

News, Views and Comments

Do Males Affect Twinning Events? A Review of Current Findings/Twin Research Reviews: Monozygotic Twins Discordant for Parkinson's Disease; Fetal Loss in Twin Pregnancies Following Prenatal Diagnosis; Uterine Rupture and Repair in an Early Twin Pregnancy; Twin Study of Affectionate Communication/Human Interest: Conjoined Twins in a Triplet Set; Identical Twin Nurses Deliver Identical Twins; Identical Twins Discordant for COVID-19 Recovery Course; Identical Twins Pass Away from COVID-19; Archeological Finds of Oldest Identical Twins

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Abstract

Research into the origins of twinning has focused mostly on contributions from the female side of the family. A review of current findings suggests that possible male contributions to twinning events have been overlooked. This section is followed by brief reviews of twin research concerning monozygotic twins discordant for Parkinson's disease, fetal loss in twin pregnancies following prenatal diagnosis, uterine rupture and repair in an early twin pregnancy and a twin study of affectionate communication. The concluding portion of this article presents human interest stories involving twins that are both informative and poignant, namely conjoined twins in a triplet set, identical twin nurses who delivered identical twins, identical twins discordant for COVID-19 recovery course, identical twins who passed away from COVID-19 and archeological finds of the oldest identical twins.

Factors affecting twinning continue to be of great interest. Much of the past and recent research in this area has focused on female contributions to twinning, especially dizygotic (DZ) twinning, with reference to maternal age, height, weight, ancestry, familial history and coital frequency (Boomsma, 2020; Segal, 2017). Given the foregoing, it was important to see what research has revealed regarding males' possible biological contributions to twinning. The idea for this article came from my knowledge of a young male who had fathered several sets of DZ twins with several different women.

Many older studies have examined the hereditary transmission of twinning, such that an exhaustive review of this topic is not possible. As such, important developments and various selected findings will be highlighted. Early studies produced mixed evidence regarding paternal factors affecting twinning. Based on studies

of individual families, Danforth (1916) asserted that both 'uniovular' and 'biovular' twinning were transmitted maternally. However, he speculated that the high incidence of twins among fathers' brothers was consistent with paternal transmission. Dahlberg (1926) believed that both mothers and fathers can be genetically predisposed toward MZ twinning, but that only mothers carried the tendency toward DZ twinning. Dahlberg's theory of MZ twinning was not widely accepted (Koch, 1966; Newman et al., 1937). Bulmer (1959) noted that the increased twinning sometimes observed among the siblings of fathers of twins reflected underreporting of singleton births on the paternal family side.

In the 1960s, Wyshak and White (1965) examined extensive family data gathered by the Genealogical Society of the Church of Jesus Christ of Latter Day Saints in Salt Lake City, Utah. They focused on the individuals who were twins themselves, noting that DZ female twins and their nontwin sisters showed increased twinning among their offspring. In contrast, this trend was not observed among males. The investigators performed analyses supporting their hypothesis that DZ twinning was transmitted via one or more female-limited alleles. Genomewide association studies that are currently underway will allow more informed assessment of this finding and others.

Rhine and Nance (1976) documented a case of familial twinning in which twin births were consistent with autosomal

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dominant transmission, represented equally in males and females. The six pairs born across four generations included a normal twin and either a macerated fetus, stillborn infant or premature infant. It was suggested that superfetation — the release of a second egg in what would be considered a subsequent menstrual cycle — was operative. By way of explanation, it was also suggested that because both parents contribute to the genotype of the placenta, either the mother's or the father's genetic background could have caused the superfetation.

Another important development was reported several years later. Khory and Erickson (1983) found that Black mothers showed a higher frequency of opposite-sex twins than Caucasian mothers. This effect persisted after adjusting for fathers' race, as well as for maternal age, parity, education and marital status, but it did not apply to same-sex twinning rates. After adjusting for mothers' race, Black fathers did not show higher rates of opposite-sex twins than Caucasian fathers, suggesting that DZ twinning is affected by maternal race only. However, St. Clair and Golubovsky (2002) documented the paternal transmission of twinning in three Scottish pedigrees, using data from 1800 to 2000. They found paternal effects for both MZ and DZ twinning, as well as twinning tendencies associated with partial male infertility.

Specific genes, gene variants and hormone levels have been linked to DZ twinning. Nick Martin, editor of *Twin Research and Human Genetics (TRHG)*, has played a pivotal role in this area since the mid-1980s, much of which has been summarized by Boomsma (2020) in *TRHG*. Female DZ twinning tendencies have been linked to elevated follicle-stimulating hormone and luteinizing levels. Montgomery et al. (2004) and Palmer et al. (2006) found that rare variants of growth differentiation factor 9 in women might be linked to DZ twinning, but common variants were not. Complex segregation analysis has shown familial DZ twinning tendencies on both the maternal and paternal family sides. Specific genes linked to DZ twinning include follicle-stimulating hormone beta subunit and SMAD family member 3, as reported by Mbarek et al. (2016). Future studies with larger twin populations will be of importance, with additional focus on paternal effects. There is a small but growing literature in this area, reviewed below (see also Segal, 2017).

