**Thesis**

# Discordant fetal anomalies in monochorionic multiple pregnancies

submitted by
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Graz, 9.9.2025

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## Zusammenfassung

### HINTERGRUND

Das Phänomen der monozygoten (MZ) Zwillingsentstehung hat stets großes wissenschaftliches Interesse geweckt, insbesondere im Zusammenhang mit monochorialen (MC) Zwillingen. Obwohl traditionell angenommen wird, dass sie einander spiegelbildlich gleichen, zeigen MC Zwillinge gelegentlich strukturelle und genetische Unterschiede. Solche Fälle werfen Zweifel an der Annahme einer vollständigen genetischen Identität (Übereinstimmung) monochorialer Zwillingen auf, und bieten daher ein einzigartiges biologisches Umfeld zur Untersuchung der dynamischen Wechselwirkungen zwischen Genetik, Epigenetik und Umweltfaktoren während der frühen menschlichen Entwicklung.

### METHODEN

Diese monozentrische Studie analysierte retrospektiv und deskriptiv 51 Fälle von MC-Zwillingsschwangerschaften mit diskordanten Anomalien, die zwischen 2013 und 2023 an der Universitätsklinik für Frauenheilkunde und Geburtshilfe in Graz betreut wurden. Die Daten wurden aus dem „MonoReg“-Register entnommen. Um unsere Befunde zu interpretieren und bestehende Hypothesen zu beleuchten, wurde eine Literaturrecherche in PubMed durchgeführt. Die Beobachtungen aus unserer Kohorte wurden mit bereits veröffentlichten Fällen verglichen, um Übereinstimmungen zu bewerten und wiederkehrende Muster zu identifizieren.

### ERGEBNISSE

Die Literaturrecherche bestätigt, dass MC-Zwillinge mit diskordanten Anomalien eine Seltenheit darstellen, wobei jedoch eine Vielzahl unterschiedlicher Fehlbildungen beschrieben wurde. Die genauen zugrunde liegenden pathologischen Mechanismen sind bislang unbekannt. Es wurden jedoch mehrere mögliche Erklärungsansätze vorgeschlagen, darunter eine ungleichmäßige X-Chromosom-Inaktivierung, postzygotische Mutationen und epigenetische Mechanismen wie eine unterschiedliche DNA-Methylierung. In unserer Kohorte traten angeborene Herzfehler, Neuralrohrdefekte und Hydrozephalus besonders häufig diskordant auf. Diese Erkrankungen gelten als multifaktoriell verursacht, was

die bedeutende Rolle des Zusammenspiels genetischer und nicht-genetischer Faktoren unterstreicht. In diesem Zusammenhang erwies sich das Schwellenwertmodell (Liability threshold model) als wertvoller Hypothese, um aufzuzeigen, wie die kumulative Wirkung kleiner genetischer und umweltbedingter Faktoren dazu führen kann, dass ein Zwilling eine pathologische Schwelle überschreitet, während der andere nicht betroffen ist.

**DISKUSSION**

MC Zwillingsschwangerschaften bieten ein faszinierendes Modell zur Erforschung der Interaktion genetischer Prädispositionen und Umweltfaktoren bei der frühen menschlichen Entwicklung und der Manifestation multifaktorieller Erkrankungen. Das Auftreten diskordanter Anomalien bei diesen scheinbar identischen Individuen fordert die traditionellen Annahmen heraus und eröffnet neue Möglichkeiten, die frühesten Einflussfaktoren individueller Entwicklung zu verstehen. Unter Berücksichtigung der limitieren Fallzahl und der teilweise unvollständigen Daten innerhalb der Kohorte, verstehen sich die Ergebnisse nicht als abschließender Nachweis kausaler Zusammenhänge. Stattdessen soll ein zunehmendes Bewusstsein für die Komplexität der Entwicklung monochorialer Zwillinge aufgezeigt werden, und die die Notwendigkeit weiterer longitudinaler und epigenetisch fokussierter Forschung unterstreicht, um die zugrundeliegenden Mechanismen der Diskordanz bei MC Zwillingen umfassend zu verstehen.

## Abstract

### OBJECTIVE

The phenomenon of monozygotic (MZ) twinning has always captivated scientific interest, especially in the context of monochorionic (MC) twins. Despite their traditional perception as representing two mirror images of another, MC twins occasionally exhibit structural and genetic discordances. These instances challenge the assumption of their identical nature and absolute genetic equivalence and present a unique biological environment for investigating the dynamic relationship of genetics, epigenetics, and environmental influences during early human development.

### METHODS

This single centre retrospective descriptive study analysed 51 cases of MC twin pregnancies with discordant anomalies managed at the Department of Obstetrics and Gynaecology at the Medical University of Graz between 2013 and 2023. Data was extracted from the "MonoReg" Registry. To help interpret our findings and explore existing hypotheses, a PubMed literature review was performed. Observations from our cohort were compared with previously reported cases in the literature to evaluate congruence and identify recurring patterns.

### RESULTS

The literature review confirms that while MC twins discordant for anomalies represent a rarity, a diverse range of anomalies have been reported. The precise underlying pathological mechanisms remain unknown; however, several potential pathways have been proposed, including skewed X-chromosome inactivation, post-zygotic mutations and epigenetic mechanisms such as differential DNA methylation. Congenital heart diseases, neural tube defects and hydrocephalus were seen more frequently in our cohort of discordant MC twins. These conditions are recognised as multifactorial diseases, highlighting the significant role of the interaction between genetic and non-genetic factors. In this context, the liability threshold model was shown to provide a valuable framework to explain how the cumulative effect of minor genetic and environmental factors can lead one twin to exceed a threshold for a pathology while the co-twin remains unaffected.

## CONCLUSION

MC twin pregnancies represent a compelling model for investigating the interaction between genetic predispositions and environmental factors in shaping early human development and the manifestation of multifactorial conditions. The presence of such discordant anomalies in these seemingly identical individuals invites a re-evaluation of traditional assumptions and offers new opportunities for understanding the earliest determinants of individuality. Acknowledging the limitations of the sample size and completeness of the cohort data, there is no claim to have definitive proven etiological pathways, but this study rather supports a growing awareness of the complexity underlying MC twin development, emphasizing the need for more longitudinal and epigenetically focused research to fully identify mechanisms driving discordance in MC twins.

## Table of contents

## Abbreviations

MZ ..... Monozygotic
AA ..... arterioarterial
ART ..... Assisted reproductive technologies
AV ..... Arteriovenous
CAKUT ..... Congenital anomalies of kidney and urinary tract
CHD ..... Congenital heart defects
CN ..... Central nervous system
CRL ..... Crown-rump length
DCDA ..... Dichorionic diamniotic twins
DZ ..... dizygotic
HF ..... Hydrops fetalis
ICSI ..... Intracytoplasmic sperm injection
IUFD ..... intrauterine fetal death
IVF ..... In vitro fertilisation
MC ..... Monochorionic
MCDA ..... Monochorionic diamniotic
MCMA ..... Monochorionic monoamniotic
MeSH ..... Medical subject headings
PGT ..... Pre-implantation genetic testing
ROS ..... Reactive oxygen species
SF ..... Selective feticide
sFGR ..... Selective fetal growth restriction
sIUD ..... Selective intrauterine death
TAPS ..... Twin anemia polycythemia sequence
TOP ..... Termination of pregnancy
TRAP ..... Twin reversed arterial perfusion
TTTS ..... Twin-to-twin transfusion syndrome
VV ..... Venovenous
XCI ..... X chromosome inactivation

## List of Figures

# 1 INTRODUCTION

## 1.1 *Motivation*

The appearance of two seemingly identical individuals has long captivated people across the globe. With advancements in scientific understanding, the biological phenomenon of monozygotic twinning, where two individuals emerge from one single zygote, has drawn increasing fascination, offering valuable insights into genetics, human development and the complex factors that influence individuality (1). Among monozygotic twins (MZ), a particular unique position is obtained by the pairs that are even further connected through their share of one single placenta, known as monochorionic twins (MC). MC twins are naturally monozygotic, accounting for approximately 66% of monozygotic twin pregnancies (2). Such an extraordinary unity, in which two individuals experience an unparalleled similarity in their developmental environment, offers a rare opportunity to explore the influence of both genetic and environmental factors on human development, though it also carries a heightened risk of complications and mortality. This close biological relationship has led to a well-established assumption that monozygotic and, by extension, monochorionic twins are "genetically identical". Nevertheless, this is deeply challenged when prenatal examinations reveal that MC twins can deviate both structurally and genetically from such expectations, not only calling into question but fundamentally undermining the core belief in their identical nature.

Hence, drawing from the general understanding of the process of twinning, there is a growing interest in discovering what occurrences or mechanisms could lie behind the discordant anomalies in MC twins. Identifying potential factors that contribute to the divergence could provide critical insights into how these twins develop distinct individual pathways and unique genomic profiles. Some literature even challenges the conventional classification of MC twins, suggesting that the distinction between DZ and MZ twinning may not be as straightforward as previously thought. For example, it has even been proposed that MZ twins may have been incorrectly classified as DZ due to their high degree of discordant anomalies (3). Additionally, it has been suggested that placentas of DZ twins could occasionally fuse during development, leading to incorrect classification as monochorionic monozygotic twins (3).

This thesis aims to examine cases of MZ MC twins with discordant structural and genetic anomalies in multiple pregnancies treated at the Department of Obstetrics and Gynaecology at the University Hospital of Graz between 2013 and 2023. It will compare these cases with existing literature and highlight theories that attempt to explain these unique findings, while also examining to what degree these align with the findings and explanatory models outlined in the literature.

## 1.2 *Theoretical Background*

### 1.2.1 The model of the twinning process

In 1955, George Corner proposed a model to explain the mechanism behind twinning. His model succeeded in being rational and plausible enough that it was quickly accepted and adopted by embryologists and obstetricians worldwide (4). At the core of this model lies the idea that MC twins result from a post-fertilisation event in which the embryo splits and that the timing of this split determines the configuration of chorionicity and amnionicity (4). The model elaborates further within the twin population and its subgroups. Dizygotic (fraternal) twins occur when two separate eggs are released during ovulation and fertilised by different sperm. Like typical siblings, these twins share approximately 50% of their DNA (5). Each twin has a unique genome and can be of the same or different sexes. However, the focus of this discussion lies in instances where a single fertilised egg splits into two, resulting in MZ twins that share nearly identical DNA (6). The extent to which these twins share structures is believed to be determined by the point in time of their splitting. If the embryo divides within the first three days, the result is dichorionic MZ twins, where each twin has its own chorion and amnion (6). However, it is generally believed that most embryo splits occur after the third day, typically at the blastocyst stage (6). In these cases, the split occurs within the inner cell mass, which has already separated from the trophoblast, which leads to the formation of MC MZ twins (24). As previously mentioned, the timing of the zygote's division is assumed to dictate how much the twins will share. As seen in Figure 1, it is presumed, that if the split occurs within the first three days, MZ twins will have separate chorions and amnions, leading to dichorionic diamniotic twins (DCDA). A split between the third- and eighth-day results in monochorionic diamniotic (MCDA) twins, who share a chorion but have individual amniotic sacs. If the division happens even later,

between the eighth and twelfth day, the twins will share both a chorion and an amnion, forming monochorionic monoamniotic (MCMA) twins (7). After twelve days, Siamese twins develop. The reasons behind why the embryo splits are still not fully understood, though various theories have been proposed, which will be discussed further on. This lack of understanding is partly because monozygotic twinning is rarely observed in animals, limiting the scope of comprehensive research (6). While scientists have managed to induce MZ twinning in species such as cattle, mice and rabbits, only the nine-banded armadillo naturally gives birth to MZ twins (8). It has been suggested that the armadillo's 2–3-month embryonic pause in the fallopian tube could contribute to the development of MC twins, though this hypothesis has been challenged, as the pause does not lead to MZ twinning in other species with a similar embryonic pause (8).

It is also worth noting that Corner's model has faced some criticism. Although it was introduced as a theory of twinning, critics argue that it was prematurely accepted as scientific fact. While post-splitting embryos have been studied, the actual moment of embryo splitting has never been directly observed (4). Although this may be a reasonable point, it must be noted, that it is simply impossible to observe it such an early development stage in vitro. This lack of direct evidence has led some to question the theory. Nonetheless, despite nearly 70 years having passed since its introduction, the model has become widely accepted, perhaps because of its rational nature and the absence of any better hypothesis to date.

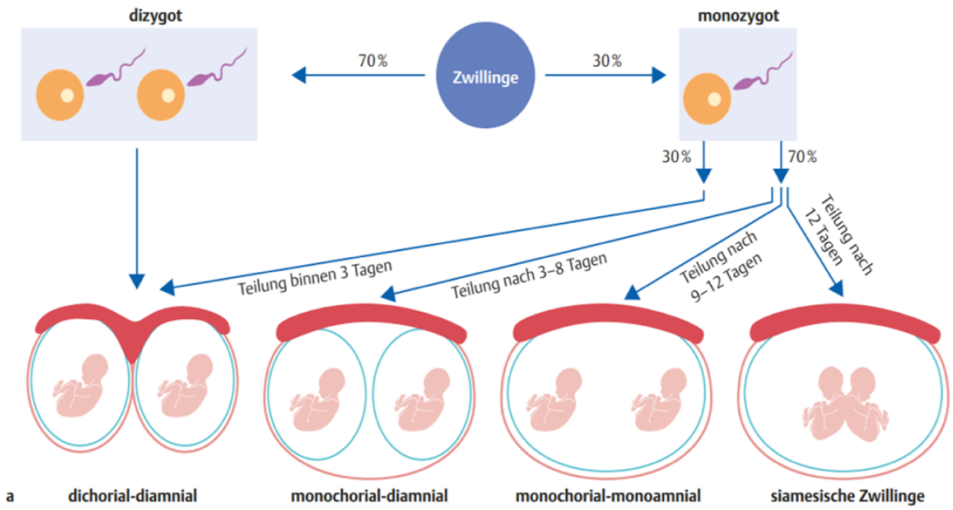



*Figure 1: Formation and Classification of zygosity, chorionicity and amnionicity in twin pregnancies (9)*

### 1.2.2 Twinning: Beyond a random occurrence

Monozygotic (MZ) twinning, unlike dizygotic (DZ) twinning, which is known to be influenced by hereditary factors, has not been conclusively linked to any genetic component. Instead, the prevailing consensus is that MZ twinning occurs randomly. At a rate of about 3-4/1000 births, the prevalence of MZ twinning appears to be constant across the globe and ethnicity. Nevertheless, some researchers have suggested the possibility of a hereditary influence also in MZ twins (9) (10). Some have even proposed that it can indeed be autosomal dominant in some instances, although the possible genes involved in MZ twinning may differ from those for DZ twinning (3). Genes that determine cell adhesion are especially of particular interest. Further analysis of these families using genotyping may lead to identifying such genes for MZ twinning (3). When one considers the possibility of MZ twinning to be familial, it is also important to point to the excess representation of females within the group of MZ twins (3). This raises the question of whether some post-zygotic events are more lethal for XY embryos. A consistent excess of female MZ twins across generations or populations would suggest a non-random pattern, hinting at genetic inheritance rather than environmental influence alone.

### 1.2.3 A quantitative breakdown on monochorionic twins

MC twinning is a rare phenomenon, occurring in approximately 3 to 4 per 1,000 live births worldwide (7). Amongst the MZ population, MC twin pregnancies make up 70%, while it is identified total 20% of all twin pregnancies (11). The frequency of twin births has shifted over the past few decades (1). In the 1970s, Africa, particularly Nigeria, displayed one of the highest twinning rates, while Asia exhibited the lowest rates (1) (5). In recent years, twin births have increased in Western countries, largely due to the rising maternal age and the growing use of IVF treatments, both of which are associated with a higher likelihood of multiple pregnancies (1). MC twins are the most common type of MZ twins, with MCDA twins, reported in 70-75% of cases, MCMA in 1-2%, and dichorionic MZ twins occurring in 25-30% of cases (12). Additionally, these pregnancies carry a high risk of complications; in fact, the risk of MCDA twins being born before 34 weeks is 60%, and in 1 out of 10 MCDA twin cases, an anomaly is detected (13). Contrary to the common assumption of their identical nature, malformations detected in MC twins affect only one twin in approximately 80% of cases, thus making them discordant for the congenital anomaly (9). Furthermore, discordant anomalies are reported to

occur 2 – 4 times more often in MC twins than in DC or singleton pregnancies siblings (9). Structural discordance is observed more frequently than genetic discordance (9) (13), yet when genetic discordance does occur, it can vary from chromosomal anomalies like common aneuploidies such as trisomy 21,13,18 and 45X (13), to point mutations, like duplications and deletions (13). The lower incidence may be attributed to the reduced likelihood of survival in a MC pregnancy with a genetic anomaly (13).

