

NEWS, VIEWS, AND COMMENTS

Zygoty Diagnosis: When Physicians and DNA Disagree/Twin Research: Sex-Discordant Chimeric Twins; Unrelated Bone Marrow Transplantation in Infant Twins With Congenital Amegakaryotic Thrombocytopenia; Twin Study of Attractiveness to Mosquitoes; Twins Coping With Crisis/Media Highlights: The Less Favored Twin; Paternity Issues; Twins With Late-Onset Tay-Sachs Disease; Triplets at MIT

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Physicians and other medical professionals do not always provide new parents with an accurate diagnosis of their twins' zygosity. An overview of this problem is presented, supplemented by an interview with a mother who recently learned that her 2-year-old 'dizygotic (DZ)' twin girls are actually 'monozygotic (MZ)'. Reviews of two case studies, one of twins with sex-discordance and chimerism and the other of twins with congenital amegakaryotic thrombocytopenia, follow. Two additional studies, one a twin analysis of attractiveness to mosquitoes and the other a study of twins coping with crisis, are also described. Several articles and letters from the popular media, concerning less favored twins, paternity issues surrounding superfecundation, twins with late-onset Tay-Sachs disease, and triplets admitted to MIT are informative and insightful.

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NOTE: Please see the July 12, 2015 issue of the *New York Times Magazine*, featuring switched-at-birth twins from Bogotá, Colombia. The twins were studied by Nancy Segal and her colleague Yesika Montoya in March–April. The link to the article is as follows: http://www.nytimes.com/2015/07/12/magazine/the-mixed-up-brothers-of-bogota.html?_r=0

Watch for a wonderful new documentary film called *Twinsters*. It tells the remarkable story of identical female twins born in South Korea, but raised separately in France and the United States. Without the Internet, they never would have known of each other. Research on these twins can be found in: Segal, N. L., & Cortez, F. A. (2014). Born in Korea — adopted apart: Behavioral development of monozygotic twins raised in the United States and France. *Personality and Individual Differences*, 70, 97–104; and in the book: Bordier, A., & Futerman, S. (2014). *Separated @ Birth: A True Love Story of Twin Sisters United*. New York, NY: G.P. Putnam's. Nancy Segal had two small parts in the film.

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Zygoty Diagnosis: When Physicians and DNA Disagree

There is considerable misinformation and misunderstanding about the nature and purpose of zygosity diagnosis for twins. The most accurate index of twin type involves within-pair comparison of (usually) 15 short tandem repeat (STR) markers. Complete concordance for all markers identifies monozygotic (MZ) twins with virtual certainty. That is because there is a very high degree of individuality in STRs — uniquely repeating patterns in specified DNA regions make it very unlikely for two non-identical twins to match across all 15 markers (National Forensic Science Technology Center, 2013). More recently, there has been increased use of Genome-Wide Association Study (GWAS) single-nucleotide polymorphism (SNP) chips for assessing twin type. This procedure involves purifying twins' DNA, placing it on tiny chips, and scanning it for selected markers (National Human Genome Research Institute, 2015).

Unfortunately, DNA analysis is not routinely offered to new parents of the same-sex twins, and the cost of the procedure may be prohibitive for some families. In addition, some mothers and fathers may prefer not to know the zygosity of their twins, concerned that it will affect their parenting in unknown ways.

Some people, including physicians and other medical personnel, often rely on placentation for clues to newborn pairs' twin type. However, misleading results can emerge from this practice. Approximately one-third of MZ twins and nearly all dizygotic (DZ) twins have separate chorions, amnions, and placentas. However, approximately two-thirds of MZ twins share a placenta and chorion, with a small fraction sharing an amnion, as well. Some MZ and DZ twin pairs (55% and 61% of deliveries at more than 35 weeks, respectively; Machin & Keith, 1997) show fused placentation, such that DZ twins might be incorrectly diagnosed as MZ. Lastly, rare cases of DZ twins with a common chorion have been described (Redline, 2003).

