Monozygotic (MZ) twins occur when one oocyte is fertilized by one spermatozoon and at some later time divides to form two embryos. The causes of this split are uncertain. Dizygotic (DZ) twins occur when two oocytes are fertilized by two spermatozoa. The frequency of MZ twinning probably is constant throughout the world, whereas DZ twinning is racially mediated with the highest rate occurring in Africans and the lowest in Asians.

When unlike sex twins are born, no question exists regarding their zygosity. They are by definition DZ. In contrast, when like-sexed twins are born, the question of zygosity arises. Unless chorionicity has been demonstrated accurately in the antenatal period and correctly interpreted at the time of delivery, it is possible to err in zygosity assignment based on placentation and membranes. In this error, it is commonly assumed that like-sexed twins are DZ because it is determined that they are dichorionic (DC) at birth. The genesis of this error is the widespread recognition that like-sexed DC twins (with fused or separate placental discs) may be DZ or MZ (approximately 25% are MZ).

The term “identical” is commonly used as a shortcut for MZ, but this is a serious misnomer. Rarely if ever are MZ twins “identical” in their phenotypic appearance even if they carry the same genetic patrimony.

WHEN TO TEST FOR ZYGOSITY

The optimal time to determine zygosity is at delivery. The obstetrician can do the assessment easily, either alone or in consultation with the pathologist. The benefits of such an assessment include: first, the ability to state with certainty that MC twins are MZ; and second, the institution of formal zygosity testing, using placental tissue, in all like-sexed DC twins, recognizing that 25% of such twins are MZ and the remainder DZ.

AVAILABLE METHODS OF ZYGOSITY DETERMINATION

Historical: In 1874, the French mathematician, Bertillon, assumed that the sex of each zygote of a pair of DZ twins would be determined independently, postulating that the number of DZ pairs was equal to twice the number of unlike-sex pairs, with the remainder of the like-sexed pairs presumably MZ. Restated in 1902 by Weinberg, this concept was criticized almost from its inception and is still subject to intense negative interpretation, as its usefulness is purely confined to statistical samples.

Physical Characteristics Assessment: Over the years, the following characteristics have been studied: biometric parameters, skeletal structures, skin, hair and dermatoglyphics, ocular and orbital anatomy, nasal and dental characteristics, and specularity (mirrored or reversed asymmetry). Many such physical characteristics are poorly developed in newborns and, later in life, comparisons are often not sufficiently robust to determine a definitive diagnosis of zygosity. Additional characteristics used, albeit with varying degrees of efficacy,
include: ear forms, patterns of ridging on the tongue and dental eruption patterns, as well as tooth morphology. Fingerprints are never completely identical, neither are the patterns in the iris of the eye. Indeed, the probability of two different irises agreeing by chance in more than 70% of their phase sequences is one in seven billion.

COMMONLY USED METHODS

Blood Groups: A commonly used and relatively inexpensive method of zygosity determination is the use of blood groups and human leukocyte antigens (HLA). Zygosity can be determined from blood by studying common population variants known as polymorphisms. These include the common blood groups, HLA types, serum proteins, enzyme polymorphisms and, most recently, DNA variations.

Using the ABO blood groups as an example, if the father of the twins is blood group AB and the mother is group O, the offspring may be either group A or group B. If one twin is group A and the other is group B, the pair is clearly DZ. If both are A or B, however, zygosity is unproven. The process can then be repeated for many other sets of polymorphisms, with the intent of establishing differences (diagnostic of DZ) or, alternatively, a high statistical probability of MZ on the basis of failing to detect differences. The higher the statistical probability desired, the more difficult it is to achieve.

DNA: DNA is undoubtedly the most sophisticated form of zygosity detection using blood and other bodily tissues as the substrate. It is commonly characterized as ‘DNA fingerprinting’. Although many authorities describe it as being most accurate, it can also be most confusing because, as of this writing, there is no gold standard for the analytic procedure. DNA testing analyzes genes rather than their protein products. As such, several genetic loci are tested simultaneously, and a pattern unique to the individual is quickly determined. Using this technology, MZ twins share identical genomic patterns in some studies. The likelihood that a DZ pair would also exhibit superimposable patterns is exceedingly low (3x10^-14).

The major problem in DNA analysis for determination of zygosity relates to the existence of small-scale mutations. This is the process by which DNA changes in specific zones, e.g. point mutations, deletions and insertions, trinucleotide repeat sequences, and tandem repeat sequences.

INCREASING POSTNATAL DISCORDANCE

The phenomenon of increasing post-natal discordance supports the concept that MZ twins are not truly “identical”. A number of post-zygotic phenomena begin at birth and affect the respective genotypes of MZ pairs. The extent to which these changes are visible depends to a great degree on the method used to analyze the DNA components. One method uses a number of microsatellite probes, also called variable number of tandem repeat markers (VNTR). The other uses restriction fragment polymorphism (RFLP). The former methodology examines DNA under a high-power microscope, as it were, whereas the latter examines segments of DNA at “lesser magnification” and provides a “broad brush stroke” diagnosis of monozygosity by failing to pick up the inevitable smaller, post-zygotic mutational differences that are now thought to be present in most, if not all, MZ twin pairs.

Case Study: The senior author (LK) and his identical twin brother (Donald Keith) exemplify some of the difficulties in accurate zygosity determination. Both are male and there were two placental discs and membranous sacs at the time of their birth in 1935. They were classified as DZ on the basis of the placentation alone by no less an authority than Dr. Irving Stein of Stein-Leventhal fame. Their childhood phenotypic appearances were remarkably similar, so much so that the diagnosis of DZ became questionable in their minds. At the age of 41, they were examined by Professors Luigi Gedda, Robert Deron and Walter Nance who requested blood samples and fingerprint analyses. Based on the results of these examinations, they were declared to be MZ with the probability of 99.6%.

When DNA analyses first became available, a mouthwash sample of both twins was obtained for an absorption spectrum. Based on a difference in one DNA zone where multiple repeat sequences were present they were reclassified as DZ by Dr. Andreas Busjahn of the Franz Volhard Clinic and the Max Delbruck Center for
Molecular Medicine of the Humboldt University in Berlin. When RFLP technology was used shortly thereafter in two different reference laboratories (Fiona Bamforth in Canada, and Catherine Derom in Belgium), they were once again classified as MZ.

Further Considerations: Because of the uncertainties just described, the use of combinations of tests has been proposed. A logical supplement to any of the standard tests is a self- or parent administered questionnaire. This concept is not new, but its use in combination with other tests is more recent. Accuracy of most reported tests is 90% or more.

IMPLICATIONS FOR CLINICAL PRACTICE
The following implications for practice derive from the recognition of the complex nature of zygosity testing, its long and continuing evolution and recent advances in ultrasound and molecular genetics.

1. All MC twins are MZ, but they may be discordant for genetic disease, malformations, etc.
2. Unlike sexed twins are DZ.
3. Not all like-sexed DC twins are DZ.
4. Not all like-sexed twins conceived using ART are DZ.
5. Not all twins who are discordant for genetic disease, chromosome constitution or a major malformation are DZ.
6. All like sex twins have the right to know their zygosity.
7. Correct terminology, i.e., MZ/DZ, should always be used rather than misleading and inaccurate terms such as identical/fraternal.

Reference