### 1.2.4 Understanding the rise in MC twins

#### 1.2.4.1 Assisted reproductive technologies

The increased use of assisted reproductive technologies (ART) as well as the trend of postponed pregnancy have been identified as key factors contributing to the rising rates of multiple pregnancies (14). ART has gained increasing popularity and acceptance in recent years, with its use becoming more widespread. Medically assisted reproduction grew significantly in the 1970s, particularly among Western populations. ART encompasses a range of treatments designed to assist in achieving pregnancy, such as in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and ovarian stimulation. So far, ART is associated with a higher rate of DZ twinning, largely due to the implantation of multiple embryos during IVF cycles and the stimulation of ovaries, leading to the release of multiple oocytes (1) (15). On the other hand, one study suggested that the risk of MZ twinning is approximately 60% higher in pregnancies conceived through ART compared to those conceived spontaneously (16). In terms of MC twinning, recent studies also propose that ART also increase the likelihood of MC twinning (16). Attali et al 2021 reported that ART could also be associated with increasing the rates of MC twinning, potentially even doubling them (17). The practice of extended embryo culture and blastocyst-stage transfers has been proposed as a factor contributing to a higher risk of MZ twinning (9) (18). On the other hand, it has also been suggested that breaching the zona pellucida, a step in IVF treatments, may trigger zygote splitting (15). In fact, a comprehensive meta-analysis conducted by Wu et al. 2002 identified growing concerns regarding the increased incidence of MZ twin pregnancies following assisted reproductive technologies. This concern prompted the initiation of the meta-analysis. However, determining the precise rate of MZ twinning remains challenging due to its relative rarity. Reported rates have varied, ranging from 0.97%

(95% CI, 0.47–1.99%) to 2.35% (95% CI, 2.07–2.67%). It also revealed that multiple studies have observed a statistically significant rise in MZ twin pregnancies associated with blastocyst transfer, as opposed to cleavage-stage embryo transfer. Furthermore, pooling of the results also demonstrated a statistically significant link between assisted hatching and MZ pregnancies. To explain the underlying mechanism, it was proposed that as part of the method of assisted hatching, a hole may be created and thus leading to the separation of blastomeres and consequently divide them. Another possibility put forward, which may stand alone or increase the risk of MZ twinning combined with such an artificial hole, included that the method of assisted hatching may disrupt the integrity of the premature zona pellucida and thereby possibly interfering with signalling pathways within the embryo. Additionally, the method of insemination—whether in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI)—has also been shown to significantly influence MZ twinning rates. However, when specifically analysing MC twin pregnancies, no significant association was found between ICSI and assisted hatching. The exact mechanisms underlying the observed association between increased rates of MZ and MC twin pregnancies and blastocyst transfer in IVF remain unclear. However, several complementary hypotheses have been proposed by Busnelli et al 2019**,** with particular focus on the role of the culture medium and the zona pellucida (19). One key consideration is the potential impact of a hardened and less flexible zona pellucida, which may increase the likelihood of the inner cell mass splitting, ultimately leading to twinning (19). This hardening is thought to result from prolonged exposure to the culture medium when embryos are transferred at later developmental stages. Additionally, extended contact with the medium may weaken intracellular bonds, further predisposing the embryo to division. Specifically, low calcium concentrations in the medium could compromise cell adhesion, making the inner cell mass more susceptible to splitting (19). Another contributing factor could be the presence of elevated glucose levels in the culture medium, which may lead to an increased production of reactive oxygen species (ROS). This oxidative stress can trigger apoptosis and promote a more linear polarization of cells within the inner cell mass, potentially facilitating embryonic division before hatching. Furthermore, the absence or insufficient levels of crucial cytokines and growth factors in the culture medium could induce cellular stress, further weaken the intercellular bonds and exacerbate the risk of MZ twinning. These effects are likely to be amplified by

prolonged culture periods. Moreover, blastocysts may exhibit greater sensitivity to mechanical stressors, such as fluctuations in pH or temperature, which could also contribute to an increased likelihood of twinning. While one might assume that a more developmentally advanced embryo would be more resilient to such external influences, evidence suggests that blastocysts may, in fact, be more vulnerable to these subtle perturbations. Taken together, these findings suggest possible multiple interrelated factors—ranging from zona pellucida integrity and culture medium composition to environmental stressors—may collectively contribute to the increased incidence of MZ twinning following blastocyst transfer (19). As current practices shift towards extended embryo culture, the MZ twinning rate and subsequently the chance of elevated frequencies of MC twinning may continue to rise (15). Interestingly, regarding pre-implantation genetic testing (PGT) with embryo biopsy, a recent meta-analysis and systematic review concluded that PGT does not increase the risk of zygote splitting compared to IVF without PGT (18). Given that MC twins are associated with higher perinatal complications than DC twins and require specialised management, it is crucial to recognise the increased likelihood of MC twinning with ART treatments (18).

#### 1.2.4.2 Maternal Age:

Particularly in singleton pregnancies, advanced maternal age, defined as being over 35 years of age, is associated with higher perinatal complications. These include a higher incidence of chromosomal anomalies such as Trisomy 21 (14), along with maternal complications, typically gestational diabetes and hypertension (14). In the context of multiple pregnancies, studies have shown that a higher maternal age is linked to a higher likelihood of twin pregnancies; however, this mainly affects DZ twins (20). The rate of MZ twinning remains constant in spontaneously conceived pregnancies but increases with the use of Assisted Reproductive Technologies (ART) (20), which is often sought after in high maternal age. On the other hand, various studies have observed that the rate of DC twins increases with maternal age. Not much can be found in the literature regarding the impact of maternal age on MC twins, yet it appears to be constant (20). However, one meta-analysis did analyse the relationship between MC pregnancies and maternal age, and made the interesting discovery, that younger maternal age (below the age of 35) is associated with a higher likelihood of MZ and MC pregnancies (19). One hypothesis proposed by the authors was that younger, presumably healthier oocytes may have a greater

tendency to divide, reflecting their higher reproductive potential. However, younger oocytes are more commonly transferred at the blastocyst stage, raising the possibility that maternal age is a confounding factor and that the observed association may instead be linked to blastocyst-stage transfers. Nevertheless, even after adjusting for this factor, the meta-analysis has found that the relationship remains statistically significant (19).

Contrary to common expectations, some studies suggest that while advanced maternal age in twin pregnancies carries similar risks as in singletons, it may even have a protective effect in women over 35, leading to more favourable outcomes (20). Although this observation has been largely attributed to the higher proportion of DC twins in older mothers, as DC twins generally present a lower risk profile than MC twins, which could contribute to such improved outcomes. However, findings remain inconsistent, with other studies reporting an elevated risk of maternal complications, such as preterm labour, hypertension, and low birth weight (14). For women over the age of 45, twin pregnancies have been associated with a 3.5 higher risk of maternal complications, including hypertension and pre-eclampsia, as well as fetal complications such as hypoglycaemia, preterm delivery, and neonatal intensive care unit (NICU) admission (14). Several large studies and meta-analyses have consistently shown that advanced maternal age significantly affects fertility and perinatal outcomes. It is linked to a higher incidence of first-trimester pregnancy loss and an increased risk of chromosomal and genetic anomalies (14). Consequently, many women of advanced maternal age require ART to conceive, which further contributes to the rising rates of twin pregnancies due to fertility treatments.

### 1.2.5 Complications in monochorionic Twins:

While MZ DC twins maintain the same perinatal outcome as DZ twins, MC twins can be confronted with distinct complications and difficulties due to the exclusive state of MC twins sharing one single placenta (12). Their connection through the placenta allows for the formation of vascular anastomoses. As such, they face unique risks of developing complications like twin-to-twin transfusion syndrome (TTTS), twin anemia polycythemia Sequence (TAPS) and selective fetal growth restriction (sFGR) (12). A union established through placental vascular anastomoses enables blood transfusion between the twins, the most significant imbalance in transfusion

being the Twin-Twin Transfusion syndrome, in which anastomoses lead to a one-sided blood flow from the donor twin to the receiving twin (Figure 2). This can be identified during ultrasound exams by a significant amniotic fluid discrepancy, caused by the donor becoming hypovolemic, followed by oliguria, and ending in an oligohydramnios (13). On the other hand, because of the excessive transfusion of blood from the donor, the acceptor becomes hypervolemic. With respect to amniotic fluid, this leads to polyuria and polyhydramnios. Meanwhile, in terms of central venous pressure, it also causes arterial hypertension, increases renal perfusion, and leads to heart failure (2). A transfer of vasoactive substances may also impact heart function negatively (13). A discrepancy in transfusion can also induce the twin anemia polycythemia sequence (TAPS), in which a chronic transfusion of red blood cells leads to an imbalance in haemoglobin (13). A closer examination of the placentas showed that the anastomose involved in TAPS is only petite, allowing primarily a chronic transfer of blood cells (13), compared to the larger anastomoses found in a TTTS placenta. The most severe form of TTTS is twin reversed arterial perfusion (TRAP) sequence, a rare complication exclusive to MC twin pregnancies. Due to delayed early heart development of one twin, in combination with venovenous (VV) and arterioarterial (AA) anastomoses of placental blood vessels, differences in blood pressure between fetuses can lead to an increase of pressure in the „pump twin“ with a reversal of blood flow through the anastomoses. As a result, the affected fetus is supplied with oxygen-poor blood by the "pump twin“. As a result of the insufficient oxygen supply, the receiving twin develops severe abnormalities, including atrophy of the upper body, which can extend to the absence of a head (acrania) or a heart (acardia). The pump twin, responsible for perfusing both fetuses, faces significant volume overload, increasing the risk of high-output cardiac failure, hydrops fetalis, preterm birth and intrauterine fetal death (IUFD) (13). Another typical complication is the selective fetal growth restriction (sFGR), which is observed in MC pregnancies, in which one twin shows restricted growth due to an unequal placental sharing, causing a significant weight discrepancy between the twins (2).

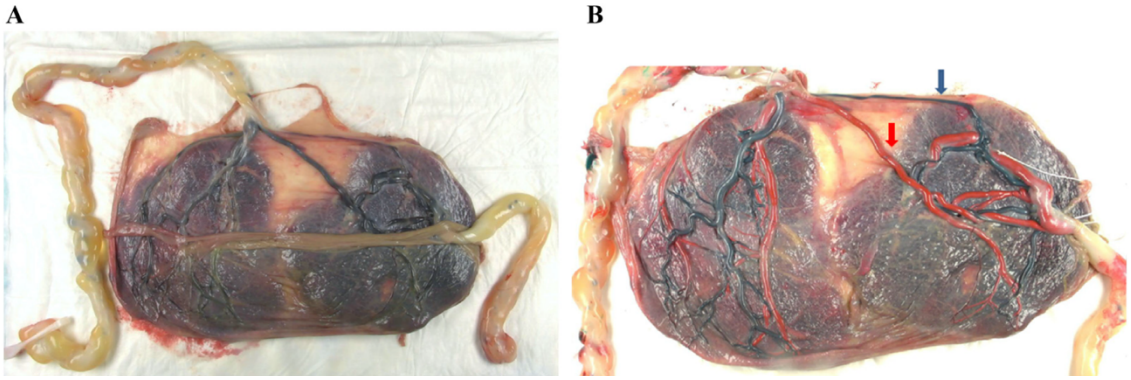



*Figure 2: MC pregnancy with abnormal configurated two masses. Red dye highlights veins, while blue dye indicates arteries. The arrows mark two large anastomosis (60*

The exceptional nature of MC twins becomes even more apparent when examining the placental vascular anastomoses present in an MC pregnancy. The literature reports that anastomoses are present in 95% of the placentas in MCDA twins. The extent to which they develop and the distribution between arteries, veins or between arteries and veins vary in each pregnancy (12). The consequence of this condition is the formation of a bidirectional blood flow, which can become problematic when it functions abnormally (12). When examining the placentas of monochorionic diamniotic twins, the structure typically reveals individual sections for each twin, along with a third shared region containing arteriovenous (AV) anastomoses, which are deeper and facilitate unidirectional blood flow. This contrasts with the more superficial AA and VV connections that allow bidirectional blood flow (13). Figure 3 illustrates such superficial and deeper vascular connections. Compared to the unidirectional flow of AV anastomosis, the bidirectional flow of AA anastomoses may serve as a protective factor against the development of transfusion discrepancies. An examination of placentas after TTTS identified that in these instances, they displayed more AV anastomoses and deficiency of AA networks (13).

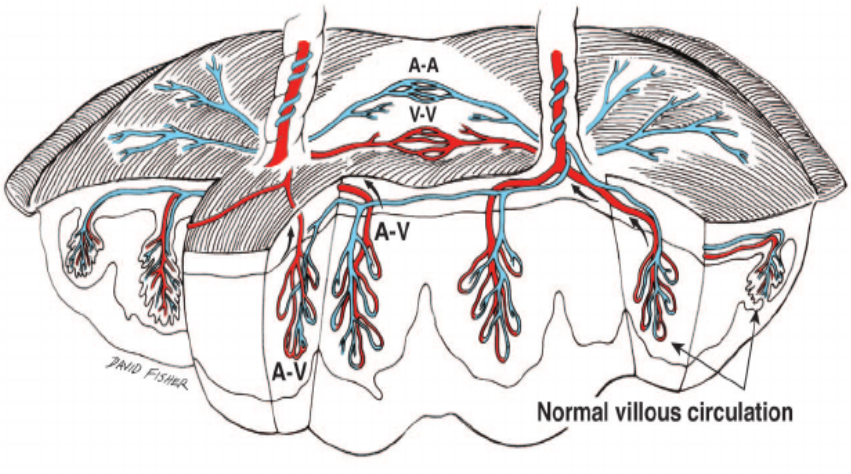



*Figure 3: An illustration of AA and VV Anastomoses in a MC pregnancy with TTTS. (II) shows the recipient, while (I) represents the donor (61)*

Their vascular communication also entails, in the case of one fetus dying, an up to 40% possibility of acute transfusion from the higher-pressure circulation of the co-twin to the low-pressure circulation of the fetus that has suffered an intrauterine death, thereby putting the living fetus at the risk for cerebral damage or death (12). Even if the weaker twin survives, a risk remains for the co-twin for neurological complications (12). To protect the healthier co-twin, in clinical practice, when one twin begins to show defects that may lead to an intrauterine fetal demise (IFUD), a selective feticide can be considered and performed (12). Selective intrauterine death (sIUD) occurs in approximately 4% of MCDA twin pregnancies after the first trimester. In 40% of these cases, the co-twin also suffers intrauterine demise, while an additional 28% die within the first 28 days after birth (13). MCDA twins are separated by the amniotic membrane, which protects them from umbilical cord entanglement (7). However, they still share a single chorion, placing them at risk of developing vascular anastomosis and subsequent placenta communication (7). In contrast, MCMA twins face significantly higher mortality rates, reaching up to 60%, with only 1-2% of surviving MZ twins being classified as MCMA twins (7). Consequently, the more twins are limited to only one variant of a chorion or amnion, the greater the risks become.

### 1.2.6 Diagnostic

Every multiple pregnancy requires a thorough evaluation, as assessment of chorionicity, amnionicity and accurate dating are critical for a successful outcome. Adequate management of multiple pregnancies begins with the precise determination of these factors, considering that perinatal morbidity in twins varies based on zygosity and chorionicity. DC MZ twins have a similar risk profile to DZ twins, as DZ twins are naturally dichorionic. In contrast, MC twins have a 50% higher risk of neonatal death and a 3.5 times greater chance of stillbirth, highlighting the critical importance of correctly identifying MC pregnancies (21).

Chorionicity should be established either through transvaginal or transabdominal ultrasound, preferably between the 8th – 10th week of gestation, but no later than 13 + 6 weeks. Delayed assessments beyond this period carry an increasing risk of misclassification, rising by 10% for each subsequent week, as placental fusion can occur after the 12th week, making precise identification more challenging

(21). Monochorionicity is indicated by a thin membrane separating the twins, which extends perpendicularly into the placenta, forming what is known as the T-sign (Figure 5) (21). When performed within the correct time frame, this finding is highly reliable, with a sensitivity of 100% and specificity of 99.8% for diagnosing MC twin pregnancies (22). In contrast, the presence of a more triangular shape, referred to as the “lambda” or “twin peak” sign (Figure 5) indicates a DC pregnancy. The lambda sign is formed when the two separate placental masses either fuse together or lie adjacent to one another (23). This occurs when placental tissue is present at the point where the thick membrane between the twins inserts into the placenta, as illustrated in Figure 5. The lambda sign resolves in about 7% of DC pregnancies after the 20th week, further emphasising the importance of early classification (21). Additional indicators of a MC pregnancy include the presence of two thin amniotic membranes instead of three. Therefore, accurate determination of chorionicity is essential, as it dictates the specific management and care required throughout the pregnancy.

Furthermore, accurate dating of a multiple pregnancy guides the correct management of the pregnancy as it progresses. In a spontaneously conceived pregnancy, dating during the earlier stages between the 11 – 13 + 6 gestational week is determined using the largest crown-rump length (CRL). After 14 weeks the most accurate method of classifying gestational age is by using the largest measured circumference of the head (21). Under the circumstances of IVF treatment, dating is based either on the age of the embryos or the date of egg retrieval (21). Finally, regarding detecting and diagnosing abnormalities, it is important that both twins are always examined, as an abnormal chorionic villous sample could overlook a healthy co-twin, while a normal sample might fail to detect the affected twin (13).

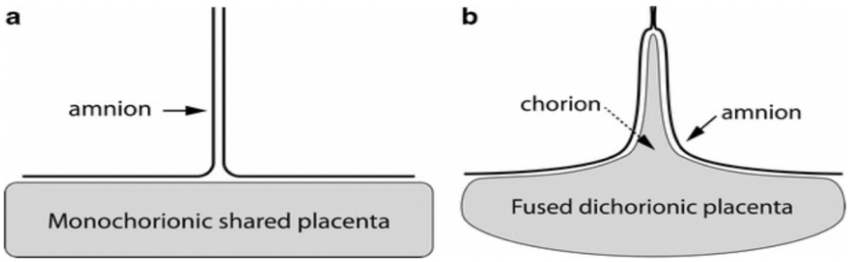



*Figure 4: a) MC - shared placenta with a thin membrane (amnion only) b) DC: Two placenta masses fused with a thicker membrane (amnion + chorion). Creating a λ shape (23).*

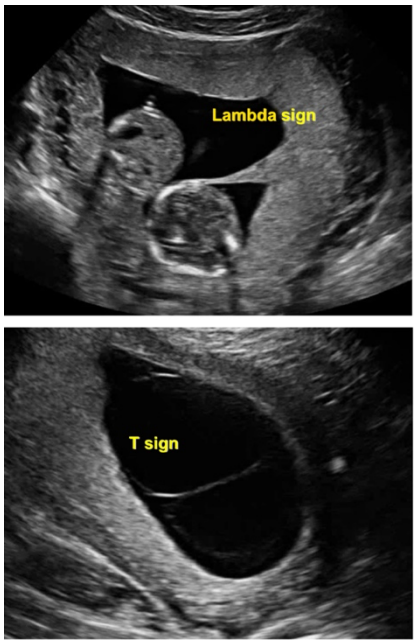



*Figure 5: Ultrasound differentiations of Chorionicity in Twin Pregnancies: Lambda vs T sign (62)*

### 1.2.7 Management

Adequate management of MC multiple pregnancies demands comprehensive and proactive care. Ultrasonography plays a key role in this process, providing a non-invasive method for monitoring fetal growth and enabling the early detection, diagnosis, and continuous evaluation of common complications, particularly twin-twin transfusion syndrome (TTTS) (12). Therefore, major medical societies recommend biweekly ultrasound examinations for all MC twin pregnancies starting from the 16th week, primarily to ensure timely detection of TTTS (13). A thorough scan in the first trimester can already detect half of the structural anomalies, although poor growth can lead to anomalies being identified later (13). Additionally,

to identify twin anemia-polycythemia sequence (TAPS) in the first trimester, routine assessments should include measuring the middle cerebral artery peak systolic velocity (MCA PSV), as an elevated velocity may indicate fetal anemia. Each ultrasound should evaluate fetal growth, amniotic fluid volume, and blood flow in the middle cerebral and umbilical arteries using Doppler velocimetry (12).