Inspection of fetal membranes by sonogram typically classifies twin type with a high level of certainty. The chorion and amnion fuse by the 16th gestational week, so a bright area surrounding the gestational sac can be seen; this image can no longer be visualized during the second and third trimesters (Nancy Corrales, personal communication, 22 January, 2015). However, errors in judgment and evaluation are still possible with this method, as revealed by Judicial Law Clerk and mother of twins, Tiffany Brown. Tiffany also astutely observed how the new knowledge that her presumably 2-year-old DZ twin daughters are really MZ has affected her parenting practices in positive ways.

Tiffany and her husband Chad reside in Benton, Arkansas, just outside Little Rock. They were parents to 2-year-old Jackson when their twins, Gabriella and Grace, were born. At 15 weeks' gestation, the perinatologist was '80% certain' that the girls were fraternal, based on the

'thick inter-twin membrane' that was evident in an ultrasound. The ultrasound technician concurred, based on the same evidence, explaining that she typically saw a thinner boundary between identical twins.

Following the twins' birth, it was easy for Tiffany to believe that the twins were fraternal. The girls differed slightly in birth weight (Gabriella was 5 pounds, 7 ounces, and Grace weighed 5 pounds, 4 ounces, a difference they have maintained), although their birth lengths were the same. She, her husband, and son never had trouble distinguishing between them, although close friends and relatives did. This is a common occurrence in the case of MZ twins because family members who spend time with twins become more sensitive than others to subtle physical and behavioral differences between them. The author has shown that her global first impressions of twin type, based on physical resemblance, indexed zygosity more accurately than did physicians, parents, questionnaires, and fingerprints, compared with serological findings (Segal, 1984). As Race and Sanger (1975) said of the late James Shields: 'We find that the blood groups practically never contradict the opinion of such a skilled observer of twins'.

Early on, Tiffany felt annoyed when people suggested that her twins might be identical, rather than fraternal. She felt they were being 'lazy' in not differentiating between the girls, due to the linking of twinship with sameness in most people's minds. However, her obstetrician (and family friend) began to suspect that the girls were identical, based on their strong physical similarities, and informed Tiffany that DNA testing was the only way to be certain. As the twins turned 2 in age, even Tiffany began questioning her initial judgment, especially when she observed the girls singing in unison, making up the music as they went along. Her pediatrician also noted that the twins' hair color, eye color, growth pattern, and tooth eruption were highly matched.

The family joined the University of Washington Twin Registry and arranged for DNA testing of the twins to be done with Affiliated Genetics, a laboratory based in Salt Lake City, Utah. The results were not what Tiffany expected, and are reproduced here because of their helpful insights for families and physicians:

Instantly, my world turned upside down; this was a case of mistaken identity . . . For so long, I had focused on all their differences instead of their similarities, although I had *longed* to see what other people had seen . . . to be able, for the first time, to see my girls as others had seen them was like discovering all over again that I was having twins . . . I felt a sense of relief receiving the news . . . On the other hand, I was rattled at the thought that my pride and stubbornness in refusing to entertain the possibility that my girls were identical *could have* resulted in my daughters not fully appreciating who they are — their very identity. I wondered if their bond would have been compromised by *my* mistaken belief that they [were] fraternal.

Research shows that parental beliefs about twins' zygosity do not affect their behavioral resemblance (Kendler et al., 1994). However, parental beliefs may affect parenting in small but significant ways. Tiffany believes she is now more focused on the twins' similarities than their differences. She noted that each girl becomes panicked upon awakening if her sister is still sleeping. Previously, she felt that her daughters were being 'cranky', but now she appreciates that this reaction may reflect their developing bond. In addition, Tiffany had previously worried that as fraternal twins, the girls might compare themselves with one another, whereas now she hopes they will take pride in each other's accomplishments. She is also more sensitive to interactions between her twins and her older son. Knowing that identical twins tend to form close bonds, she makes certain that Jackson receives the attention he deserves.

There are many other reasons for twins and their families to have accurate zygosity information. Many of these reasons are documented in an upcoming debate to be published in the *British Journal of Obstetrics and Gynaecology* (Craig et al., in press). Included among them are improved management of twins' prenatal difficulties, understanding of their developmental events, and evaluation of their medical life histories. The point that DNA testing should be routinely offered to parents of same-sex twins is underlined. Clearly, knowledge of twin type should be welcomed by twins and their families, as well as by the medical and educational professionals working with them.