An Israeli study found an older father influence on twinning, but the effect was slight and was only observed for opposite-sex twin pairs and male–male twin pairs (Kleinhaus et al., 2008). Explanations for these findings are unknown. Subsequent research on over 8 million births in the United States found that fathers in their forties were more likely to have twins (both MZ and DZ) than younger fathers. However, the elevated twinning rate for fathers was lower than that for mothers. In addition, paternal age had the greatest effect on twins born to Caucasian, non-Hispanic fathers (Abel & Kruger, 2012).

There are other clues as to how males may influence twinning, but they are not limited to older fathers or to DZ twinning. Specifically, an increased level of insulin growth factor-2 (IGF-2) is associated with cell growth and division, and an individual's IGF-2 level is partly heritable. The *IGF-2* gene transmitted by father to offspring is active, whereas the same gene transmitted by mother is inactive. It is, therefore, possible that MZ twinning is elevated in some families by the paternally transmitted level of IGF-2 (Steinman, 2003).

Danish studies of semen quality and twin pregnancy are revealing (Asklund et al., 2007; Storgaard et al., 2006). Storgaard et al. (2006) conducted a twin study showing a substantial genetic component to plasma levels of Sertoli cell function and to sperm

cell chromatin stability and morphology. (Sertoli cells play a leading role in the formation and development of the testis in fetal and early postnatal life. They also provide the environment and support for germ cells during spermatogenesis after puberty; Hutchison et al., 2008). Environmental effects, including the prenatal environment, were largest for sperm count.

Asklund et al. (2007) found that the fathers of DZ and MZ twins' percentages of motile sperm were 11.5% and 12.5%, respectively, higher than those of fathers of singletons. (The twins' zygosity was confirmed by DNA analysis.) In addition, fathers of DZ and MZ twins' percentages of morphologically normal sperm were 3.6% and 4.6%, respectively, higher than those of nontwin fathers. Fathers of DZ twins had a 24.7% higher sperm concentration and a 21.8% higher total sperm count (albeit not statistically significantly different from that of nontwin fathers), whereas fathers of MZ twins had a 17.7% higher sperm concentration and a 16.4% higher total sperm count relative to fathers of nontwins. Maternal age and parity did not affect these findings. The authors of this study were somewhat surprised by the extension of their findings to MZ twins, as well as DZ twins. They also noted that their study supported prior research showing a decline in DZ twinning among subfertile males.

The results regarding semen quality are especially interesting. It would be of great interest and significance if researchers were to obtain such measures on males who fathered more than one set of DZ twins. The original studies did not indicate whether these fathers had conceived more than one DZ twin pair, or whether they had conceived twins with more than one woman; however, had such males been included it is likely that this information would have been provided. It would also be of importance to know whether women who conceive DZ twins do so subsequently, but with other males.

This article has concentrated on DZ twinning, but evidence of familial MZ twinning in specific places around the world is being studied. Specific genes linked to MZ twinning have been proposed, although transmission on the paternal or maternal side has not been indicated. Information about these studies is summarized in Segal (2017) and at the website for the Reverade Laboratory (2020) in Singapore.

A Collaborative Maternal Factor and Twinning

Multiple birth findings from Zambia, Pakistan, India, Guatemala, Kenya and Argentina have been reported by an international research team (Marete et al., 2014). It was found that while maternal age and parity were significantly higher for multiple birth mothers (across all countries combined) than for nontwin mothers ($M = 26.10 + 5.40$ years and $24.5 + 5.1$ years, respectively; $Mdn = 2$, range 0–3, and 1, range 0–3, respectively), maternal age was considerably younger than the typical mid-thirties age and beyond found in Western nations (see Segal, 2017). The young age of these mothers suggests a different female physiological background in those populations. As suggested, (1) studying males who fathered twins with different women, and (2) following these women to determine whether they conceive additional twins with different males would bring needed insight to this area.

Twin Research Reviews

Monozygotic Twins Discordant for Parkinson's Disease

The monozygotic (MZ) twin concordance rate for Parkinson's disease (PD) is low, at just 20%. Therefore, factors affecting the

differential expression of this condition in genetically identical individuals are of interest. An international European team recruited five pairs of MZ twins discordant for idiopathic PD (Dulovic-Mahlow et al., 2021). Their aim was to examine mitochondrial DNA (mtDNA) changes that might align with the within-pair disease differences. Skin biopsies were used to establish fibroblast (cells in connective tissue) cultures from which mtDNA was extracted and whole genome sequencing was performed. It was concluded that subtle molecular variations in mitochondrial integrity are of clinical significance and that somatic mtDNA variants might serve as modifiers or biomarkers of disease expression. Genome sequencing did not yield genetic differences.