#### 1.2.7.1 Management of MC with structural anomalies:

When confronted with a case of discordant MC twins, three potential approaches can be considered. It is possible to pursue expectant management (EM), yet this approach bears the risk that the healthy co-twin may be exposed to the intrauterine demise of the affected twin or suffer neurologic loss. This is reported to occur in about 24% – 25% of cases (9). Another course of action would be to undergo a selective feticide (SF*),* which carries about a 5% risk of resulting in the loss of the entire pregnancy (9). Lastly, the pregnancy can be fully terminated (TOP).

### 1.2.8 European surveillance of congenital anomalies

As the European Surveillance of Congenital Anomalies, EUROCAT is a register that collects data on congenital anomalies across European countries, including the United Kingdom, Germany, France, Sweden and numerous more. The anomalies reported between 2013 – 2022 were 259,24 per 10.000 births, meaning about 2,6% of registered births in this time period were affected by an anomaly. While common defects such as congenital heart defects (CHD), particularly Ventricular septal defects and congenital anomalies of kidney and urinary tract (CAKUT) dominate, the distribution shows a broad spectrum of severe conditions (24).

# 2 CASES OF DISCORDANT ANOMALIES REPORTED IN THE LITERATURE

The perception of MZ twins as identical by society and medical professionals extends beyond mere expectations of physical resemblance or unity. It is rooted in the assumption that MZ twins are born with an identical genetic makeup. While this belief offers a straightforward and satisfying explanation for the intrigue surrounding identical twins, it may oversimplify reality. Therefore, contrary to expectation, increasing literature reports describe discordant structural and genetic anomalies in MZ twins, revealing the true complexity underlying this phenomenon. Studies of MZ twins discordant in disease phenotypes have shown that such cases offer valuable insights into the diversity and spectrum of diseases (5). A similar observation was made in a case of twins with Frijns syndrome, where they exhibited discordant clinical presentations regarding the diaphragmatic defect, which was previously believed to be a constant feature of the disease (5). A review of reported cases was conducted, highlighting such diversity and how discordance can manifest, including chromosomal abnormalities (Figure 6), variations in phenotypic expression (Figure 7) and instances where Mendelian disorders are confined to only one twin (5). Examples of cases with discordant genetic and structural discordance will be highlighted. Concerning some of the reported cases, attempts to explain the mechanisms and possible triggers underlying the discordance, have been made and will be discussed (5).

**TABLE III. Overview of Publications on Monozygotic Twin Pairs of Whom Only One Twin Is Considered to Have a Chromosomal Abnormality**

| Chromosomal abnormality | Refs. |
|---|---|
| 45,X | Gentilin et al. [2008], Nieuwint et al. [1999], Reiss et al. [1993], Rohrer et al. [2004], Uchida et al. [1983], Weiss et al. [1982] |
| del(10) | Juberg et al. [1981] |
| der(11) | Bourthoumieu et al. [2005] |
| Trisomy 1 (partial) | Watson et al. [1990] |
| Trisomy 13 | Taylor et al. [2008] |
| Trisomy 21 | Cheng et al. [2006], Dahoun et al. [2008], Gilgenkrantz and Janot [1983], Nieuwint et al. [1999], O-'Donnell et al. [2004], Rogers et al. [1982], Sethupathy et al. [2007] |

del, deletion; der, derivative chromosome.

*Figure 6: Publications in MC Twins with discordant chromosomal abnormality (5)*

**TABLE II. Overview of Publications on Monozygotic Twin Pairs With a Monogenetic Disorder That Report a Difference in Phenotype Between Both Twins**

| Disorder | MIM number[a] | Refs. |
|---|---|---|
| 22q11-deletion syndrome | #611867, #192430, #188400 | Fryer [1996], Goodship et al. [1995], Hillebrand et al. [2000], Rauch et al. [1998], Singh et al. [2002], Vincent et al. [1999], Yamagishi et al. [1998] |
| D-2-hydroxyglutaric aciduria | #600721 | Misra et al. [2005] |
| Alagille syndrome | #118450 | Kamath et al. [2002] |
| Alport syndrome | #301050 | Matsukura et al. [2004] |
| Anophtalmia-esophageal atresia | #206900 | Zenteno et al. [2006] |
| Cystic fibrosis | #219700 | Mekus et al. [2000], Picci et al. [2007] |
| Crouzon syndrome | #123500 | Lajeunie et al. [2000], Sher et al. [2008] |
| Dravet syndrome | #607208 | Miyama et al. [2008] |
| Familial amyloid polyneuropathy | +176300 | Holmgren et al. [2004], Munar-Ques et al. [1999], Saporta et al. [2009] |
| Fragile X syndrome | #300624 | Helderman-van den Enden et al. [1999], Kruyer et al. [1994] |
| Frijns syndrome | %229850 | Vargas et al. [2000] |
| Facioscapulohumeral muscular dystrophy (FSHD) | %158900 | Tawil et al. [1993] |
| G syndrome | %145410 | Young et al. [1988] |
| Gaucher disease | #231000 | Lachmann et al. [2004] |
| Gerstmann–Straussler disease | #137440 | Webb et al. [2009] |
| Huntington disease | #143100 | Friedman et al. [2005], Panas et al. [2008], Anca et al. [2004], Georgiou et al. [1999] |
| Joubert syndrome | %213300 | Raynes et al. [1999] |
| Kallmann syndrome | +308700 | Hipkin et al. [1990] |
| Keratoconus | #148300 | McMahon et al. [1999], Weed et al. [2006] |
| Langerhans histiocytosis | 604856 | Chen et al. [2004] |
| LEOPARD syndrome | #151100 | Rudolph et al. [2001] |
| Lymphedema-distichiasis | #153400 | Kumar et al. [2007] |
| McCune–Albright syndrome | #174800 | Endo et al. [1991] |
| Neurofibromatosis type 1 | #162200 | Kelly et al. [1998], Detjen et al. [2007] |
| Neurofibromatosis type 2 | #101000 | Baser et al. [1996] |
| Goldenhar syndrome | %164210 | Ryan et al. [1988], Satoh et al. [1995] |
| Pai syndrome | 155145 | Al-Mazrou et al. [2001], Guion-Almeida et al. [2007] |
| Photosensitive epilepsy | %609569 | de Haan et al. [2005] |
| Primary ciliairy dyskinesia | #242650 | Noone et al. [1999] |
| Severe combined immunodeficiency (SCID) | #102700+ | Niehues et al. [1996] |
| Sickle cell anemia | #603903 | Amin et al. [1991] |
| Thanatophoric dysplasia | #187601/#187600 | Corsello et al. [1992], Horton et al. [1983], Horton et al. [1983] |
| Tibial hemimelia with ectrodactyly | %119100 | Dayer et al. [2007] |
| Tricho-rhino-phalangeal syndrome | #190350 | Naselli et al. [1998] |
| Tuberous sclerosis | #190350/#191100 | Humphrey et al. [2004], Kondo et al. [1991], Martin et al. [2003] |
| WAGR syndrome | #194072 | Bremond-Gignac et al. [2005] |
| Williams syndrome | #194050 | Castorina et al. [1997], Maurer et al. [1979], Murphy et al. [1990], Oorthuys [1984], Pankau et al. [2001] |
| Wilson disease | #277900 | Czlonkowska et al. [2009] |
| (X-linked) adrenoleukodystrophy | #300100 | Di et al. [2001], Sobue et al. [1994] |

Symbols preceding a MIM number: #, a descriptive phenotype, not necessarily representing a unique locus; +, description of a gene of known sequence and a phenotype; %, a confirmed Mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known; no symbol before a MIM number generally indicates a description of a phenotype for which the Mendelian basis, although suspected, has not been clearly established.[a]Mendelian Inheritance in Man (MIM) numbers correspond to Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) (November 1, 2009). World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/.

*Figure 7: Publications on MZ twins with a monogenetic disorder that are discordant in phenotypic expression (5)*

**TABLE IV. Overview of Publications on Monozygotic Twin Pairs of Whom Only One Twin Is Considered to Have a (Possibly) Mendelian Disorder**

| Disorder | MIM[a] | Refs. |
|---|---|---|
| Aglossia-adactylia | 103300 | Robinow et al. [1978] |
| Aicardi syndrome | %304050 | Costa et al. [1997] |
| Asplenia syndrome | %208530 | Hwang et al. [2006], Wilkinson et al. [1979] |
| Bladder exstrophy | %600057 | Bugge [1981], Reutter et al. [2003] |
| Body stalk anomaly | 230750 | Daskalakis and Nicolaides [2002], Vidaeff et al. [2005] |
| Beckwith–Wiedemann syndrome | #122470 | Berry et al. [1980], Bose et al. [1985], Chien et al. [1990], Clayton-Smith et al. [1992], Leonard et al. [1996], Litz et al. [1988], Olney et al. [1988], Orstavik et al. [1995], Weksberg et al. [2002], Bliek et al. [2009] |
| Caudal duplication anomaly | #607864 | Kroes et al. [2002], Oates et al. [2006] |
| Congenital hypothyroidism | #275200/#218700 | Rettig et al. [1980] |
| Colorblindness | +303800 | Jorgensen et al. [1992] |
| Complete congenital heartblock | 234700 | Cooley et al. [1997] |
| Congenital bilateral perisylvian syndrome | #300388 | Lenti and Triulzi [1996] |
| Cornelia de Lange syndrome | #122470 | Carakushannsky and Berthier [1976], Carakushansky et al. [1996] |
| Darrier's disease | #124200 | Sakuntabhai et al. [1999] |
| Duane's retraction | #604356, +%126800 | Kaufman et al. [1989], Rosenbaum and Weiss [1978] |
| Duchenne muscular dystrophy | #310200 | Burn et al. [1986], Chutkow et al. [1987], Gomez et al. [1977], Lupski et al. [1991], Pena et al. [1987], Richards et al. [1990], Tremblay et al. [1993], Zneimer et al. [1993], Bonilla et al. [1990] |
| Epilepsy with structural brain abnormalities | | Briellmann et al. [1998], Brodtkorb et al. [2000], Kuzniecky et al. [1995], Sisodiya et al. [1999], Supprian et al. [2000] |
| Fabry disease | #301500 | Redonnet-Vernhet et al. [1996] |
| Fragile X syndrome | #300624 | Kruyer et al. [1994], Tuckerman et al. [1985] |
| Frontonasal dysplasia | 136760 | Mohammed et al. [2004], Wu et al. [2007] |
| Facioscapulohumeral muscular dystrophy (FSHD) | %158900 | Griggs et al. [1995], Tawil et al. [1993] |
| Goldenhar syndrome | %164210 | Boles et al. [1987], Stoll et al. [1984], Touliatou et al. [2006], Verona et al. [2006], Wieczorek et al. [2007] |
| Growth hormone deficiency | +139250 | Simpson et al. [1999] |
| Hemihypertrophy | %235000 | West et al. [2003] |
| Hemophilia A | +306700 | Bennett et al. [2008] |
| Hemophilia B | #306900 | Kitchens [1987], Revesz et al. [1972] |
| Holoprosencephaly | %236100 | Machin et al. [1985], Peng et al. [2007] |
| Humero-radial synostosis | %236400/143050 | McCredie [1975] |
| Hunter syndrome | +309900 | Winchester et al. [1992] |
| Idiopathic torsion dystonia (ITD) | #128100/%602124 | Chan and Tsui [1997] |
| Kabuki syndrome | %147920 | Shotelersuk et al. [2002] |
| Kearns–Sayre syndrome | #530000 | Blakely et al. [2004] |
| Klippel–Feil syndrome | %118100 | Toyoshima et al. [2006] |
| Klippel–Trenaunay syndrome | %149000 | Hofer et al. [2005], Oduber et al. [2010] |
| Landau–Kleffner syndrome | 245570 | Feekery et al. [1993] |
| Leber's hereditairy opticus atrophy | #535000 | Biousse et al. [1997], Johns et al. [1993] |
| Lesch–Nyhan syndrome | #300322 | De et al. [2005] |
| Mayer Rokitansky Küster syndrome | %277000 | Heidenreich et al. [1977], Duru and Laufer [2009] |
| McCune–Albright syndrome | #174800 | Peleg et al. [2009] |
| Ocular cicatricial pemphigoid | 164185 | Bhol et al. [1995] |
| Oculo-oto-radial (IVIC) syndrome | #147750 | Elcioglu and Berry [1997] |
| Oto-palato-digital syndrome | #311300 | Robertson et al. [2006] |
| Oral–facial–digital syndrome | #311200 | Shotelersuk et al. [1999] |
| Primary lateral sclerosis | %611637 | Sorenson [2006] |
| Primary progressive aphasia | #607485 | Doran and Larner [2004] |
| Proteus syndrome | %176920 | Brockmann et al. [2008] |
| Retinitis pigmentosa | #180100 + others | Bernstein and Aptsiauri [2003] |
| Rett syndrome | #312750 | Carter et al. [2008], Migeon et al. [1995], Subramaniam et al. [1997] |
| Rubinstein–Taybi syndrome | #180849 | Kajii et al. [1981] |
| Say syndrome | 181180 | Ashton-Prolla and Felix [1997] |

(*Continued*)

*Figure 8: Publications of MZ twins discordant for a Mendelian Disorder (5)*

Another study, conducted by Bhutia et al.2024 between 2011 and 2021, examined twins with discordant anomalies, including MC pairs. Figure 10 shows the spectrum of discordant anomalies in MC twins that was observed in this study and their outcome (25). Among 83 twin pregnancies, discordant anomalies were observed in 1,76% of DC and 1,4% of MC twins, however, the statistical significance of this difference was not reported. The study also compared the affected systems between DC and MC twins, as shown in Figure 9 (25). Notably, among the MC twins, the most affected system was the central nervous system, accounting for 35,7% of anomalies (25).

| System | Chorionicity | |
|---|---|---|
| | Dichorionic ($N = 69$) | Monochorionic ($N = 14$) |
| Musculoskeletal system | 18 (26%) | 3 (21.4%) |
| Circulatory system | 14 (20.2%) | 4 (28.5%) |
| Urinary system | 8 (11.59%) | 1 (7.14%) |
| Central nervous system | 7 (10.14%) | 5 (35.71%) |
| Face and neck | 7 (10.14%) | 1 (7.14%) |
| Respiratory system | 5 (7.24%) | 0 |
| Gastrointestinal system | 4 (5.8%) | 0 |
| Multisystem abnormality | 6 (8.69%) | 0 |

*Figure 9: Comparison between discordant DC and MC twins (25)*

| Types of system-wise anomalies | Number of cases *n* (%) | Perinatal outcome |
|---|---|---|
| Malformation of the central nervous system | | |
| Anencephaly | 2 (14.29) | 1 SFD, 1 NND |
| Arnold–Chiari II malformation | 1 (1.45) | SFD |
| Holoprosencephaly + ventriculomegaly | 1 (7.14) | NND |
| Diastematomyelia | 1 (7.14) | Miscarriage after RFA |
| Malformation of the face and neck | | |
| Cystic hygroma | 1 (7.14) | SFD |
| Malformation of the circulatory system | | |
| Common arterial trunk + atrioventricular septal defect | 1 (7.14) | Operated, alive and healthy |
| Ventricular septal defect | 1 (7.14) | Under follow-up |
| Right aortic arch + ventricular septal defect | 1 (7.14) | Operated, alive and healthy |
| Right isomerism (heterotaxy) | 1 (7.14) | Live birth, death after 45 days |
| Malformation of the urinary system | | |
| Unilateral multicystic dysplastic kidney | 1 (7.14) | Under follow-up |
| Malformation of the musculoskeletal system | | |
| Talipes equinovarus | 1 (7.14) | Spontaneous miscarriage |
| Congenital diaphragmatic hernia | 1 (7.14) | Operated, alive and healthy |
| Exomphalos | 1 (7.14) | NND |

*Figure 10: Spectrum of affected systems in discordant MC twins (25)*

## 2.1 DISCORDANT CONGENITAL HEART DEFECTS:

As the most common congenital anomaly, CHD represent a significant condition that may deliver new insights into the mechanisms underlying the differences seen in MC twins (26). The general perception upholds, that if a CHD is detected in one MZ twin, their monozygotic nature would indicate that the co-twin must also be affected. Though various reported cases of MC twins discordant with CHD challenge this. While the rate in singletons of CHD is around 0.9%, it is notably higher in MC twins, affecting approximately 59 out of every 1,000 live births (5,9%). A study assessed CHD in 163 twins and reported that out of 23 MC twins, 18 were discordant for CHD, a striking 78% (26). Figure 11 demonstrates the spectrum of CHD diseases in MZ twins found in this study (26). This calls for one to look at the prevalence reported by the EUROCAT (European surveillance of congenital anomalies), which collects and reports birth defects across Europe (24). As shown in Figure 11, the study reported that septal defects were the most common CHD lesion observed among discordant MC twins, occurring in 45.5% of cases. Right heart lesions followed as the second most frequent defect, present in 18.2% of the discordant cohort. EUROCAT also reports that CHD are the most prevalent birth defects overall, with septal defects being the most frequent heart lesion among all congenital malformations across Europe, including in singletons (25). This would indicate that CHD are not a malformation that are unique to MC twins but instead reflects the overall prevalence.

| Diagnosis[1,2] | Concordant CHD Cohort (n = 10)[3] | Discordant CHD Cohort (n = 22)[3] |
|---|---|---|
| Septal defects | 6 (60%) | 10 (45.5%) |
| Systemic venous anomalies | 0 (0%) | 1 (4.5%) |
| Right heart lesions | 4 (40%) | 4 (18.2%) |
| Left heart lesions | 0 (0%) | 2 (9.1%) |
| Transposition of the great arteries | 0 (0%) | 2 (9.1%) |
| Thoracic arteries/veins | 0 (0%) | 3 (13.6%) |
| CHD severity category[4] | 1 (0) | 1 (0)[5] |

Data are no. (%) or median (IQR).

