "Research with Twins on Psychophysiological Characteristics," we have compiled the questions posed to the authors during the Twin Studies in Behavioral and Health Sciences event, extending the discourse from the preceding sections. This event brought together students, professionals, and researchers for a dynamic exchange of ideas. The questions included in this section aim to enrich the reader's understanding of the topics explored, thereby fostering a deeper engagement with the material.

Question 1: The first question, from an undergraduate in Psychology, is for Dr. Assary. Hello, my name is Mariana. I'm a freshman at the University of São Paulo, so please excuse me if my question isn't particularly sophisticated—I'm still new to these academic discussions. I have a twin sister, and we are monozygotic. While we appeared virtually indistinguishable in childhood, we have become increasingly dissimilar in appearance during adolescence to the point that people often doubt we're twins. Given that we have shared a remarkably similar environment throughout our lives – living together, sharing meals, participating in the same sports, and generally engaging in all activities together – this divergence in our appearances is quite intriguing. This makes me wonder: in cases like ours, could a genetic factor have predicted or predetermined such differences early on? Or is it more likely that an environmental

factor caused these changes? And if so, what kind of environmental factor could have such a profound and rapid impact in such a short span of time? (Audience Question)

Answer: *Thank you, Mariana. Your question is insightful and far from simple. It's crucial to distinguish between making predictions and explanations at the level of the individual versus the population. Our research often employs an epidemiological approach, analyzing large populations to draw general inferences. While these insights can be applied to individual cases, it's important to acknowledge the limitations of this approach and the assumptions underlying such models. Monozygotic twins, despite their identical genetic origins, can exhibit phenotypic differences due to environmental factors encountered even before birth. For instance, whether they share the same chorion can significantly impact their development and subsequent phenotypic outcomes. Similarly, the in-utero nutritional environment, particularly if they share a placenta, can play a role. These are environmental factors that may not always be adequately captured in studies primarily focused on genetic influences. To illustrate, unless a substantial proportion of the 20,000 twin pairs in a study shared a similar in-utero environment, such correlations might not be readily apparent in the data. This can pose a challenge when attempting to generalize findings to specific individual cases like yours. Another potential explanation lies within the realm of genetics. Although monozygotic twins are genetically identical at conception, post-zygotic mutations – genetic changes occurring after conception – can arise. These mutations could introduce subtle genetic differences between twins, potentially manifesting later in life and contributing to observable phenotypic variations. In summary, identifying the exact cause of phenotypic differences in a specific individual case is challenging. It likely involves a complex interplay of factors, including environmental sensitivities – an area where monozygotic twins offer invaluable research opportunities – and genetic factors such as post-zygotic mutations.*

Question 2: What is the current understanding of sleep cycle maturation in utero? Specifically, I am interested in the probability of distinct sleep cycle developments occurring during gestation. (Audience question)

Answer: *This question delves into some very interesting and complex issues (see Lunshof et al., 1997, Mirmiran et al., 2003, Serón-Ferré et al., 2001, Tendais et al., 2013, Wong et al., 2022). Rhythmic patterns in fetal breathing movements,*

limb activity, and heart rate emerge in the human fetus from mid-gestation onwards. The early development of the suprachiasmatic nucleus (SCN) in gestation, coupled with the observation of circadian rhythms in both the fetus and newborn, suggests the presence of a functional fetal circadian system. This system may be influenced by maternal circadian inputs, potentially contributing to the regulation and synchronization of fetal rhythms. Supporting this hypothesis, a study of twins observed a correlation between the diurnal rhythm of fetal heart rate and the maternal heart rate rhythm in intact fetuses, but not in an anencephalic fetus, suggesting a crucial role for the fetal brain, and likely the SCN, in generating and maintaining fetal circadian rhythms. (Emma Otta)

Question 3: The following question is addressed to Dr. Patricia. What kind of neurological disorders could be predicted by studying a baby's cry? (Audience Member)

Answer: *Extensive research has been conducted in this area (e.g., Ben-Sasson et al., 2024; Lawford et al., 2022; Manigault et al., 2023; Unwin et al., 2017). For instance, studies have identified links between infant cry characteristics and conditions such as autism spectrum disorder and maternal postpartum depression. Furthermore, cry analysis shows promise as an early diagnostic tool for developmental disorders and vocal tract abnormalities. The ultimate goal is to leverage cry patterns as a tool for early, proactive diagnosis of various developmental concerns, enabling timely interventions.*

Question 4: The initial question directed to Dr. Alice, is: 'How does the heritability of sleep duration change over time?' (Audience Member)

Answer: *Thank you for that question. There have been several meta-analyses and reviews on this topic, and the results have been mixed. One reason for this is that some meta-analyses focus on participants over the age of six. I was actually an author on one such study. We chose this age cutoff because young infants still have irregular sleep patterns and frequent naps, making it difficult to assess consistent sleep duration. By focusing on older children, we aimed to capture more stable sleep patterns. In contrast, another review that looked at the entire lifespan found that the genetic influence on sleep duration is lower in very young children. This suggests*

that shared environmental factors—like being raised in the same family—play a more significant role in early childhood. While parenting can also introduce non-shared environmental influences, the primary shared environment impact is more pronounced during the earlier stages of life. This difference in focus could explain the varied findings across studies. (Alice Gregory)