Fetal Loss in Twin Pregnancies Following Prenatal Diagnosis

A collaborative effort by researchers from Italy, England and Russia compared fetal loss in twin pregnancies in which mothers had or had not undergone chorionic villus sampling or amniocentesis (Di Mascio et al., 2020). The meta-analysis included 16 studies in which the prenatal diagnostic procedures had been used in 3419 twin pregnancies and had not been used in 2517 twin pregnancies. No significant group difference was evident when comparing fetal loss before 24 weeks and within 4 weeks of having had the procedures. The rate of fetal loss was lower than the rates of 3.07% and 3.84% reported in previous studies, but was closer to those reported by the American College of Obstetricians and Gynecologists, in which the rate of pregnancy loss linked to amniocentesis in twins is approximately 2%.

Uterine Rupture and Repair in an Early Twin Pregnancy

It is rare for a woman pregnant with twins to experience early uterine rupture, followed by a partially successful outcome. Such a case was initially published in 2002 by Chinese researchers, but a recent case prompted these researchers to revisit their case for comparison with a recent Japanese report (Takahashi et al., 2020; Yao & Jin, 2020). In both instances, the women became pregnant with twins via assisted reproductive technology — in the 2002 case by in-vitro fertilization and embryo transfer, and in the 2020 case by artificial insemination and gonadotropin therapy. In both instances, surgical repair allowed the pregnancy to continue, but only single remaining fetuses were delivered at approximately 35 weeks' (+2 weeks) and 34 weeks' gestation, respectively. Yao and Jin (2020) noted that the distance between gestational sacs in a dichorionic-diamniotic twin pregnancy plays a key role in the continuation of the pregnancy following removal of the inviable fetus.

Twin Study of Affectionate Communication

Possible genetic effects on affectionate communication were investigated using a twin research design (Floyd et al., 2020). Affectionate communication as a trait was defined as the purposeful and apparent enactment of expressions of closeness, care and fondness toward another. The twins, drawn from the Washington State Twin Registry (WSTR), included 229 MZ pairs and 235 DZ same-sex and opposite-sex pairs. The WSTR twins' zygosity is assessed by responses to questions about childhood similarity. Twin participants completed a 10-item Trait Affection Scale. It was determined that 46% of the variance in expressed affection and 21% of the received variance were explained by genetic effects. Female twins and twins 50 years of age and older were mostly responsible for these findings.

Human Interest

Conjoined Twins in a Triplet Set

Eighteen-year-old triplets, Mackenzie, Macey and Madeline Garrison from Iowa, are unique in that Mackenzie and Macey were originally part of a conjoined twin pair (Goldstein & DeSantis, 2020). The three sisters, who appear to be identical from photographs, differ considerably in height — Mackenzie and Macey are of similar stature, but only reach as high as Madeline's shoulders. The two were joined at the pelvis and separated in 2003, at age 10 months, by Dr James Stein, the chief medical officer at Children's Hospital in Los Angeles, California. The operation required 24 hours to complete. The surgery was successful, but both triplets were left with one leg so must rely on prosthetics for mobility. Since then, they have undergone spinal surgery to correct scoliosis. The triplets were adopted by Darla Keller and her family soon after the first surgery; the Kellers also have three biological sons. The triplets plan to graduate from high school and attend college, although scholarships will be required.

A recent essay on the unknown paths our lives might have taken, due to personal decisions or chance events, is worth reading in this regard (Rothman, 2020). I would assert that there is an exceptional class of individuals who *are* able to see themselves living an alternative life — they are identical twins reared apart from birth and identical twins discordant for life-changing circumstances, as is true of these triplets. Many illuminating examples of this phenomenon can be found within this rare population (Segal, 2012; Seltz et al., 2004).

Identical Twin Nurses Deliver Identical Twins

Twenty-six-year-old identical twins, Tori Howard and Tara Drinkard, are nurses at the Piedmont Athens Regional Medical Center in Athens, Georgia (Pelletiere, 2019). Tori works in the Neonatal Intensive Care Unit (NICU), while Tara works in labor and delivery. The twins were both assigned to be present for the delivery of identical twins, Addison and Emma Williams, on September 25, 2019. Addison was born 3 minutes ahead of her twin sister. Like twins in many other pairs, these twins were born prematurely at 32 weeks, 6 days. They remained in the hospital's NICU until they were healthy enough to be discharged. The twins' parents, Brannan and Rebecca, were delighted that another set of identical twins — Tori and Tara — were involved in the twins' delivery and newborn care. This exciting story also means a great deal to Tori and Tara.