[1]Based on the International Nomenclature for Congenital Heart Surgery.

[2]p = 0.53.

[3]Includes only neonates affected by CHD.

[4]Category 1 = low risk of hemodynamic instability in the delivery room, Category 2 = minimal risk of hemodynamic stability but requiring postnatal surgical intervention, Category 3 = likely hemodynamic instability requiring immediate specialty care, Category 4 = expected hemodynamic instability requiring immediate surgical intervention.

[5]p = 1.

https://doi.org/10.1371/journal.pone.0251160.t001

*Figure 11: Congenital heart defects in MZ Twins (23)*

Regarding the cause of these findings, studies have not identified any strong genetic influences in these discordant MC twin cases so far. Any genetic differences discovered later in their life have mainly been attributed to epigenetic mechanisms rather than any true germline mutations, with teratogens being put forward as a possible reason to alter epigenetic processes and thereby causing differences in gene expression. A comprehensive study examining MC twins discordant for CHD was conducted by Imany-Shakibai et al. 2012 aiming to showcase the variety and possibly find underlying pathomechanisms (26). For example, differentially methylated regions in the promoters of genes related to cardiac development were identified. However, it remains unclear whether these differentially methylated regions are the cause or a consequence of developmental processes. The relationship between gene expression and epigenetic modifications may be circularly dependent, making it challenging to determine the primary initiating factor. Further studies are required to elucidate whether these methylation patterns drive cardiac development or result from it. This idea can be connected to the “Butterfly Effect”, a concept that illustrates how even the smallest, seemingly trivial, or unnoticed changes in a nonlinear system can lead to profound, far-reaching consequences over time. In biological systems, this effect is particularly relevant, as it can explain how minute molecular alterations, such as genetic mutations, can set off a chain reaction with significant outcomes. For example, in the context of cancer, a single genetic mutation can dramatically alter a cell’s behaviour, leading to uncontrolled growth and the eventual development of malignancy. This underscores how subtle shifts in genetic or epigenetic factors might play pivotal roles in complex biological processes (27). On the other hand, microRNA involved in molecular transport and adrenergic signalling within cardiomyocytes was found to be elevated in twins with CHD compared to those without. Regarding environmental factors, no differences in the concordant and discordant cohort were discovered relating to maternal co-morbidities such as diabetes, hypertensive disorders or pre-pregnancy BMI. Although this is not surprising, as such maternal factors should affect both twins equally. Notably, all five in-vitro fertilisation pregnancies in the cohort were part of the discordant group.

Genetic differences in the discordant group of this study were particularly low. Although one discordant case of this cohort included a MC twin pair, in which the

affected twin with a septal defect and left inguinal hernia, their microarray had copy gains on 7q21.21 and Xp22.2 and 1.5Mb copy loss of 15q13.2q-13.3. While the significance of the copy gains is unknown, the copy loss on chromosome 15 has also not been associated with cardiac deformities but has been associated with neurodevelopment defects. Although the amount of genetic testing and finding CHD-specific mutations in discordant twins is low, the low genetic findings in these cases support this assumption that epigenetic mechanisms and a different environment, as it is found in TTTS, in MC twins may be an underlying factor in discordant CHD (26).

On the other hand, a 2011 study reported that a significant 41% of all discordant CHD cases were associated with placenta-related pathophysiology. Consequently, relative hypoperfusion of one twin has been suggested as a possible cause of discordance in CHD (26). A state that can develop on the grounds of a MC pregnancy, leading to possible vascular anastomoses, differences in relative placental share and different cord insertions. Especially TTTS is being considered as a potential cause, since the recipient twin with a TTTS can experience an increase in blood flow and higher aortic velocity, which carries a higher risk of structural anomalies. As such, MC twins are not guaranteed to experience the same environment, which impacts their individual growth. In cases of TTTS, the frequency of CHD increases to 9.3% in MC pregnancies and it was diagnosed in twice as many discordant cases, as in the concordant cases, which again highlights the possible critical role of hemodynamics in the twins‘ growth and perhaps cardiac development, even past the heart’s initial formation (26). Another study also found that the recipient of MC twins with TTTS, showed at all Quintero stages, the strain of the left ventricular was in fact reduced. The right ventricular strain was decreased in the third and fourth Quintero stages (26). Another striking discovery revealed that MC twins with TTTS have a 70 times higher relative risk of developing right ventricular outflow tract obstruction than compared to singletons (26).

## *2.2 VACTERL*

VACTERL association describes the presence of at least three of the following malformations: Vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula, renal anomalies and limb anomalies (28). The literature

also describes cases of MZ twins discordant for VATER/VACTERL malformation. A review reported, that 11 out of 13 such twin pairs were discordant for the syndrome (28). Given the high rate of discordance and the limited number of familial cases reported, as seen with VACTERL, it can be assumed that hereditary factors may play a minimal role in most cases. Instead, differences in patterns in DNA Methylation likely led to variations in gene expression or somatic mutations (28). As described by Machin 2009, it was even suggested that the discordance in MC twins may be a result of transfusional occurrences, even if they were not severe enough to be TTTS. This may have caused a hypotension and hypoperfusion of major territories (3).

## *2.3 BODY STALK ANOMALY*

As a rare anomaly presenting with severe kyphoscoliosis, rudimentary umbilical cord and substantial abdominal wall defect, Body-Stalk anomaly occurs in the first trimester in about 1 in 7500 pregnancies (29). Due to a substantial number of spontaneous abortions and termination, the birthing rate is only 1 in 14 000 births. In the fifth week of embryogenesis, a maldevelopment of the lateral, caudal and cephalic embryonic folds occur, the abdominal organs lie outside the abdominal cavity and are nearly attached to the placenta, the umbilical cord can be absent or shortened (29). The precise cause is still unclear, although due to its severe and variable nature a multifactorial aetiology is presumed. The anomaly has been related to particularly MC twins, triplet pregnancy, young maternal age and cocaine abuse of the mother. Survival rate is minimal, as only few survival cases can be found in the literature (29). Interestingly, the recurrence rate appears to be very low, with only a single case reported in the literature. The literature describes cases of MC twins discordant for body stalk anomaly, including two cases reported by Daskalakis (30). This anomaly involves a significant abdominal wall defect, an underdeveloped umbilical cord and severe kyphoscoliosis. It is an exceptionally rare developmental defect, occurring in approximately 1 in 7,500 pregnancies, with even fewer instances in MZ twins (23). One reported case involved a 20-year-old woman with a monochorionic twin pregnancy, in which one twin was diagnosed with a large abdominal wall defect, femoral abduction, and severe kyphoscoliosis at 14 weeks, consistent with body stalk anomaly. Following preterm labour at 33 weeks, an emergency caesarean section was performed. The affected twin died 30 minutes

after delivery. Another case involved a 25-year-old woman with a monochorionic monoamniotic (MCMA) twin pregnancy discordant for this malformation (30). At 14 weeks, one twin was noted to have severe kyphoscoliosis, bilateral talipes, a large abdominal wall defect with extensive exomphalos of bowel and liver, and lower limbs positioned outside the amniotic cavity. At 35 weeks, an elective caesarean section was performed, and the affected twin similarly passed away 30 minutes post-delivery. In both instances, to reduce the risk of fetal demise in the healthy twin, expectant management was chosen, and one healthy twin was delivered in each case. Overall, the sonographic findings typical for body stalk anomalies in these twin cases were consistent with those observed in singleton pregnancies (23). Some theories aspiring to explain the pathogenesis of discordant body stalk anomaly in twins have been proposed, including premature amnion rupture, abnormal germ disc and abnormalities in blood flow (30).

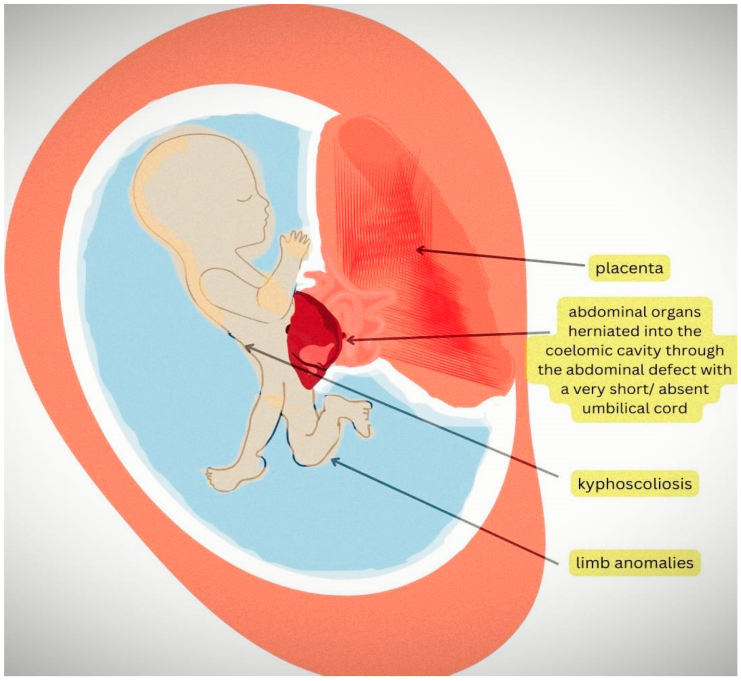



*Figure 12: Body-stalk-anomaly (31)*

### 2.3.1 PREMATURE AMNION RUPTURE:

Considering the observation that in these described cases a significant part of the bodies of the abnormal twins was in the coelomic cavity, the most likely cause of body stalk anomaly in both singleton and twin pregnancies is suggested to be an early rupture of the amnion before the coelomic cavity fully closes (30). This rupture allows parts of the fetal body to herniate from the amniotic cavity through the membranes into the coelomic cavity, leading to structural defects in the abdominal wall and spine. In some instances, the formation of amniotic bands can further contribute to additional malformations, such as limb amputations, encephalocele and short umbilical cords. The length of the umbilical cord is affected by the tension exerted on it; as the amniotic bands restrict fetal movement and whether the fetus closely to the placenta, the cord's growth is significantly limited (30).

### 2.3.2 ABNORMAL GERM DISC:

Aligning with the observation in the above-described cases, the abnormal germ disc theory describes the anomaly arising from a failure in normal embryonic folding in the 5th week of gestation. At this critical stage, the trilaminar embryo should undergo folding along the cephalic, lateral, and caudal axes to form a cylindrical shape. When this process is disrupted, the coelomic cavity fails to close properly, and the amniotic sac does not form as it should. Recent research by Paul et al. further supports this theory, suggesting that with the damage occurring prior to the 6th week of gestation, the abnormal position of the fetus in the exocoelomic cavity could be a key characteristic of this anomaly (30).

### 2.3.3 BLOOD FLOW

Another theory mentioned in the literature is supported by animal studies and the observed association between body stalk anomaly and cocaine use, a drug known for its vasoconstrictive effects (30). A generalised reduction in embryonic blood flow in early stages may result in an incomplete closure of the ventral body wall, leaving the coelomic cavity open. This lack of closure compromises the support of the amnion, making it prone to rupture and potentially causing the formation of amniotic bands (30).

# 3 HYPOTHESES ON THE GENESIS OF DISCORDANCE: THEORIES EXPLORED

A review of the literature uncovers multiple theories attempting to explain the origins of genetic and structural discordance in MC twins. Although some have challenged this viewpoint, the prevailing understanding is that the twinning process occurs randomly, with any subsequent differences between the individuals developing independently (5). In contrast, others suggest that differences between twins, whether genetic or structural, do not arise after the splitting but may instead serve as the primary trigger for twinning (31). This perspective directly contradicts the 'All-or-Nothing Principle,' which asserts that malformations cannot be induced within the first two weeks after conception. According to this principle, any damage to the embryo during this period would either be repaired by the pluripotent cells, or, if the damage is too severe, the embryo would be terminated (32). One must change one ‘s perspective to truly be able to delve into this topic, in the sense of considering that mechanisms of a genetic and epigenetic nature, as well as environmental processes, ensure that MZ twins become their unique individuals. Machin 2009 went even so far as to imply that it is truly remarkable, that some pairs of monozygotic twins remain so similar, despite the abundance of post zygotic environmental and epigenetic events (3). Within the collective of MZ twins, the majority, about two-thirds, hold an even more significant position, considering that they share a single placenta (3). For any speculations of the genesis of such discordances, one must acknowledge the exposure to such an incredibly unique environment that a single shared placenta upholds. The environment of the MZ twins may also not be as similar, possibly leading to structural differences (5). The amount of placenta allocated to each twin may differ, the number of cells allocated and the timing of the twinning may be an underlying cause of discordance (5). Geoffrey Machin suggests that the initial changes may occur moments after the zygote forms, potentially involving alterations in foundational elements such as germ cells. These changes could arise around the time of, or in tandem with, the determination of organ classification, tissue differentiation and axis formation (3). Another aspect concentrates on a possible cascade of events during sensitive phases of development, which could also be the underlying cause of discordance in complex diseases. It may begin with a single recessive mutated gene, which is then

succeeded by a further somatic mutation in the other allele (5). Trauma to the inner cell mass, such as during events like hatching, has also been proposed to trigger a splitting of the zygote (31). This could explain the rise of twins in ART and may even be a contributing factor in possible hereditary twinning (5). Further theories discuss disproportional blastomere distribution, X inactivation, chromosomal mosaicism and point mutation.

## *3.1 DISPROPORTIONAL BLASTOMERE DISTRIBUTION*

It has been proposed that a possible disproportion in blastomere distribution between the twins during the twinning process, would imply that they begin to diverge in their development from the moment of twinning. On the other hand, such an imbalance may even be the trigger to initiate the twinning process. Nevertheless, this disproportion may form the foundation for further differences between the twins as they continue to develop (31). Blastomeres are cells that arise from the cleavage of a fertilised egg, a process in which cells repeatedly divide without increasing in size (33). A particular pair of female twins inspired this theory: one twin exhibited the expected random X-inactivation, while the other was found to express a pathological skewed X-inactivation. This finding suggested that, in very early embryogenesis, the distribution of stem cells to each twin may not always be precisely equal or as meticulously balanced as expected. A smaller initial pool of stem cells for one twin could lead to developmental disparities, impacting both prenatal and postnatal growth. Successful development requires a cascade of normal transcriptional events to allow the orderly progression of cell lineage and differentiation (31). Additionally, it has been proposed that unequal blastomere allocation might necessitate an extra mitotic cycle for MZ twins to reach the birth weight of singletons or even DZ twins. A cell clone with fewer blastomeres would require more mitotic cycles to achieve comparable birth weight (34). Unequal blastomere distribution could also have vascular implications. A twin with fewer initial cells might receive a smaller portion of the shared placenta, leading to growth impairments. As a result, the intrauterine environment of MC twins may even be less similar than that of DZ twins. This discordance could extend beyond X chromosomes to other genes that may or may not undergo the same methylation patterns in each twin, leading to phenotypic differences.

## 3.2 X-INACTIVATION AND IMPRINTING

In order to maintain control over the amount of genetic information in each diploid cell, not every gen expresses both alleles (34). Two prime examples of epigenetics are X chromosome inactivation (XCI) and genomic imprinting (34). Females possess an X chromosome from each parent, while males hold a single maternal X chromosome. Thereby, to avoid an excess of genetic information, the greater amount of genetic information on the X chromosome, compared to the Y chromosome, requires a random inactivation of the X chromosome in female cells. Ultimately, this should result in a random inactivation between maternal and paternal active X chromosomes. If this does not occur randomly but instead one X chromosome is preferentially inactivated, and for instance, results in a 90/10 ratio, then a skewed X-inactivation manifests. Much of the coordination of the choice, number and counting of X - chromosome inactivation is done by a X-inactivation centre, which also silences around 1,000 genes of the X chromosome (34). This occurs quite early during embryogenesis, specifically in the peri-implantation embryo within the 10 – 20 cell epiblast lineages, a time when somatic cells emerge. This eventually ends in a mosaic of the X-chromosomes in females, which will maintain their inactivation allocation in further mitosis. Cases of a skewed X inactivation have been reported, as illustrated in Figure 13.

**TABLE II. Discordant Phenotypes With Unequal X-Inactivation in Female MZ Twin Pairs**

| Disorder | Refs. |
|---|---|
| Fabry, 231 Asp->Asn | Redonnet-Vernhet et al. [1996] |
| Lesch-Nyhan, IVS8 + 4 A->G | De Gregorio et al. [2005] |
| Acardius, skewed in acardius | Masuzaki et al. [2004] |
| FRAX, full mutation | Willemsen et al. [2000] |
| Hemophilia A | Bennett et al. [2008] |

*Figure 13: Female MZ Twins with unequal X-inactivation with discordance in phenotypic expression (3)*

In such instances of female twins demonstrating a discordance in phenotypical expression in X-chromosome-linked diseases, skewed X-inactivation has been discussed as a causing factor**.** This was drawn from the observation, that each case of reported MZ twins with a X-linked disease, have always been discordant for the

disease. At no time has it been reported that both or neither of them was affected (31). Evidence demonstrated, that in the affected pairs the healthy twin showed either a random X-inactivation or an inactivation of the X chromosome with the mutated gene, while the clinically impacted twin displayed a non-random inactivation (31). Interestingly, in some of these pairs, an unbalanced distribution of blastomeres was also detected. Two possible mechanisms are proposed to explain how the skewed X inactivation may have transpired. For one, within the inner cell mass, two clones of cells with dissimilar XCI segregate and demonstrate mutual aversion from the clones with inversed suppression. The X-inactivation would proceed and may subsequently even trigger the splitting and finish as a reciprocal skewed X-inactivation (34). However, this remains a highly theoretical scenario, as it is unlikely that maternal and paternal X-inactivation would occur in a perfectly balanced 50:50 ratio. Moreover, this theory would imply the existence of individuals with exclusively maternal or paternal X-inactivation, a phenomenon that has not been documented. Another possibility entails a functioning random X-inaction but draws back to the unbalanced allocation of blastomeres. It is then likely that the twin with the fewer portion of blastomeres, despite it occurring by chance, would conclude with a skewed X-inactivation. The larger count of blastomeres in the co-twin would allow for, at least, a nearly balanced inactivation (34). Lee et al. 2013 reflect on the potential significance of XCI in female MZ twins, suggesting that if skewed XCI is indeed a common occurrence in this population, then it may even represent a crucial factor in the twinning process, possibly even playing a role in its initiation (34). This could account for the observed higher number of females among MZ twins (34). It was even suggested that singleton females with secondary skewed XCI and resulting X-linked diseases may be a surviving twin of a MZ pair (34). On the other hand, genomic Imprinting follows a similar principle but dives a little deeper, as it concerns the coordinated regulation of either the maternal or paternal allele of distinct domains in genes (31). Cases of discordant imprinting can be seen in Table 8, each lead to discordant phenotypes. When looking at XCI and imprinting, although both different in their nature, they collectively impact 5%- 10% of genes in the mammalian genome. As a result, they are crucial in the context of congenital diseases and malformation, since mutations in these genes cannot be compensated by a silenced wild-type allele. Thereby this regulation plays a key role in some

congenital diseases such as Fragile X syndrome, Rett, Prader-Willi/Angelman and Beckwith-Wiedemann Syndrome (34).