Twin Research

Sex-Discordant Chimeric Twins

A recent case report of sex-discordant monochorionic (MC) twins promises to renew debate over the biological mechanisms underlying DZ twinning (Rodriguez-Buritica et al., 2015). A male co-twin underwent medical evaluation at the age of 2 months, following detection of aortic stenosis (narrowing of the heart's aortic valve) and hypospadias (opening of the urethra on the underside of the penis). The pregnancy was unremarkable and the twins were delivered by cesarean section at 39 weeks' gestation. Prenatal ultrasound indicated a single placenta, but no symptoms of fetal transfusion. Placental analysis identified a monochorionic/diamniotic placenta, characteristic of MZ, not DZ gestations.

Analysis of a blood sample from the male co-twin revealed two cell lines of similar percentage: 46, XY/46, XX. The skin karyotype also showed the two cell lines, and blood interphase fluorescence in situ hybridization (FISH) analysis showed the 46, XY cell line in 43% of the cells, and the 46, XX cell line in 57% of the cells. SNP analysis indicated five track combinations, consistent with two different genomes, whereas three track combinations are observed in samples with a single genome.

The phenotypically normal female co-twin also showed two cell lines, 46, XX/46, XY. Thirty-two of her skin cells showed the normal female 46, XX cell line, while blood interphase FISH analysis showed the 46, XX cell line in 44.5% of her cells and the 46, XY cell line in 55.5% of her cells. Other molecular genetic procedures were also performed for both twins.

Several key findings and issues emerged from this case study. First, the presence of two different cell lines in different tissues showed that the sharing of cells between the twins was not confined to blood, as was the case in previous reports. Second, the developmental origin of this particular case (MC/DZ twinning, with discordant phenotype and single placentation) remains at issue. The investigators presented a number of fascinating biological scenarios, but settled on one involving embryo amalgamation. Specifically, they posited that the twins were originally two separate embryos (46, XY and 46, XX) that amalgamated, exchanged cells, and twinned between days 4 and 8 after conception, eventuating in an MC pregnancy. This process, also called tetragametic chimerism, would be consistent with the different phenotypes reflecting different proportions of cell lines in the gonads. Given this possibility, the female co-twin would be expected to have Y chromosomal material in tissues other than blood, although such evidence was not detected. The investigators called for additional research on events giving rise to such unusual twinning, and suggested that MC/DZ twins may occur more commonly than suspected.

Unrelated Bone Marrow Transplantation in Infant Twins With Congenital Amegakaryotic Thrombocytopenia

Monozygotic female twins with congenital amegakaryotic thrombocytopenia (CAMT) were referred to physicians at the Mayo Clinic in Rochester, Minnesota for evaluation and treatment (Rao et al., 2015). CAMT is a rare inherited autosomal recessive condition that involves thrombocytopenia (low blood platelet count). If untreated, this disorder evolves into bone marrow failure. CAMT is usually recognized within the first month of life, although the twins in question were seen at the Mayo Clinic at the age of 3 years.

Congenital amegakaryotic thrombocytopenia is best treated in long term by allogenic stem cell transplantation (transplantation of stem cells from one person to another). Successful transplants most often involve genetically related donors and recipients, although CAMT has been managed effectively with transplants between non-relatives. The twins in question lacked a suitable related donor, so an unrelated donor was sought. The first twin received her transplant 2.6 years ago and is doing well with 100% donor chimerism. The second twin received her transplant 1.5 years ago and is also doing well with 100% donor chimerism. The preparatory regimens differed between the co-twins,

given their different symptoms and severity, although the donated stem cells were matched.

Further research into the treatment of this condition is encouraged. Unfortunately, the method by which the twins' zygosity was assessed was not reported. However, tests conducted to determine their shared compatibility with the same donor, and their success following the receipt of matched cells are consistent with monozygosity.

Twin Study of Attractiveness to Mosquitoes

Individual differences in attractiveness to mosquitoes have been reported scientifically and observed anecdotally. A self-report comparative rating questionnaire was used to assess genetic influences on mosquito attraction and mosquito bite frequency in a sample of 197 MZ and 26 DZ Australian twin pairs (Kirk et al., 2000). Genetic effects on mosquito attraction as measured by an ordinal rating scale were reported, but showed low reliability. However, the comparative rating measure in which participants indicated which twin was bitten more often yielded strong genetic effects.