Identical Twins Discordant for COVID-19 Recovery Course

In a previous issue of *Twin Research and Human Genetics*, I surveyed ongoing and planned twin studies of COVID-19 infection (Segal, 2020). Since then, several media reports of twins who contracted the virus have appeared and deserve mention for possibly generating future hypotheses and questions. Identical male twins from Italy developed COVID-19 symptoms on the same day, in March 2020. They were 60 years of age, resided at the same address and were employed at the same automobile repair shop (Remaly, 2020). Despite these life history similarities, the twin brothers followed dramatically different paths toward recovery. One twin developed fairly mild symptoms and was discharged from the hospital after a 12-day stay. In contrast, his co-twin developed a more serious form of the infection that required a 22-day hospital stay and mechanical ventilation. Speculation as to why the twins showed these marked discrepancies include prenatal events that

may have differentially affected their immune response, unexamined differences in their gut microbiome or their initial viral load (quantity of virus present in a given volume of fluid).

Additional details about this case are available in the original case report (Lazzeroni et al., 2020). It is important to note that the researchers classified the twins as identical based on their appearance and other personal characteristics, rather than DNA analyses or a standard physical resemblance questionnaire. In the event that the twins are truly fraternal, the results and conclusions of this case report would require considerable revision. Media reports are not held to these standards, yet readers should view twin type with some caution if classification is not scientifically demonstrated.

Identical Twins Pass Away from COVID-19

Twin brothers, Rosendo and Rogelio Mendoza, passed away from COVID-19 just hours apart (Diaz, 2020). The 56-year-old twins, who appear to be identical based only on inspection of their photographs, were cared for at the University Medical Center in Lubbock, Texas. They worked together as welders on oil rigs and were considered inseparable. It is unclear how they contracted the virus, but both twins developed symptoms during the weekend prior to Thanksgiving, 2020. Both twins had been placed on ventilators. Rogelio suffered from an unnamed pre-existing medical condition that affected his breathing and his condition quickly became serious. Rosendo did not suffer from this condition, but his heart weakened and he succumbed to the disease approximately 16½ hours after his twin brother, Rogelio. These twins pose a strong contrast to the Italian twins described above, highlighting the complex and unpredictable nature of COVID-19.

Archeological Finds of Oldest Identical Twins

In an exciting discovery, Viennese archeologists, in collaboration with international scholars, identified the remains of the oldest known identical twin pair (Nature, 2020; Teschler-Nicola et al., 2020). The twin boys, estimated to date back to over 30,000 years ago, were buried beneath the shoulder blade of a mammoth at the Austrian site of Krems-Wachtberg. Their identical twinning was established by genomewide ancient DNA analyses. Based on study of the twins' maxillary secondary incisors, isotope measurements and barium distribution in enamel (a biomarker of breastfeeding), the twins were considered to be full-term, although one twin appears to have passed away at birth, while his co-twin survived for 50 days. The remains of a third male infant were discovered nearby and is likely to be the twins' third-degree relative. This conclusion was based on the increased and nonoverlapping coefficient of relatedness between this third infant and any other individual discovered at the site. It was also noted that the three infants had the same Y chromosome and mitochondrial haplogroups. (Maternal haplogroups consist of mitochondrial DNA that extends back to a single common ancestor.) There are over 4000 known haplogroups; see van Oven & Kayser, 2009).

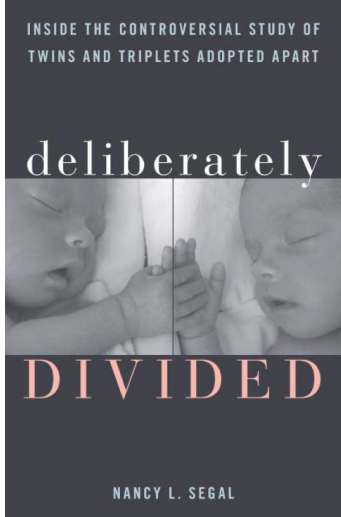
In a related finding, University of Saskatchewan bio-archeologist, Angela Lieverse, described what she had believed was the earliest record of human twins (Lieverse et al., 2015; CBC Radio-Canada, 2015). A study of a Siberian female skeleton revealed the presence of two full-term fetuses in the womb. The positioning of the bones suggested that one twin was breech and

the other was in the process of being born. The remains were estimated to be 7700 years old.

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Dr. Nancy L. Segal's newest book, ***Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart*** (published by Rowman & Littlefield) is now available for pre-orders on most websites. The book presents an in-depth look at the 1960s-70s New York City study of separated twins whose development was covertly tracked by psychoanalytic psychiatrists and assistants — without telling the parents they were raising a reared-apart twin. The study was featured in the recent documentary films, *The Twinning Reaction* (2017) and *Three Identical Strangers* (2018).