## *3.3 CHROMOSOMAL MOSAICISM*

Genetic mosaicism occurs when an individual has two or more cell lineages with differing genotypes, all originating from a single zygote (35). On the other hand, when separate cell lines come from different zygotes, this condition is referred to as chimerism. Mosaicism results from mutations that occur after the zygote stage, known as postzygotic mutations (35). As a post–zygotic genetic event, chromosomal mosaicism has also been introduced in the literature to account for genetic and structural discordance in MZ twins. This observation is based on studies of MZ twin pairs with aneuploidies, including the four most frequent observed presentations: 45X (Turner syndrome) and trisomies of chromosomes 21 (Down syndrome), 18 (Edwards syndrome) and 13 (Patau syndrome), who showed significant phenotypic differences. In these instances where the twins exhibit differing expressions of aneuploidy, mosaicism has been identified (31). Although it did not present itself the same in all cases. In some cases, fibroblast cell lines between the embryos showed dichotomy, suggesting the potential of blood chimerism via vascular anastomoses in MC twins (34). In other instances, fixed tissue cells have also demonstrated mosaicism, potentially on account of non-disjunction at the first post-zygotic division. For example, the literature reported on MZ twins with 45X and 47XXY, as well as another female pair with a 45X and 47XXX cell line, in both a 46XY or 46XX was not detected (34). Non-disjunction may the underlying cause. Further instances of X-chromosome mosaicism can be found, which are listed in Figure 14 below. Such a non-disjunction of the X-chromosomes can be followed with the affected female twin with 45X to clinically show signs of Duchenne muscular dystrophy. For example, this was seen one twin pair, the symptomatic twin showed 45X/46XX mosaicism in lymphocytes, none in fibroblasts, the other twin's chromosomes in lymphocytes were 46XX. The son of the unaffected female twin was diagnosed with Duchenne later in life. Additional instances of non-disjunction were observed in MZ twin pairs discordant for trisomy 21, who presented with 46XY/ 47XX +21, chimerism in blood but a normal set of chromosomes in fibroblasts (3). A different case demonstrated a MZ pair with a discordant phenotype of the Fragile X Syndrome with discordant trinucleotide expansion. The unaffected

twin was a mosaic for the full and premutation while in the other twin, the full mutation was identified (3). Mosaicism was not limited to the quantity of chromosomes, but structural anomalies were also identified. The literature reveals various examples, one included one MZ pair which was discordant for a 46XY, del (7) (q32-> qter) chromosome constitution with chimerism in lymphocyte (24). It must be noted that chorionicity was not always included in the documentation of the cases (31).



TABLE I. X-Chromosome Aneuploidy Mosaicism*

| Author | Twin A fibroblasts | Twin B fibroblasts | Blood chimerism | MZ testing |
|---|---|---|---|---|
| Edwards et al., 1966 | UT 45,X | NM 45,X | 45,X/46,XY | |
| Karp et al., 1975 | UT 45,X/46,XY | NM 2 | 45,X/46,XY | BGA |
| Schmidt et al., 1976 | UT 45,X | NM 46,XY | None | BGA |
| Reindollar et al., 1987 | UT 45,X/46,XY | NM 45,X/46,XY | ? | MC |
| Arizawa et al., 1988 | UT 45,X | NM 46,XY | None | |
| Turpin et al., 1961 | UT 45,X | NM 46,XY | 2 | MC |
| Perlman et al., 1990 | UT 45,X | NM 46,XY | ? | MC |
| Wapner et al., 1990 | UT 45,X | NM 46,XY | 2 | MC |
| Dallapicolla et al., 1985 | UT 45,X | NM 3 2 (triplets) 46,XY | | MC |
| Kurosawa et al., 1992 | UT 45,X/47,XXY | 45,X/47,XXY | | RFLP |
| Deacon et al., 1980 | TRAP, features UT 45,X | NF pump twin 46,XX | 45,X/46,XX | MC |
| Pedersen et al., 1980 | UT 45,X | NF 2 | | BGA-9932 |
| Ross et al., 1969 | UT 45,X/47,XXX | NF 45,X/47,XXX | | BGA 70-99 |
| Kaplowitz et al., 1991 | UT 45,X | NF 46,XX | 45,X/46,XX | RFLP |
| Neilson et al., 1982 (case 3) | UT 45,X | NF 46,XX | | MC |
| Reiss et al., 1993 | UT 45,X | NF 46,XX | | RFLP |

*NF, normal female phenotype; NM, normal male phenotype; UT, Ullrich-Turner phenotype; BGA, blood group antigens; RFLP, restrictive fragment length polymorphism.

*Figure 14: X - Chromosome Mosaicism (25)*

## *3.4 POINT MUTATION*

There is limited documentation in the literature of postzygotic single-gene mutations in MZ twins. Although one notable case, reported by Machin 2009 involved MZ twins discordant for Van der Woude Syndrome, where a nonsense mutation in the IRF6 gene was found in one twin but not the other (3). Even though discordant single-gene mutations are rare, differences in clinical presentation have been observed more frequently. For example, one twin pair with neurofibromatosis showed significant variation in the condition's expression: one twin had a much higher density of café-au-lait spots and developed an optic glioma by age two. Although, it is important to recognise that neurofibromatosis is characterised by variability in phenotypical expression. However, this case raised questions about whether the differences might also reflect an uneven early distribution of cells, as other similar cases have shown more consistent phenotypes between twins.

## *3.5 Intermediate Twins*

In some cases, the hypotheses proposed thus far have not provided a satisfying explanation, prompting some researchers to entertain the concept of "intermediate"

twins (3). Such intriguing cases would include twins that are genetically and phenotypically too similar to be classified as DZ yet they also do not fully meet the criteria for MZ twins. For instance, MC twins that are of opposite sexes or who significantly differ phenotypically would fall into this category (5). Although highly theoretical, some attempts have been made to explore this phenomenon further including polar body fertilisation, dispermic fertilisation and fusions of trophoblasts.

## *3.6 Polarbody*

The leading hypothesis centre on the role of polar bodies formed during meiosis. For heterozygous maternal alleles, the first polar body represents the exact reciprocal of the secondary oocyte and ovum due to chromosomal segregation, which may involve recombination (3). If the first polar body, containing 46 chromatids and 23 chromosomes, is fertilized instead of degenerating, a triploid twin can result. This phenomenon has already been documented in a MC twin pair derived from the same oocyte, where the healthy chimeric twin was diploid (46XY) and the other was triploid (69XXX). Even if a mechanism existed to reduce the first polar body to a haploid state before fertilisation, any resulting diploid twins would be less genetically similar than typical DZ twins, due to the reciprocal distribution of heterozygous maternal alleles between the secondary oocyte and the first polar body. In cases where the second polar body is fertilised, recombination would cause reciprocal differences in heterozygous alleles within the recombined segment, resulting in additional variability in the maternal genetic contribution (3).

## *3.7 Dispermic Fertilisation*

Another proposed explanation for certain twin cases involves dispermic fertilisation followed by the diploidisation of a triploid zygote (23). In this scenario, two distinct cell lines develop from the dispermic triploid zygote: one cell line expels an X-bearing male nucleus, while the other discards a Y-bearing male nucleus. The subsequent fusion of these cells results in chimeric twinning (3).

## *3.8 Fusion of Trophoblasts in DZ twins*

The practice to conclude that any multiple pregnancies with a single placenta (MC) must also be MZ has been put into question by documented cases of MC pregnancies that are, in fact, of DZ twins. In some cases that were initially classified

as MC, dizygosity was discovered when twins were of opposite sexes, exhibited unusual phenotypes, or were simply identified through routine twin care (3). Machin, 2009 compiled data on 13 cases of MC dizygotic twins, all of whom exhibited blood chimerism but no chimerism in solid fetal tissues, confirming their dizygosity. Although one case occurred naturally, most were conceived via IVF/ICSI. In these cases, trophoblast fusion led to blood-restricted chimerism and possibly also placental chimerism. However, since the cells did not fully integrate into one body, the twins developed separately, resulting in MC placentation in DZ twins (3). This concept is visualised in Figure 15. Although it must be noted, that the zone pellucida, which would prevent any fusions, is not depicted and considered in this instance.

The formation of an MC placenta enabled blood vessel connections between the twins, leading many DZ pairs to display blood lymphocyte chimerism and/or blood group chimerism. However, the absence of chimerism in solid fetal tissues suggests that the cells forming the inner cell masses did not mix before the twinning process began. In some examined cases, even trophoblastic mixing was minimal. One particular case involved the implantation of three blastocysts via IVF, two of which developed into opposite-sex twins with distinct genitalia. After birth, pathology confirmed they shared a single chorion with interconnected blood vessels. Although no chimerism was detected in amniotic cells or skin fibroblasts, blood lymphocytes of both twins showed chimerism (46XY and predominantly 46XX) by three months of age. Early DNA studies initially suggested they were monozygotic. However, in situ hybridisation later revealed X signals in the female twin's placental area and Y signals in the male's, confirming their dizygosity (3).

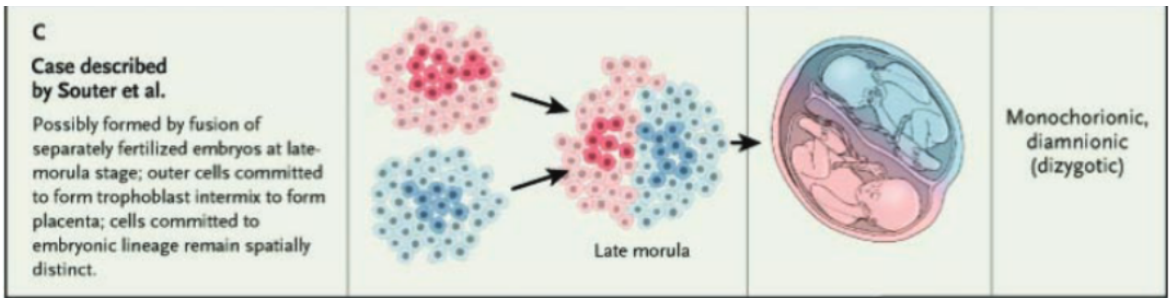



*Figure 15: Possible Fusion of Trophoblasts (3)*

## *3.9 Reevaluating the true frequency of MC twins*

To gain a complete understanding of MC twins, it's essential to recognise that these twins are frequently misclassified or overlooked, potentially leading to an underestimation of their actual frequency (3). Misidentification often begins with an initial, overly simplified assumption about twin status. Terms like "monozygotic twins" and "identical twins" are commonly used interchangeably, even by medical professionals, fostering an expectation among parents that MZ twins will be indistinguishable. As a result, MZ twins who naturally differ may be incorrectly categorised as DZ and excluded from MZ statistics. Similarly, twins deemed "not identical enough" are often misclassified as DZ. Such misclassification has serious implications, as identifying a multiple pregnancy with MZ twins requires specific management due to potential unique complications. Additionally, MZ twins are expected to be similar in size, so discrepancies in growth, affecting both facial proportions and appearance, may lead to mistaken DZ classification (3). Conversely, certain conditions can mask differences in MC monoamniotic (MCMA) twins, where one twin may compensate for the other's developmental issues. For example, if one twin has renal agenesis and develops oligohydramnios, the healthy twin may offset this effect. Vascular connections in MC twins can even allow for the transfusion of TSH and thyroid hormones to a twin with thyroid dysgenesis, protecting the affected twin from prenatal hypothyroidism. However, because postnatal TSH screening is typically missed in such cases, this can have long-term health implications. For this reason, the literature recommends a second TSH screening 14 days after birth for all same-sex twins. Additionally, it's hypothesised that certain anomalies in one twin may lead to a "vanishing twin," where the twin does not survive past the first trimester. Together, these factors suggest that the true rate of discordance among MZ twins may be underestimated (3).

# 4 Materials and Methods

## *4.1 Research question*

This thesis aims to investigate the spectrum of discordant anomalies reported in MC twins, the proposed theories explaining these discrepancies and how these align with clinical cases. Specifically, it conducts a retrospective analysis of MC multiple pregnancies with discordant anomalies in Graz from 2013 to 2023 to assess the degree of concordance between observed cases and existing explanatory models in the literature.

## *4.2 Data collection*

All data were obtained from the local registry "MonoReg“, which has been maintaining records of patients with multiple pregnancies at the University Hospital in Graz, Austria. The registry had received prior approval from the ethics committee (EK 29-105 ex 16/17). It was created and is continuously managed by Prof. Dr. Philipp Klaritsch. Patient information was retrieved from medical records stored in the hospital information system, including “openMEDOCS” and “Pia ViewPoint.” These sources contain medical reports, discharge summaries, clinical notes and diagnostic findings. Relevant data was systematically extracted to complete the following categories:

- Patient demographics: Date of Birth
- Pregnancy characteristics: Chorionicity/Amnionicity, fetal anomaly, genetic findings (if diagnostic procedures conducted and results available)
- Diagnosis and intervention details: Time of diagnosis (antenatal/postnatal), Gestational age at diagnosis, Type of intervention, Gestational age at intervention, Procedure date
- Pregnancy outcome: Gestational age at birth or miscarriage, Date of birth/miscarriage, Location of referral
- Maternal factors: Assisted Reproductive Technologies, any pre-existing maternal medical conditions

However, not all categories could be fully completed for every patient. Especially, genetic findings were highly restricted due to confidentiality regulations. Additionally, most patients were only referred to the hospital for consultation and potential

treatment but ultimately continued their care and delivery at their local hospital. All available data was compiled in a Microsoft Excel database, which is continuously updated with new cases as they arise. However, for the purpose of this thesis, cases between 2013 and 2023 were included.

Comprehensive literature search was conducted using PubMed to identify relevant articles. The search strategy included amongst others, Medical Subject Headings (MeSH), combining them with boolean operators such as AND and OR. As such, the search with "Twins, Monozygotic"[MeSH] AND "Congenital Abnormalities"[MeSH]) AND (discordance OR discordant)" provided 411 results. These were manually screened for relevance, while additional sources with key e terms such as „twins“, „monochorionic“, „congenital abnormality“, „phenotype“, „zygote“, „mutation“ were also manually reviewed. Furthermore, pre-selected literature provided by the supervisor was also incorporated. Finally, the reference lists of all included articles were assessed to identify additional relevant studies.

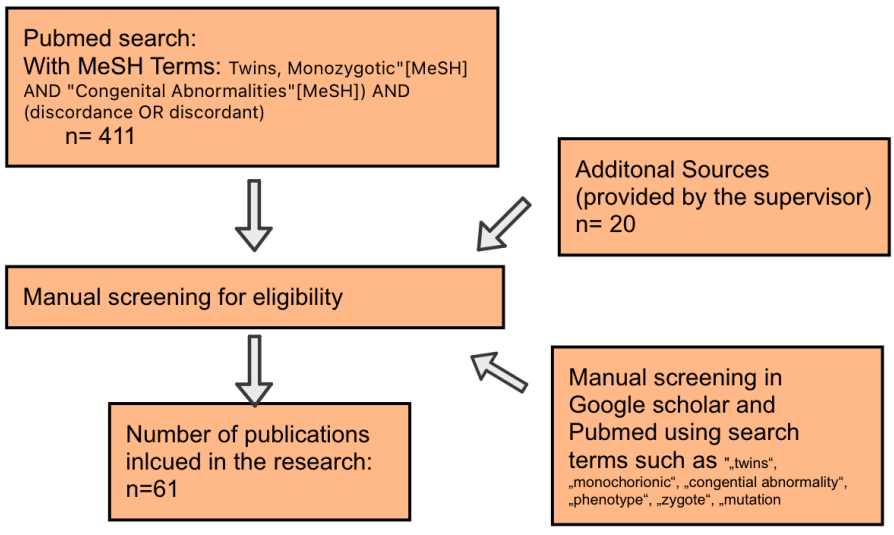



*Figure 16: Flow chart*

## 4.3 Study design

The study is a descriptive, retrospective and monocentric study conducted at the Department of Obstetrics and Gynaecology at the Medical University of Graz. Data was collected from the register between the years 2013 and 2023. Data analysis was performed using descriptive statistical methods to showcase the findings and identify patterns in the data and discuss to what extent this aligns with the findings in the literature. All data were anonymised to ensure patient confidentiality.

## 4.4 Selection of patient cohort

As discussed in „Data collection“ the data was obtained from the register „MonoReg“, which consisted of data collected from the hospital’s information system „openMEDOCS“ and „Pia View Point“ of women with MC multiple pregnancies who were consulted at the Department of Obstetrics and Gynaecology at the Medical University of Graz.

## 4.5 Inclusion criteria

To be included in the “MonoReg” registry, patients needed to receive care at the Department of Obstetrics and Gynaecology in Graz with a multiple pregnancy and suspicion of discordant genetic or structural discordance diagnosed. Genetic discordances, such as differing karyotypes or structural discordance, such as the presence of a congenital anomaly in one fetus and not the other, as confirmed via prenatal imaging (e.g. ultrasound) or invasive testing (e.g. amniocentesis, chorionic villus sampling). All women had to be at least 18 years of age and be seen at the centre between the years 2013 and 2023. Cases involving triplet pregnancies were also included, provided they had a DC configuration and thereby contained a MC placenta between at least two out of three fetuses. Since the aim of this thesis is to provide a descriptive overview of the cases seen and managed at the centre, rather than to perform statistical comparisons or identify significant differences, patients with incomplete follow-up data were still included in the report.