The mechanisms underlying mosquito bite attraction and frequency have been at issue. It has been suspected that individuals' particular body odors predispose some people to be bitten more frequently than others. The first twin study to assess the heritability of this trait by exposing mosquitoes to human body odors was undertaken recently (Fernandez-Grandon et al., 2015). Participants included 18 female MZ and 19 DZ female twin pairs from the TwinsUK database in London. All twins were post-menopausal to avoid hormonal effects from the menstrual cycle. The mosquitoes were groups of 20 *A. aegypti* females between 5 and 7 days of age. Prior to the study, the mosquitoes were fed glucose solution only.

The mosquitoes were exposed to the twins' hands through a Y-shaped olfactometer made of plexiglass. One dataset included a test of each individual twin's susceptibility when paired with clean air. The second dataset included co-twins paired with each other. Ten replicates of each treatment set were completed. Narrow-sense heritabilities were substantial for both relative attraction (0.62) and flight activity (0.67). When twins were tested together, a higher value emerged for relative attraction (0.83). The authors concluded that genetic influence on attractiveness to mosquitoes was demonstrated, but noted that the small sample limited the precision of their genetic estimates. In addition, the mechanisms underlying their results remain uncertain. For example, whether the differences between the MZ and DZ twin pairs are best explained by the presence or absence of attractants or repellants is uncertain. However, the findings increase the likelihood that more effective insect repellants may be available in the future.

Twins Coping With Crisis

Emotorics is a movement observation system for the analysis and interpretation of emotive behavior. It is based on

a paradigm, developed by dance-movement therapist Y. Shahar-Levy in 1996, which regards human motor behavior as expressing individuals' emotive features such as perceptions, feelings, and memories (Specktor, 2015). Based on personal observations and relationships with twins, Specktor (2015) concluded that all co-twins, regardless of zygosity, can be classified as either TT1 (individuals characterized by competency so are seen by others as mature, relative to their co-twin) or TT2 (individuals seen as childlike in comparison with their co-twin, yet are characterized by superior emotional competencies, making them better able to cope with crises.) Specktor further asserted that events responsible for these within-pair differences occur during the very early years, and persist. She then applied the Emotorics system to two MZ male pairs, one DZ female pair, one DZ male pair, and one opposite-sex pair, between 5 and 7 years of age. Twins in all five pairs were classifiable as TT1 or TT2.

Greater attention to the origins and nature of intra-pair twin dynamics is very commendable and clearly needed. However, the classification system (TT1 and TT2) was based on impressions rather than on systematic study, the hypothesized link between the twins' very early experiences and childhood was not substantiated, and the Emotorics system was applied to a very limited number of pairs whose zygosity determinations were undocumented. Hopefully, these issues can be addressed in future research.

Media Highlights

The Less Favored Twin

The Sunday *New York Times* publishes a column titled 'Social Q's', to which readers can send relevant questions about socially difficult issues (Galanes, 2015). Answers to selected inquiries are published each week. An entry labeled 'A Twin in Need' came from a high school senior and (apparently) fraternal twin: 'I have to deal with my twin sister who is smarter, prettier, and cooler than me'. She also wrote that her parents 'barely look' at her because her sister is at the center of all things. She sought advice on how to make the months prior to leaving for college more tolerable.

The multi-part answer given was a good one: Everyone feels like the overlooked sibling on occasion; spend time away from the twin sister by (hopefully) attending a different college; apply for a summer camp counseling job away from home; for a 'vicarious thrill', see *Hand to God*, a Broadway play about a shy teenager whose puppet, attached to his left hand, shouts out every mean comment the boy would love to say.

I believe that other fraternal twins will resonate to the question and answer presented above. I also suspect that fraternal twins' relationship and identity issues are overlooked because behavioral differences between them are expected. Greater parental and professional attention to these twins' developmental trajectories and outcomes should be encouraged.

Paternal Issues

In May 2012, Judge Sohail Mohammed of Paterson, New Jersey ruled on a case involving superfecundated twins with different fathers (Correction: Twin-2 Dads, 2015), known as heteropaternal superfecundation. (Superfecundated twins, conceived at different times within the same menstrual cycle, can also have the same father.) It was decided that the man from whom the twins' mother sought child support would be responsible for only one of the children. Initially, the woman had sought child support from this individual whom she assumed was the father of both twin daughters. However, DNA evidence showed that from this individual whom fathered only one of the twins.