Question 5: The next question was addressed to Dr. Alice by an undergraduate psychology student at the University of São Paulo regarding a practical issue. 'You mentioned that publishing articles, and books for children, and running a dissemination program requires much work. Could you estimate how much effort is involved in publishing and these outreach activities?' (A member of the audience referencing Professor Alice Gregory's book The Sleepy Pebble and Other Stories)

Answer: *That's a great question. I'm not entirely sure about the situation in Brazil, but in the UK, the system isn't particularly designed to extensively support these types of initiatives. While there is encouragement to engage with the public, there's also significant pressure to demonstrate the impact of your work, which can involve navigating various challenges. For me, it was a personal initiative—I wanted to do it, so I decided to take the leap. One of the biggest hurdles was finding a publisher. Many publishers are interested in children's books, so I reached out to several. At first, I didn't receive any responses, but eventually, I connected with someone who saw the potential in addressing the topic of sleep. They even referenced the success of books like The Snail Who Went to Sleep, which piqued their interest and opened the door to further discussions. From there, we collaborated with a colleague experienced in writing children's stories and a talented artist to bring the book to life. Collaboration was essential for this project because no single person has all the skills needed to execute it successfully. Working with skilled, like-minded individuals not only made the process smoother but also a lot more enjoyable and rewarding.*

Question 6: Thank you, Alice. One more question—how did you measure distorted beliefs in your study? (Audience Member)

Answer: *Thank you for asking! For that study, we used the Dysfunctional Beliefs About Sleep Questionnaire as a key measure. Additionally, we included several other scales, such as the Pre-Sleep Arousal Scale and mindfulness-related items from an abbreviated questionnaire. Collecting this data was quite an undertaking.*

Twin studies often require collaboration, which means you rarely get to include all the measures you might ideally want. However, I eventually secured funding to conduct a dedicated study on cognitive factors and sleep. We distributed booklets containing these questionnaires to participants in twin samples and analyzed the data longitudinally.

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14. CONCLUDING REMARKS AND FUTURE DIRECTIONS

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The book TWIN STUDIES IN BEHAVIORAL AND HEALTH SCIENCES has presented a multidisciplinary investigation of twin development, encompassing a wide range of topics, from prenatal development and childhood to adolescent and adult life. It explores a wide range of twin research methodologies, extending beyond the classical twin studies and including various theoretical and methodological frameworks. The successive chapters provided an opportunity to engage with conceptual and methodological issues, as well as research findings on twins, presented by: (i) psychologists with expertise in conducting behavioral research in human ethology, development, and cognitive psychology; (ii) medical professionals specializing in fetal medicine and neonatology, with experience in monitoring high-risk pregnancies, such as those involving selective fetal growth restriction; and (iii) professionals in pediatric dentistry and forensic dentistry. The chapters in the first section provided insights into the unique aspects of twin pregnancies, the challenges faced by high-risk twin newborns, and the intricate development of fetal sensory and motor abilities. The focus then shifted to the unique aspects of dentofacial development in twins, highlighting the interplay between genetic and environmental factors in shaping oral health outcomes. Finally, the book delved into the psychophysiological characteristics of twins, examining their sleep patterns, emotional expressions, and the impact of early experiences on their mental health.

This comprehensive approach underscores the importance of considering both genetic and environmental factors in understanding twin development. While genetic similarities play a crucial role in shaping twin characteristics, the unique intrauterine environment, early interactions, and individual experiences contribute significantly to phenotypic variability. The findings presented in this book have important implications for clinical practice, research, and our broader understanding of human development.

Several promising avenues for future research emerge from the findings presented here. One critical area is the more in-depth exploration of how prenatal factors influence twin development. For example, investigating the long-term effects of specific prenatal conditions, such as growth restriction, on cognitive, social, and emotional outcomes is imperative. Another promising direction involves analyzing fetal facial expressions in twins, which may offer unique insights into early neurodevelopmental processes and their variability. Furthermore, studying the role of epigenetic mechanisms in mediating the impact of prenatal experiences on twin development holds considerable potential. These investigations could explain the complex interplay between genetic, epigenetic, and environmental factors, significantly enhancing our understanding of how early-life conditions shape developmental trajectories in twins.

The book is collaboratively organized by professors from USP in psychology, dentistry, and medicine, who are responsible for the FAPESP Thematic Project "USP Twin Panel." Studies on behavior, health, and well-being from an ontogenetic perspective using twins as a model represent an active area of research. The book introduces readers to both well-established and emerging knowledge, substantiated by research. It fosters opportunities for interdisciplinary knowledge-building—both theoretical and applied—and possibilities for interdisciplinary interventions. The rising twin birth rate is a significant public health issue, considering the associated risks to both children and mothers. Comprising 15 chapters, the book is divided into three sections: the early development of twins, dentofacial development, and twin research on psychophysiological characteristics. The organizers are confident that students and professionals will be inspired by the authors´ enthusiasm and will discover new avenues for professional practice and research.