## 4.6 Exclusion criteria

Patients who received care at the centre with singleton pregnancies or multiple pregnancies without discordance (i.e. where genetic or structural abnormalities

were present in both fetuses) were excluded from the registry. Any cases consulted before 2013 or after 2023 were not taken into consideration.

# 5 Results

## *5.1 Traits of the cohort*

In total, the registry included 51 patients between the years 2013 – 2023. The ages range between 20 and 43 years of age, resulting in an age span of 23 years. The median is 30 years of age, while the mean age is also 30 years of age.

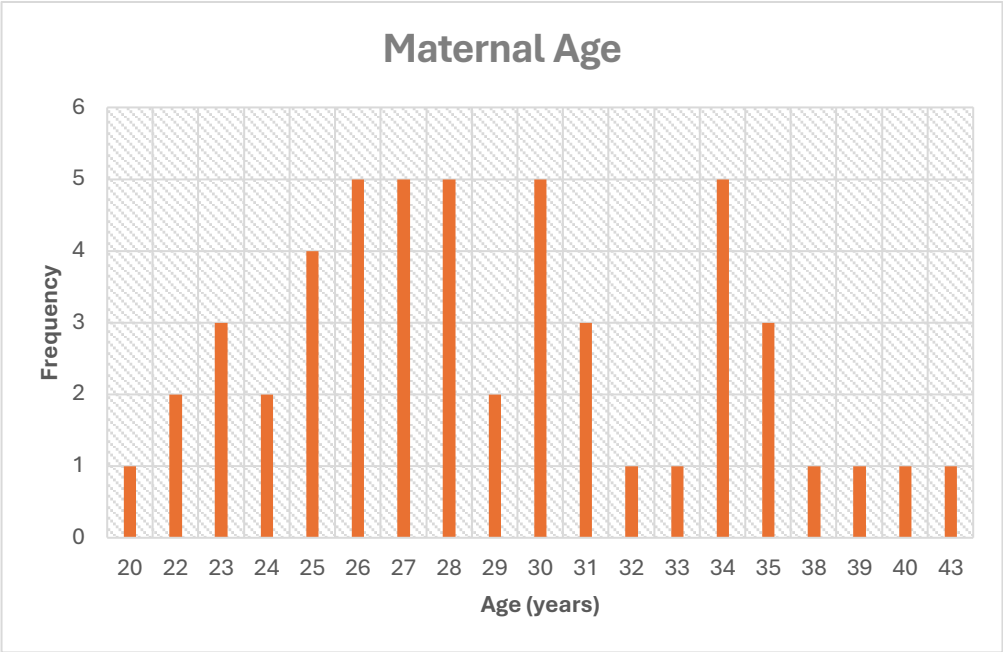



*Figure 17: Age distribution*

## 5.2 Overview of Anomalies

An overview of the discordant anomalies seen in Graz between 2013 – 2023 is showcased below. The most common discordant anomalies were hydrocephalus (9,8%), anencephaly (9,8%), body-stalk-anomaly (9,8%), spina bifida (7,8%) and CHD (5,8%).

| Anomaly | Frequency |
|---|---|
| Hydrocephalus | 5 |
| Anencephaly | 5 |
| Spina bifida | 4 |
| Body-stalk-anomaly | 5 |
| Congenital heart defect | 3 |
| Hydrops fetalis | 2 |
| Omphalocele | 3 |
| Renal agenesis | 2 |
| Ventriculomegaly | 2 |
| LUTO (Lower Urinary Tract Obstruction) | 2 |
| Ascites | 1 |
| Cantrell's Pentalogy | 1 |
| Cephalopagus | 1 |
| CPAM, ARSA, sFGR Type 2 and suspected TAPS | 1 |
| Brain anomaly and diaphragmatic hernia | 1 |
| HLHS (Hypoplastic Left Heart Syndrome) | 1 |
| Hygroma Colli | 1 |
| Caudal regression syndrome | 1 |
| Cloacal exstrophy | 1 |
| Congenital emphysema | 1 |
| Complex malformations | 1 |
| Multiple malformations (renal agenesis, occipital cephalocele) | 1 |
| Complex malformation syndrome (acrania, exencephaly, sternal defect, omphalocele) | 1 |
| Esophageal atresia | 1 |
| Pericardial effusion | 1 |
| Bilateral retinoblastoma | 1 |
| Rhombencephalosynapsis | 1 |
| Schimmelpenning-Feuerstein-Mims syndrome | 1 |
| Skeletal anomalies | 1 |
| VACTERL | 1 |
| Vein of Galen aneurysm | 1 |
| CNS malformations (Dandy-Walker malformation) | 1 |

*Figure 18: Overview of anomalies in Graz between 2013 - 2023. Individuals with more than one anomaly are represented multiple times.*

## 5.3 Time of diagnosis

Apart from a single postnatal diagnosis of oesophageal atresia with a type IIIB fistula, all remaining discordances of the twins were diagnosed antenatally.

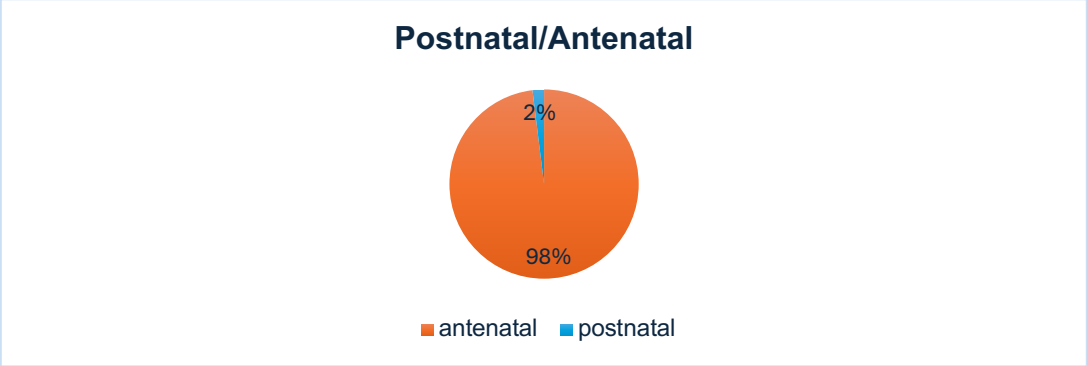



*Figure 19: Time of diagnosis - antenatal/postnatal*

## 5.4 Gestational age at the time of diagnosis

The gestational age at which the discordant anomaly was diagnosed ranged from 9+2 weeks to 29+4 weeks of gestation. In the majority of cases (84% n=43), the diagnosis of discordant anomalies in MC twin pregnancies was made during the second trimester (14+0 to 27+6 weeks of gestation). Only six cases (12%, n=6) were diagnosed in a first trimester screening (≤13+6 weeks), while a single case (2%, n=1) with a late onset of Lower Urinary Tract Obstruction was diagnosed in the third trimester. Only one single case of an oesophageal atresia with a Type IIIB fistula was diagnosed postnatally (2%, n=1). The median gestational age at diagnosis was 18+2 weeks.

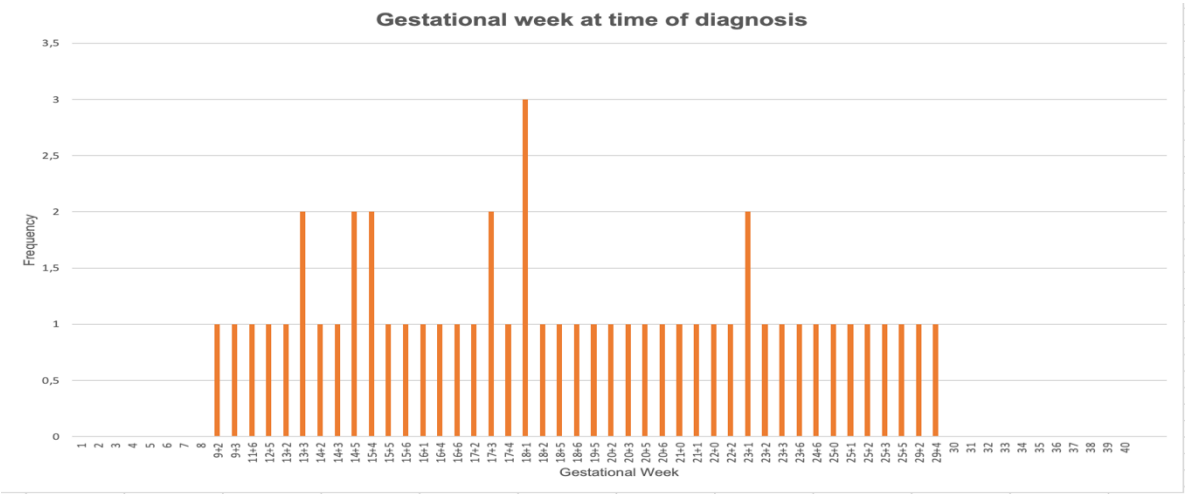



*Figure 20: Gestation week at the time of diagnosis*

## 5.5 Interventions

Out of the 51 MC twins with discordant anomalies, 52,9% (n=27) underwent cord occlusion (CO) for a selective feticide on parents' request. Conservative management was chosen in 29,4% (n=15) of cases. Radiofrequency ablation and laser coagulation were performed in 4 cases each. Potassium chloride injection was used in only a single case.

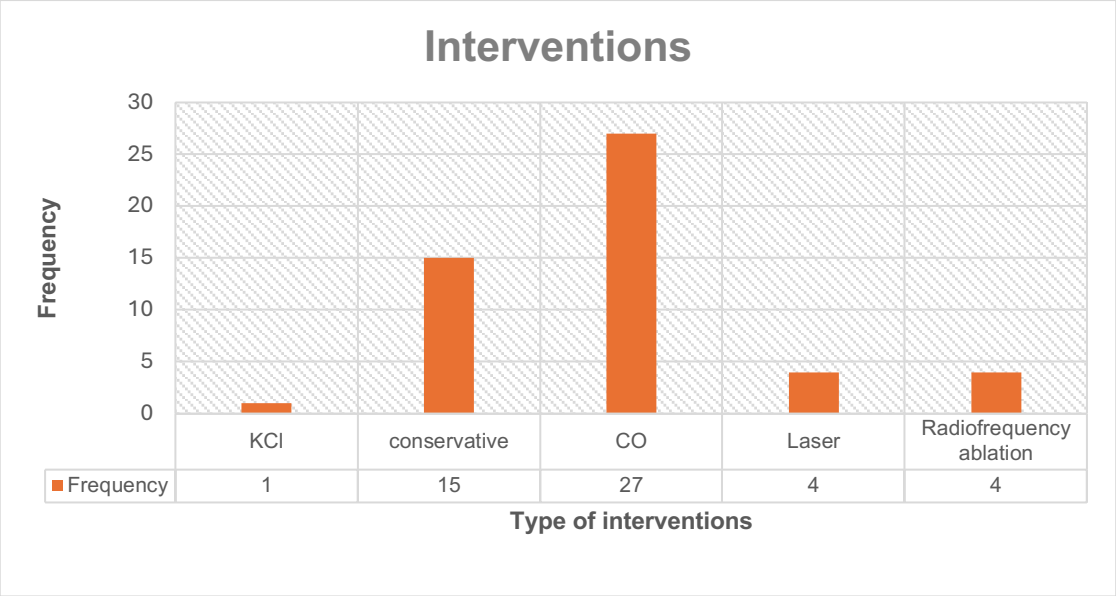



*Figure 21: Types of interventions*

## 5.6 Assisted Reproductive Technologies

In this cohort, 88,2% (n=45) of the pregnancies were conceived spontaneously, indicating that assisted reproductive technologies (ART) played a minor role in this population. Among the 51 patients, only 11,8% (n=6) resulted from ART, of which 4 were conceived via IVF while 2 via ICSI.

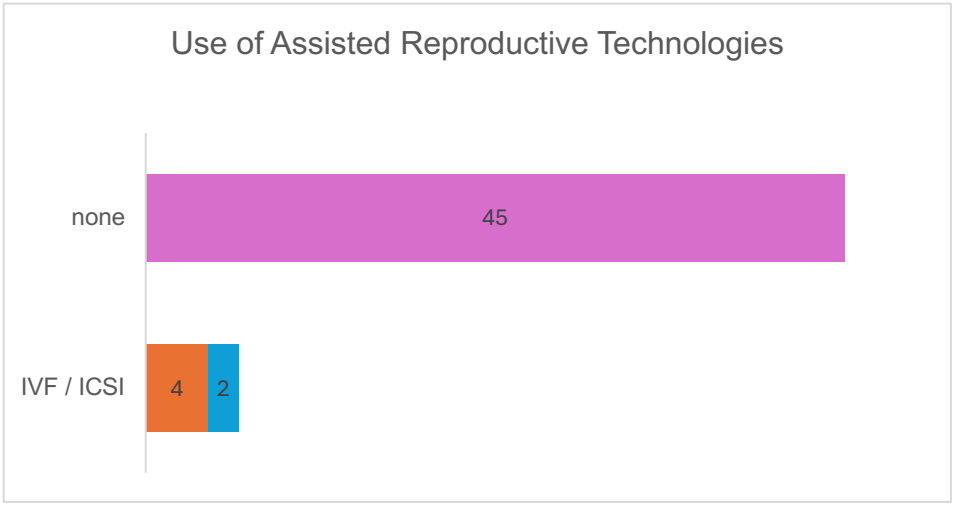
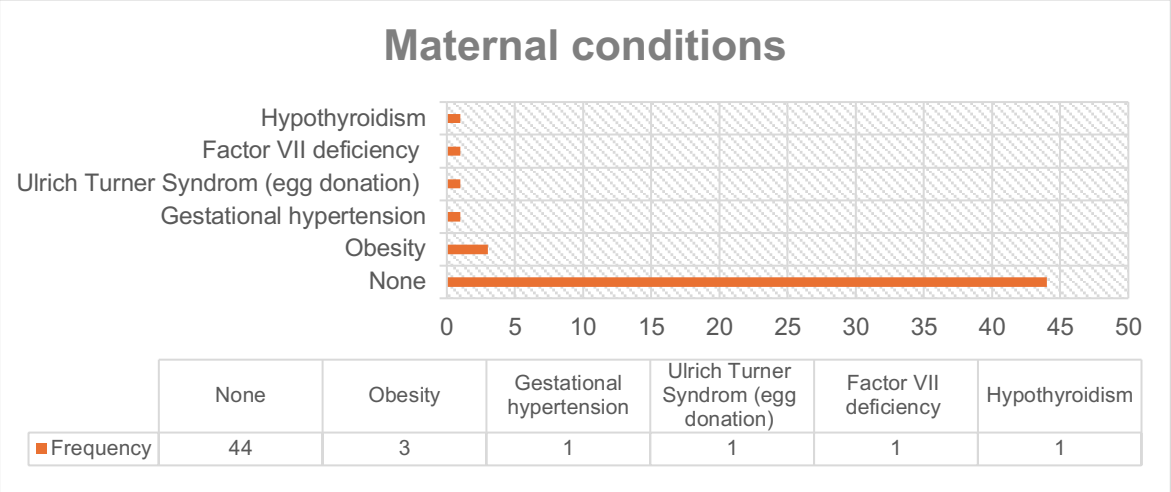



*Figure 22: Use of Artificial Reproductive Technologies*

## 5.7 Maternal co-morbidities

In most cases (n=44, 86,3%), no maternal comorbidities were reported. Among the remaining patients, obesity (≥30 BMI) was the most frequently documented condition (n=3, 5.9%), followed by a single case of gestational hypertension, Ulrich Turner syndrome (pregnancy conceived following egg donation), factor VII deficiency and hypothyroidism (each n=1, 2%). Overall, maternal conditions were rare in this cohort, with this only 7 women (13,7%) presenting with any pre-existing medical conditions.




| | None | Obesity | Gestational hypertension | Ulrich Turner Syndrom (egg donation) | Factor VII deficiency | Hypothyroidism |
|---|---|---|---|---|---|---|
| Frequency | 44 | 3 | 1 | 1 | 1 | 1 |

*Figure 23: Maternal conditions*

## 5.8 TTTS/TRAP

In the predominant proportion of cases (n=46, 90%), neither TTTS nor TRAP sequence was observed. TTTS was diagnosed in 4 pregnancies (7,8%), while TRAP sequence occurred in 1 case (2%). These findings indicate that severe vascular complications were relatively rare in this cohort.