Judge Mohammed's written opinion indicated that the situation of twins having different fathers is very rare. Some medical investigators have recently underlined the uniqueness of this event, but have cited literature dating to the early 1990s (Peigné et al., 2011). I would take exception to this conclusion for several reasons. A number of superfecundated twin cases have been reported in the medical literature, albeit as scattered case studies. Even collectively, the number is likely to underestimate the true frequency of superfecundation because not all cases are discovered. It is more likely that the actual frequency of superfecundated twins with different fathers approaches that of extramarital births, in general. In 2010, 40.8% of births in the United States were to unmarried women (The Fraying Knot, 2013). Of course, non-paternity rates vary considerably across populations and regions, from 2% in a representative Mormon sample to 30% in a southeastern British sample; the British data were based on blood group analyses from an unpublished study of residents living in one town (Olson, 2007). A link between a greater frequency of sexual activity and fraternal twinning has also been reported, based partly on an elevated frequency of illegitimately conceived fraternal twins (James, 1992); however, an earlier study did not find differences in coital frequency among parents of MZ twins, DZ twins, and singletons (Bonnelyke et al., 1990). The foregoing is generally consistent with higher rates of heteropaternal superfecundation than have been reported.

A 1992 review reported that the frequency of heteropaternal superfecundation in fraternal twins whose parents were involved in paternity suits was 2.4% (Wenk et al., 1992). Judge Mohammed's opinion identified only two previous court cases, a number I have confirmed. However, not all questions of non-paternity involving twins are handled in the courts.

Twins With Late-Onset Tay-Sachs Disease

Tay-Sachs disease is a recessive genetic condition with symptoms developing in affected infants at about 6 months of age. It involves the absence of an enzyme that breaks down

fatty substances. These fatty substances then accumulate in the brain, affecting the nerve cells. Loss of many body functions follows, including eyesight, hearing, and movement. There is no known cure for this condition (Mayo Clinic, 2014). There is also a rare, late-onset form of this disease that has received little attention until recently.

Identical twin, Katie Buryk, was in college when she found it challenging to climb stairs (Buryk, 2015). Eventually, she had difficulty walking, standing up from a sitting position, and performing everyday tasks. She consulted the Mayo Clinic in Rochester, Minnesota before she, her twin sister Allie, and her parents underwent genome sequencing. The procedure showed that both she and her twin have late-onset Tay-Sachs disease, while their parents are both carriers of the relevant gene form. According to the National Tay-Sachs and Allied Diseases Association, fewer than 100 late-onset cases worldwide have been reported.

There is no known cure for this form of Tay-Sachs disease, and little research has been conducted to find one, most likely due to its rarity. Consequently, the Buryk twins have appealed to the public by establishing the Katie and Allie Buryk Research Fund. After only two months, by February 2015, they raised over \$120,000. On May 7, 2015, their case appeared in the *New York Times* in the 'Think Like a Doctor' column, in which readers are given details of a medical case and asked to render a diagnosis; the first person to submit the correct solution receives a book about medical reasoning and diagnosis written by the physician who described the case.

Triplets at the Massachusetts Institute of Technology (MIT)

Fraternal triplets, Christopher, Claire, and Edward Goul, of Newport Beach, California, will be attending MIT in the Fall 2015 (Annear, 2015). This competitive university accepted less than 8% of this year's applicants, making the triplets' success even more remarkable. An MIT official noted that triplets have attended the school in the past, but that such events are rare. It is, therefore, worth noting that more than one set of quadruplets have been admitted to Yale University (Fitzgerald, 2009; Steinberg, 2010)

Genetic influence on general intelligence and special skills has been well documented by scores of twin and adoption studies, with identical twins showing greater similarity than fraternal twins (see Segal, 2012). However, some fraternal twins and triplets are expected to be alike. In spite of their overall matched academic success, the Gouls have chosen different majors, including electrical engineering (Christopher), biology (Claire), and math and physics (Edward). Initially, the triplets had planned to enroll in different schools, but because of anticipated stresses associated with college study, they are pleased that they will be attending MIT together.

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