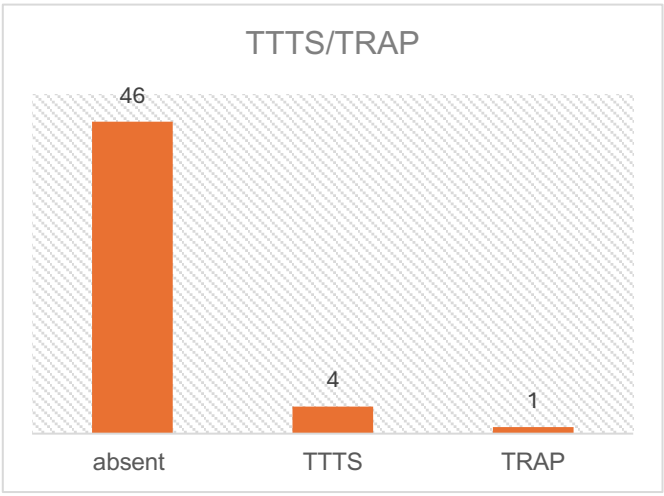
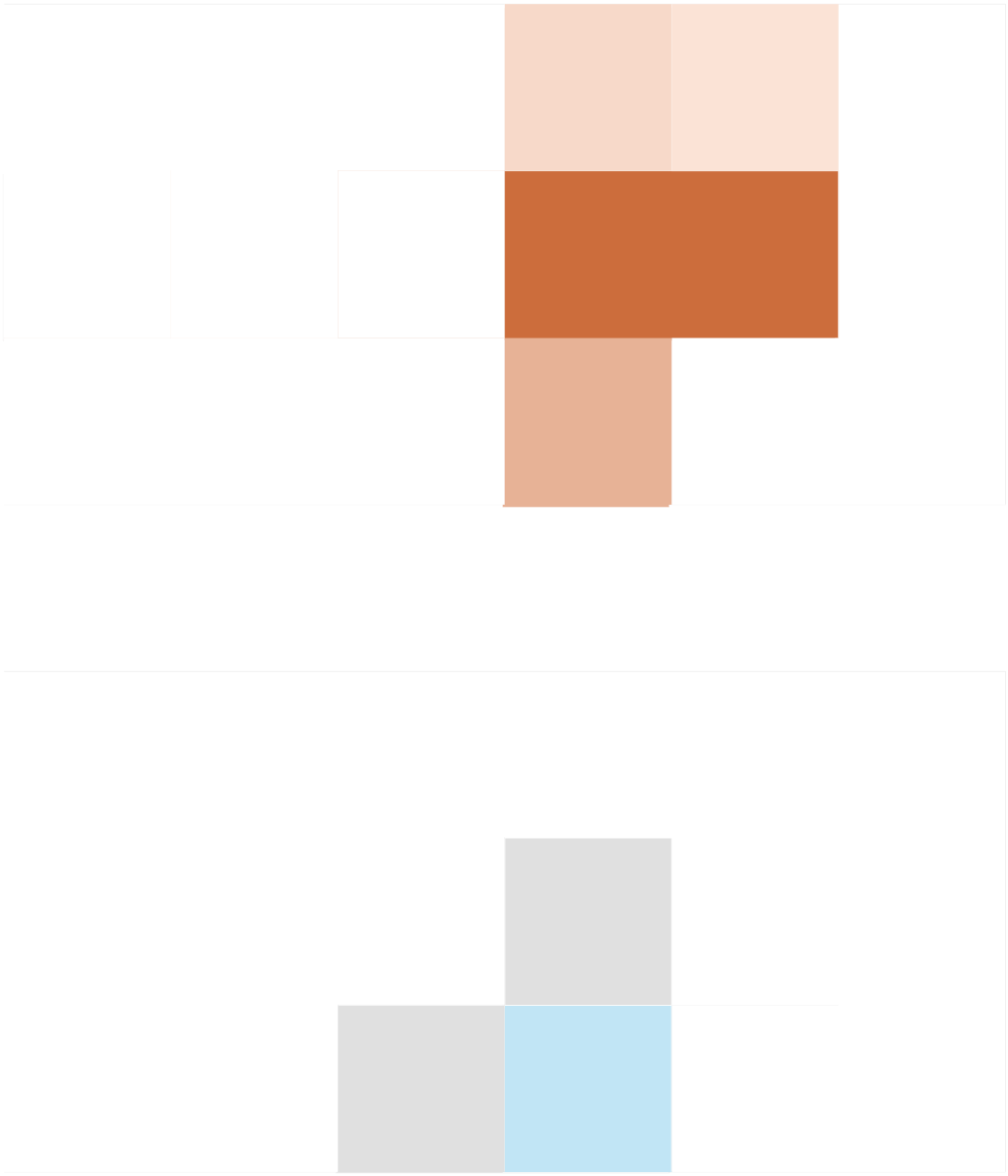



*Figure 24: Presence or absence of TTTS/TRAP*

## *5.9 Regions of Referral*

Most patients in this cohort were referred from within Austria. The highest number of referrals came from Vienna (n=16), followed by the local province of Styria (n=13), Carinthia (n=6) and Salzburg (n=5). Additional Austrian provinces included Upper Austria (n=2), Tyrol (n=1) and Lower Austria (n=1). Cross-border referrals included Slovenia (n=3), Hungary (n=1), Romania (n=1) and Bosnia (n=1).



## *5.10 Genetic results*

Genetic testing was offered to families of affected individuals, with 10 choosing to proceed with genetic analysis of the affected twin. Among these, 8 cases showed no detectable genetic abnormalities. One case, showed a heterozygous KRAS c.35G>A mutation, representing a postzygotic event that produces somatic mosaicism and accounts for the varying clinical phenotype of Schimmelpenning-Feuerstein-Mims syndrome. In another case the detection of only one twin having an omphalocele, lead to the parents deciding to undertake some further genetic testing, resulting in the detection of a microdeletion on chromosome 17p11.2, thereby a Smith-Magenis-Syndrome.

# 6 Discussion

This thesis aimed to investigate cases of MC twins with structural or genetic anomalies, for which they are also discordant for, as well as to discuss what theories can be found in the literature to explain these manifestations. Relevant cases that were seen at the Department of Obstetrics and Gynaecology in Graz between 2013 and 2023 were also showcased.

## *6.1 Key findings*

The literature describes numerous cases demonstrating a relatively broad spectrum of anomalies in which MC twins have been found to be discordant of, highlighting that, although rare, such occurrences do indeed happen. Several hypotheses have been proposed, each attempting to pinpoint possible causes and the timing of genetic or developmental divergence in discordant MZ twins. Some of the theories appear to be more plausible than others, such as disproportionate blastomere allocation at the very earliest of the zygotic stages. It was even suggested that these occurrences may be a possible trigger for the embryo to split. Theories about skewed X-chromosome inactivation provide compelling insights, especially considering that the literature reported a higher incidence of female monozygotic twins. Also, many hypotheses in the literature focus on MZ twins, neglecting and not specifically addressing MC twins, overlooking the unique vascular environment of MC pregnancies. While some publications have considered the unique vascular situation, it was often in the context of CHDs in discordant MC twins, particularly TTTS being proposed as a major contributor to discordant twins' anomalies. Although, in this cohort, only in 5 cases the vascular components of TTTS/TRAP were diagnosed, each not in relation to a CHD. At first glance, this may challenge the prevailing assumption that such vascular complications, unique to MC twins, are a major driver of discordant anomalies, but given the limited sample size of this cohort, the data should be interpreted with caution. On the other hand, more speculative theories, such as polar body fertilisation and dispermic fertilisation, are intriguing and theoretically possible, yet remain highly unlikely and rather implausible in practice. More feasible are hypotheses that have been proposed in direct relation to documented anomalies, such as in cases of VACTERL association and body stalk anomaly. These theories are based on consideration of the specific

anomalies themselves, their underlying pathomechanisms and the potential timing and nature of the developmental disturbances that could have led to their manifestation.

Rather than reaching a definite conclusion, the purpose of this analysis is to be thought-provoking, while also highlighting new angles and inspire new outlooks on MC twins. This thesis was able to report on 51 cases in the matter of 10 years, offering a foundation for some comparison with published studies found in the literature, from which certain points arise that allow for some further reflection. The literature reveals ongoing discussion on whether maternal age influences the rate of MC twinning. While some advocate for the fact that higher maternal age increases the likelihood of MC twinning, others argue the opposite, proposing that higher maternal age of ≥35 years may potentially be even a protective factor. One such a study that indicated that higher maternal age was not a characteristic feature of MC twinning, was a cohort of MC twins with discordant anomalies in which the mean maternal age was 27.9 years, compared to 30.9 years for DC twins in the same cohort (36). Both are below the age that is considered be higher maternal age. Regarding the showcased cohort of Graz, 30 years of age was both the median as well as the mean maternal age, thereby both representing the average maternal age at childbirth across the European Union in 2022, which was 30.9 years (37). This suggests that the cohort is broadly representative of the general maternal population. Given this, our findings do not show an overrepresentation of either younger or older maternal age among MC twin pregnancies. Thus, this cohort does not support either side of the conflicting views reported in the literature, suggesting that maternal age alone may not be a reliable predictor of chorionicity, and further research is needed to better understand the complex factors influencing the type of twin pregnancy.

In most instances, the discordant anomalies were found or confirmed in the second trimester. This agrees to the typical timing of prenatal structural anomaly screening, which is done in the second trimester (20+0 – 21+6 SSW). The median gestational week of diagnosis was 18+6, a little earlier than in a study by Rustico et al. 2018 reporting a median of 19.1 gestational weeks (38). This indicates that some anomalies may manifest early, thereby pathological processes that lead to

discordances may be present and detectable earlier. This corresponds with some of the theories discussed, as the proposed pathological processes would also take place in the very early stages of human development. However, this must also be interpreted with thoughtfulness, as it may not only reflect early manifestation of anomalies, but as a specialised centre, also centre-specific factors such as high vigilance and access to early screening. Additionally, the diagnosis of a MC twin pregnancy alone triggers a more extensive and careful screening. Consequently, the early detection rate cannot be generalised to a broader clinical setting, where early intensive monitoring is not routinely done. This may, however, emphasise the importance of early evaluation and careful management in such a high-risk group.

The distribution of interventions indicates that cord occlusion (CO) was clearly the preferred management strategy in this cohort, possibly reflecting the centre's specific expertise or preference for this method. Conservative management also played a substantial role. The low frequencies of more specialised procedures suggest that these were reserved for highly specific clinical scenarios. Regarding the outcomes of the cohort, no interventions guaranteed a perfect outcome; double IUFD still occurred after an intervention. Together, these findings illustrate that although CO was the most frequently performed intervention, conservative management was associated with the most favourable outcome in this very small sample. However, it is important that this interpretation is approached with caution due to the extremely small numbers and likely selection bias, as patients undergoing interventions such as RFA or CO likely presented with more severe or lethal anomalies, thus inherently carrying a poorer prognosis independent of the intervention choice (39).

CHD was amongst the more frequent anomalies in our cohort (3 out of 51 – 5,8%), although this finding must be interpreted with caution, since that it was the most common anomaly recorded in Europe between 2012 and 2022 (24). As such, CHD is among the most prevalent congenital anomalies in the general population and its occurrence in our cohort may simply reflect its high baseline prevalence and multifactorial aetiology. Nonetheless, the already discussed specific theories have proposed that the unique vascular and hemodynamic environment of MC twins may contribute to an elevated risk of CHD. For example, a study by Springer et al. 2014

reported that MC twins with TTTS, were identified to have a higher prevalence of CHD (9,3%) compared to those without (4,7% p< 0,03) (40). Hence, while a direct association with MC twins cannot be established based on our data, the interplay between general population risk factors and MC-specific mechanisms calls for further investigation.

An interesting aspect is that the central nervous system was affected the most frequently in MC twins (41), especially by neural tube defects (42). Supporting this observation, anencephaly and spina bifida, both CNS anomalies, were among the most prevalent anomalies in our cohort. Although some authors argue that they did not find twins, including MC twins, to have a higher risk of having spina bifida. Notably, when compared to the EUROCAT registry, which encompasses anomalies across all births, including singletons, spina bifida was ranked only 16th in frequency, and hydrocephalus, the most common anomaly in our cohort, was ranked 15th. This discrepancy suggests that central nervous system (CNS) anomalies may occur disproportionately more frequently in MC twin pregnancies compared to the general population. It remains unclear whether this reflects a true biological tendency in MC twin pregnancies or arises from factors such as intensified prenatal screening, referral bias or reporting practices specific to specialised centres.

Even though the use of ART is known to elevate the probability of MZ twinning, its effect on MC twinning is less certain. In this context, the observation that in this cohort, merely 12% reported conception via ART, is particularly noteworthy. As such, this indicates that at least in this showcase population, ART did not appear to have played a substantial role. Therefore, considering that this cohort only includes MC twin pregnancies with discordant anomalies, it suggests that while ART may increase the rate of MZ twins, it does not appear to be significant contributing factor to discordance in MC twins. Nonetheless, caution is advised when drawing conclusions due to the small number of cases, and further studies are needed to investigate this in more detail.

The literature also suggests that maternal comorbidities, such as hypertensive disorders, diabetes or obesity, may contribute to discordance in MC twins. However,

in the present cohort, maternal co-morbidities were largely absent in the present cohort, with 86% of cases reporting no previous medical conditions. Among the few documented comorbidities in seven patients, there were three cases of maternal obesity, a condition that is also common in the general population. This limited occurrence reduced the likelihood that maternal obesity can be attributed to be a major factor in discordance twins. Furthermore, as previously mentioned, maternal health factors would typically be expected to affect both fetuses equally, it appears unlikely that maternal comorbidities are a major driver of discordant anomalies in MC twins.

For the remaining results, any further meaningful interpretation or analysis is limited due to the small sample size. Nevertheless, these findings offer preliminary insights into potential trends and hypotheses that could be further explored in larger, more comprehensive studies.

## *6.2 Multifactorial disorders*

When discussing discordant anomalies in MC twins, it is also important to differentiate between monogenetic and multifactorial conditions, the latter being conditions where genetic and environmental elements interplay. Although it is intriguing to look at all the various proposed theories, each attempting to shed some light on the pathophysiological mechanism lying behind discordant MC twins, it is important to acknowledge, only a fraction of human diseases is monogenetic and thereby caused by a mutation in a single gene.

Examples of monogenetic disorders include Cystic Fibrosis, Marfan Syndrome and Huntington's disease (43). These single-gene conditions are attributed to a single mutation with a large effect. In contrast, an oligogenic disorder like osteogenesis imperfecta can be attributed to more than a few genetic loci, although each one with a large effect. If more genes are involved, each of them having only a small effect, then they are referred to as polygenetic disorders (43). In circumstances where multiple genetic factors act simultaneously or are also supported by environmental attributes, these conditions are known as multifactorial disorders. Rather than one single gene or one environmental factor being the cause of a condition, multifactorial diseases like CHD or neural tube defects (anencephaly, spina bifida) require more

than one genetic loci or environmental factor to manifest, each acting as an additive factor. As a result, various phenotypic possibilities arise, which should nearly follow a Gaussian distribution (43).

Diseases are often perceived as dichotomous, either present or absent. While this perspective fits well with monogenic disorders that follow Mendelian inheritance, it falls short with more complex diseases. This is especially relevant in the context of MC twins. Drawing from the assumption that genetic differences are not the primary trigger for the splitting, they begin with the same genetic constitution. However, from the moment of separation, each twin undergoes their own unique developmental and environmental journey. This may include epigenetic changes, asymmetric placental sharing and vascular anastomoses, all of which can lead to unequal exposures. Therefore, despite their shared genetic origin, MC twins do not necessarily represent an identical sum of genetic *and* environmental factors, contributing to phenotypic discordance, including a discordant manifestation of anomalies (43).

Applying the understanding of multifactorial diseases to this context showcases that the presence of certain conditions and the manifestation of specific phenotypes often result from the cumulative effect of numerous minor contributing factors, whether genetic or environmental. For a particular phenotype or disease to present similarly in both MC twins, a multitude of these small factors would need to align and accumulate in each twin. Consequently, while one twin in an MC pair might be exposed to the full complement of these additive factors, their co-twin might only experience some. This concept is further emphasised by the unique intrauterine environments that MC twins are exposed to, particularly those who develop vascular anastomoses within their shared placenta. These vascular connections can lead to an unbalanced blood flow, further supporting the likelihood that environmental factors do not uniformly accumulate in both twins and, consequently, may not result in the same phenotypic outcome. For instance, the presence of a developmental disruption affecting one twin but not the other could contribute to the manifestation of a condition like body stalk anomaly in only one member of the pair. Therefore, errors in development should not always be attributed to a singular causative event. Instead, the development of a pathogenic condition often arises from the additive

effect of multiple subtle alterations, whether of genetic or environmental origin, ultimately exceeding a threshold for disease manifestation.

Multifactorial diseases are thought to operate under a liability threshold (43). This threshold model entails that a certain accumulation of predisposing factors, whether genetic or environmental, must be surpassed for a condition to manifest phenotypically. While the foundational genetic loci involved in these diseases may still adhere to Mendelian inheritance patterns, the multifactorial model introduces additional principles. Firstly, it proposes that multiple genetic loci interact together, with each locus contributing to or taking from the likelihood of the trait's manifestation. Secondly, it suggests that while numerous loci may be involved, their number is limited. Lastly, the model explicitly acknowledges the significant influence of environmental factors in shaping the ultimate phenotype. Consequently, the interplay of these numerous genetic loci and environmental factors creates a spectrum of potential phenotypes. As seen in Figure 27, this variability helps to explain why even genetically near-identical MC twins, experiencing differing intrauterine environments and thus potentially accumulating a different combination and quantity of these contributing factors, can present with markedly different clinical outcomes, with one twin potentially crossing the liability threshold for a specific anomaly while the other does not.

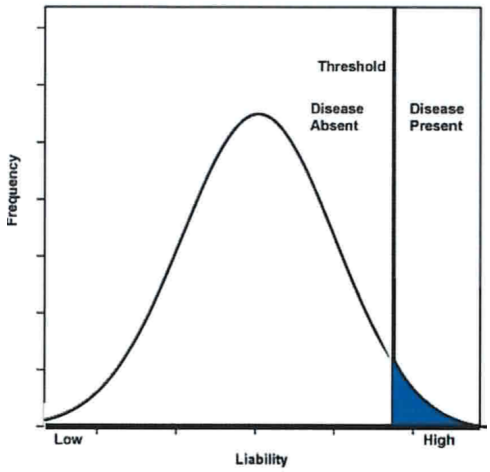



*Figure 27: Threshold of liability (43)*

### 6.2.1 Congenital hydrocephalus

Congenital hydrocephalus, characterised by an abnormal accumulation of cerebrospinal fluid in the cerebral ventricular system at birth, affects approximately 1 in 500 live births (44). A distinction is made between obstructive and non-

obstructive forms. Prenatal cases are usually obstructive and are caused by impaired cerebrospinal fluid circulation. In MC twins, hydrocephalus can occur during TTTS or other intertwin vascular complications leading to ischemia or cerebral haemorrhage. However, in general it is a condition of complex and not yet fully understood aetiology (45). It may occur as an isolated anomaly or in association with other congenital defects as part of a broader syndrome, in which case identifying the underlying cause becomes even more challenging. It may be considered a multifactorial disorder, environmental influences such as prenatal infections (e.g., Rubella, Toxoplasma gondii, Cytomegalovirus) and deficiencies in folic acid or vitamin B have been associated with its development (45). On the other hand, genetic factors are estimated to account for approximately 40% of congenital hydrocephalus cases (45) (44), although specific causative genes have only been identified in about 5% of these cases. Remarkably, two X-linked genes, L1CAM (L1 Cell Adhesion Molecule) and AP1S2 (Adaptor-Related Protein Complex 1 Subunit Sigma 2), have been associated with the condition (44). This gene encodes a transmembrane glycoprotein involved in key neurodevelopment processes, including neuronal adhesion, migration, growth cone morphology, neurite extension and myelination (44). On the other hand, mutations in AP1S2 are linked to Fried-Pettigrew syndrome, another X-linked disorder characterised by hydrocephalus, intellectual disability and iron or calcium deposition in the basal ganglia. As discussed previously in this thesis, X-linked genetic conditions have been proposed in the literature as potential contributors to discordant phenotypes observed in MC twins. Since they are located on the X chromosome, mutations in these genes follow X-linked inheritance pattern. In these for example, in female carriers, one of their two X chromosomes undergoes inactivation. If one twin exhibits skewed inactivation of the healthy X chromosome, she may have insufficient functional L1CAM or AP1S2 protein, leading to symptoms of L1 or Prettigrew syndrome. On the other hand, her co-twin, with a more balanced or favourable X-inactivation pattern, may remain asymptomatic or exhibit milder symptoms despite harbouring the same mutation in each cell. This is particularly notable, given that mutations in L1CAM are responsible for 5–15% of hydrocephalus cases and define a subgroup known as L1 syndrome (45). Congenital hydrocephalus serves as a compelling example of a multifactorial disorder where the interplay of both genetic and environmental factors is crucial in determining its manifestation. It remains questionable whether the

observation of discordant congenital hydrocephalus in MC twins, especially in the absence of typical complications like TTTS, TAPS or sFGR, could therefore be less about a unique aetiology arising from the MC state itself, and more reflective of one twin surpassing the liability threshold.

### 6.2.2 Neural tube defects

Cases of neural tube defects were observed in the cohort in Graz. Neural tube defects result when any disruptions occur during the neurulation process. It is unclear what exactly is causing it, although as a typical malformation with a multifactorial aetiology, genetic risk factors and environmental exposure to toxins, are thought to play a role (46). Especially anti-epileptic drugs such as valproate and carbamazepine affect folate absorption, leading to less folate available. Adequate folate levels are essential for proper neural tube development. Also, maternal conditions like diabetes are recognised as contributing factors, as hyperglycaemia may impair organogenesis by disrupting protein folding and inducing apoptosis in embryonic cells (46). It is known that there is a higher incidence of neural tube defects in twins, with the risk being particularly elevated in MC twins (47) (48). For example, a study by Joó et al. 2011 found that out of all the investigated twin pregnancies with neural tube defects, notably 68% affected MZ twins (49). This proportion was considered excessive given that MZ twins are rarer.

Anencephaly is a frequently occurring neural tube defect, affecting 1 to 5 out of 1000 births. It's a result of a failed neural tube closure on its rostral end during the 4th week of gestation (46). As a condition incompatible with life, its mortality rate is 100% (48). The association rate between MC and anencephaly was reported to be 33%, while this study also mentioned higher numbers by other studies up to 46% (49). Twin pregnancies that were conceived by ART have been associated with a higher risk of anencephaly (50). Spina bifida, on the other hand, the caudal end of the neural tube fails to close in the 4th week post-fertilisation, resulting in the exposed neural tissue to degenerate with consequently neurological deficit (51). This condition affects approximately 1 out of 1000 births, making it one of the most common congenital malformations (51). Genetics are thought to contribute 60-70% (51). Regarding environmental factors, while folate is a key factor in spina bifida, maternal obesity has also been particularly linked to the condition. However, BMI

data was not considered in this study, and research on obesity rates in mothers of discordant MC twins with spina bifida is limited.

It is suggested that a combination of predisposing genotype and environmental factors influences neural tube closure (52). For example, the loss of function of Pax3 gene 34, leads to neural tube defects in mice, which may lose its function because of hyperglycaemia in diabetic mothers. Interestingly, while there is agreement on the fact that central tube defects are common in twins, especially in MC twins, a different study has argued that while they agree on general neural tube defects, spina bifida, particularly, is less common in twins compared to singletons. However, this study did not consider chorionicity and was not published recently, it remains to be further investigated whether MC twins are disproportionately affected (52).

### 6.2.3 Congenital Heart Defects

Congenital heart diseases (CHDs) represent one of the most common types of birth malformations, with an estimated incidence of approximately 10 per 1,000 live births. However, this figure likely underrepresents the true prevalence because registries typically include only clinically significant cases, excluding milder defects that may be asymptomatic and subsequently go undiagnosed. CHDs are widely recognised as multifactorial disorders, influenced by both genetic predispositions and environmental exposures (53). To date, around 100 genes have been associated with various forms of CHD. Nevertheless, large-scale whole-genome sequencing studies have identified a clear genetic cause in only about 30% of cases, highlighting that a purely monogenic explanation is insufficient in more than two-thirds of the cases. This suggests that environmental factors play a substantial role in the aetiology of CHD (53). Among the most influential environmental contributors are maternal health conditions, particularly diabetes and obesity, as well as exposure to teratogens during critical periods of fetal development. Agents such as thalidomide, retinoic acid, and excess vitamin A have been specifically implicated in disrupting early cardiac morphogenesis, underscoring the vulnerability of heart development during the embryonic phase (53). In the context of twin pregnancies, additional complexities arise. A study by Hay et al. 2970 observed that CHDs appeared more frequently in

same-sex twin pairs than in opposite-sex twins or singletons (54). While zygosity and chorionicity were not explicitly reported, it was presumed that the majority of this cohort consisted of MZ twins, raising questions about genetic and shared environmental influences. However, the lack of precise classification limits the strength of conclusions, particularly in relation to MC twins, where shared placental circulation may introduce distinct hemodynamic factors. Supporting this complexity, an earlier study by Uchida and Row 1961 found that out of 1,125 individuals with CHD, only 14 were twins, most of whom were discordant for CHD (55). This high rate of discordance led the authors to emphasise the importance of considering each twin as an individual case, rather than assuming a shared causative mechanism. Other reports have echoed these findings, suggesting that while genetic background is important, individual intrauterine conditions may have an even greater impact on CHD manifestation in twins (40) (26).

### 6.2.4 Hydrops fetalis

Hydrops fetalis (HF) is defined as the pathological accumulation of fluid in at least two different fetal compartments (e.g. peritoneal cavity, pleural space, pericardium and skin) and is divided into two major types: immune and non-immune. Immune HF, caused by maternal antibodies due to Rh incompatibility, has become increasingly rare due to effective anti-D immunoglobulin prophylaxis in Rh-negative mothers. As a result, non-immune hydrops fetalis (NIHF), which occurs without maternal antibody-mediated haemolysis, now accounts for approximately 85–90% of all HF cases (56). NIHF has a high mortality rate of 40–50% and is associated with a wide range of aetiologies, including cardiovascular anomalies (21.7%), hematologic disorders (10.4%), chromosomal abnormalities (13.4%), genetic syndromes (4.4%), lymphatic dysplasia (5.7%), inborn errors of metabolism (1.1%), infections (6.7%), thoracic anomalies (6.0%), urinary tract malformations (2.3%), extrathoracic tumours (0.7%), twin-to-twin transfusion syndrome and placental causes (5.6%), gastrointestinal anomalies (0.5%), miscellaneous causes (3.7%), and idiopathic cases without identifiable origin (17.8%) (56). Genetically, both monogenic and chromosomal causes have been increasingly recognised, with at least 131 genes now associated with

NIHF, further emphasising its multifactorial nature (57). While monogenic causes play a significant role, the diagnostic complexity remains high due to overlapping phenotypes and variability in clinical presentation. Additionally, in MC twin pregnancies, immune causes would typically affect both fetuses equally; thus, discordant presentation of HF in MC twins strongly suggests a non-immune aetiology (57).

### 6.2.5 Renal agenesis / Lower urinary tract obstruction

Congenital anomalies of the kidney and urinary tract (CAKUT) are among the most common birth defects. As a recognised multifactorial condition, CAKUT can result from a combination of environmental, epigenetic and genetic influences (58). This complexity is reflected in the observation that even within a single family, affected individuals may present with different manifestations of CAKUT (58). One specific anomaly, renal agenesis, is frequently linked to delayed or defective development of the Wolffian duct or improper induction of the ureteric bud. This condition not only affects kidney formation but also creates significant intrauterine environmental changes, as bilateral renal agenesis will ultimately lead to anhydramnion, which in turn may impair fetal lung development. Bilateral renal agenesis is notably more prevalent in males (58). While phenotypic variability observed in CAKUT is often attributed to mutations in single genes that regulate the early development of the kidneys and lower urinary tract, the precise contribution of genetic versus non-genetic factors in many cases remains challenging to fully uncover, especially given that information regarding genetic testing, as it was also the case in this registry, is not always available (58). While a genetic predisposition to CAKUT may be present in both MC twins, the specific environmental or epigenetic factors experienced by each twin during critical developmental windows can result in one being affected while the co-twin is not or presenting with a different spectrum of urinary tract anomalies.

### 6.2.6 Omphalocele

Omphalocele is a congenital malformation of the anterior abdominal wall, characterised by herniation of abdominal organs, most commonly the

intestines, with sometimes the liver being also affected. Furthermore, an association has been observed between omphalocele and extremes of maternal age, particularly in mothers younger than 20 or older than 40 years. Despite its complexity, prognosis is generally favourable, with survival rates around 80%, and even up to 90% in cases of isolated omphalocele without accompanying anomalies. It is the most frequent abdominal wall defect, occurring in approximately 3.4 per 10,000 pregnancies and it has also been reported more frequently in twin pregnancies (59). The underlying cause is a developmental dysfunction during organogenesis. Normally, during the physiological midgut herniation between the 9th and 11th weeks of gestation, the intestines return to the abdominal cavity by the 12th week. If this retraction fails, the herniation persists and results in a pathological omphalocele. While omphalocele may present as an isolated anomaly, it is frequently associated with chromosomal abnormalities, most notably trisomies 13, 18 and 21 and is particularly common in infants with Beckwith-Wiedemann syndrome (59). Therefore, the discordance of omphalocele in MC twins is not simply a consequence of their shared chorionicity but rather a complex outcome of various interacting factors that can affect each twin differently during critical stages of development.

Considering that MC twins originate from one single zygote, their genetic profile is thought to be mainly the same. The difference in manifestation of a condition, may be due to a cumulative of small changes that together have a major effect later down the road. Especially regarding the unique environment of MC twins, these shared but potentially unequally distributed environmental factors can act as the non-genetic contributors in the development of multifactorial anomalies.

## *6.3* ***Limitations***

The interpretation of these findings should be done within the context of the following limitations.

Firstly, it must be acknowledged that this specific research focus, discordance in MC twins, has not been the subject of substantial, dedicated studies to date, constraining the volume and depth of available data. The cohort consisted of only a small number of cases, with 51 individuals being recorded in the registry in the time frame of 10 years, even though it included many national and international referrals. Studies reported in the literature, often also consisted only of a small cohort. This recurrent limitation is closely linked to the rarity of such cases: twins who are simultaneously monozygotic, MC *and* discordant for genetic or structural anomalies represent a particularly uncommon and specialised subset. These are quite unique and specialised cases, naturally case numbers are not as high. Even with recognition of the need to do some in-depth research of this topic, because of the limited case numbers, it makes it difficult to conduct any large-scale studies with enough statistical power to find differences in MC cohorts and thereby also being able to draw definite conclusions. Also, many twin studies investigating associations with congenital anomalies have not adequately accounted for chorionicity, thereby limiting the extent to which their conclusions can be applied to the unique intrauterine environment of MC pregnancies.

Concerning the presented data, it must be acknowledged that the data was limited, especially regarding detailed pregnancy outcomes, limiting gaining deeper insight and understanding into these cases. This is not unique to the current register but has been repeatedly identified as a broader issue in the study of MC twins, as highlighted in previous publications (60). Many patients decline genetic investigations for personal or cultural reasons, and even when testing is performed, access to detailed results is often highly restricted due to confidentiality policies. However genetic analyses are crucial for identifying potential underlying genetic or chromosomal abnormalities that may contribute to the observed anomalies. Without this information, the ability to interpret the aetiology and differentiate between genetic and environmental influences, as well as the ability to systematically analyse genetic discordance in MC twins, remains limited.

Furthermore, some cases of sex discordance in MC twins were described, which challenges the common practice that ultrasound detects sex discordance in twins automatically implies dizygosity. Similarly, the ultrasonic findings of a single placenta do not necessarily confirm their monochorionicity. These limitations underscore the importance of exercising caution when drawing definitive conclusions about monozygosity based solely on the presence of a single placenta. Such misclassification can lead to incorrect inclusion of discordant MC twin cases, which may impact the true prevalence and affected anomalies. It is also important to note that the registry exhibits selection bias. On the one hand, women with MC twin pregnancies with anomalies who seek the centre in Graz, even from foreign countries (including Slovenia, Bosnia, Hungary and Romania), are represented. On the other hand, it's a highly specialised centre that treats complex cases. This also hinders the ability to obtain reliable outcome data, as thorough follow up-is often difficult. In our cohort, for example, some patients were referred from neighbouring countries and subsequently continued care abroad, making comprehensive postnatal follow-up difficult or impossible. This limits external validity, thereby no conclusions regarding the prevalence or incidence of MC twin pregnancies with anomalies in the general population can be drawn based solely on the cases seen here.

Another aspect to consider when assessing the true prevalence of MC twins with discordant anomalies, is the potential underreporting of stillborns or intrauterine deaths associated with MC pregnancies. These cases are often excluded from statistics, leading to a possible underrepresentation. Also, the degree of investigation in the cause of death can vary significantly, due to variations in standard practices, resource limitations or parental wishes, further complicating data collection.

## *6.4 Implications*

An understanding and awareness of the potential for discordance in MC twins, as well as more detailed research into its various manifestations, holds promise to enhance on prenatal diagnostic techniques and refine management strategies. Accurate early determination of chorionicity and detailed second-trimester assessments of both fetuses are therefore crucial for the correct interpretation of

twin discordance and thereby appropriate pregnancy management. This awareness calls for a vigilant approach, demanding an equal and individual assessment of both fetuses, thereby avoiding not drawing conclusions about one based on the other twin. It is also vital for clinicians to exercise caution against automatically assuming monozygosity based on the presence of a single placenta, as DC fused twins can occasionally mimic this presentation. Therefore, discordance in MC twins may warrant more individualised and frequent monitoring protocols, even in the absence of obvious complications like TTTS.

The identification of discordance in MC twins also carries significant ethical implications. The diagnosis of discordance in MC twins, may call for difficult decisions regarding pregnancy management, including selective termination. A better understanding of this occurrence and outcomes of both twins, can in the future, provide a better and more evidence-based approach for navigating these decisions.

Based on the gained observations, further studies in this area may provide a better understanding of the very early genetic and epigenetic processes that occur in the very first days, showcasing possible factors that play a crucial role in early development, beyond the initial genome. It further illustrates, because of the distinct environment that MC twins are exposed to and the unique vascular and hemodynamic situation, how much they represent an extremely unique subset of twin pregnancies. As such, the influence of early cellular events, gene expression variability and placental influence on phenotype can be studied. Considering the findings from the literature, it is particularly relevant to investigate whether placental characteristics correlate with discordant anomalies in MC twins. It is also worth exploring whether the presence of for example TTTS can predict the likelihood of discordant multifactorial anomalies and whether there are any epigenetic markers that are expressed differently between an affected or unaffected twin. Some additional aspects, especially related to maternal factors, demonstrate further reasons that call for additional study. This includes data regarding whether and what kind of Artificial Reproductive Treatments were utilised, considering it is seen as a possible factor increasing the likelihood of MC twinning. Such information could further clarify whether this cohort may even have discordant anomalies more often,

even though the data of the presented cohort suggests otherwise. The sex of the twins should also be considered going forward. Regarding multifactorial diseases, which were among the more frequent diseases in this cohort, the maternal factors such as the body mass index (BMI), medical history, particularly the presence of diabetes, require a more detailed exploration, as these were associated with being possible contributors to multifactorial diseases.

## *6.5* ***CONCLUSION***

Although cases of MC twins discordant for anomalies are rare, they represent a real clinical scenario that practitioners may encounter and are expected to continue observing. This body of evidence challenges the traditional assumption that monozygosity and *monochorionicity,* equate to genetic and phenotypic uniformity. Instead, it invites for a deeper reflection on the interaction between genetic, epigenetic and environmental factors in the earliest stages of development. Despite the numerous hypotheses proposed, the precise pathophysiological process underlying discordance in many cases remains poorly understood. Furthermore, so far, the literature has primarily focused on monozygotic twins, without consistently distinguishing between placental configuration (MC or DC), and it also lacks longitudinal studies that follow the reported cases over extended periods.

When comparing the presented data with the literature, it's important to remember that the cohort consisted of a limited set of samples. Additionally, the bias in the data makes it difficult to draw definitive conclusions. The data in this registry would not prove or disprove anything but rather serve as thought-provoking prompts for further discussion. Given that the specific anomalies observed in our cohort of MC twins, such as congenital hydrocephalus, hydrops fetalis and CHD, are themselves established as multifactorial diseases in the general population, it suggests that the occurrence of these conditions in MC twins, particularly with observed discordance, may not necessarily indicate a unique aetiological pathway caused solely by the MC state. Instead, the presence of these typical multifactorial diseases within this unique environment offers an opportunity to further investigate the complex interplay of genetic predisposition and environmental influences in the manifestation of these conditions. The observed discordance between genetically near-identical twins, who are exposed to the unique environment of shared yet often asymmetrical

intrauterine environment of a MC pregnancy, highlights the significant contribution of non-genetic factors. Also, epigenetics serves as a crucial link between genes and the environment. While the DNA sequence might be identical, epigenetic modifications such as DNA methylation can cause differences in gene expression between the twins. In this instance, also subtle environmental differences can trigger epigenetic changes, impacting developmental pathways. Therefore, it provides a powerful way for perhaps uncovering the subtle and cumulative effects of various contributing factors that ultimately determine phenotypic expression. By keeping this in mind and simultaneously considering the model of liability threshold, it shows that MC pregnancies provide an unparalleled opportunity to gain a better understanding of the complex dynamic of genetics, epigenetics and environmental factors.

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