

GENETICS

Chromosome abnormalities and selected genetic syndromes

Modes of inheritance

Autosomal dominant (AD) inheritance

- These disorders are encoded on autosomes and will manifest when a single copy of the mutant allele is present (i.e. in heterozygotes). The disease/mutant allele is dominant to the wild-type allele.
- Males and females are equally affected and may both transmit the disease with a 50% risk of transmission to any offspring.
- Penetrance is the variability of clinical manifestation of an autosomal disorder. Many conditions (Huntington disease) show delayed or age-dependent penetrance in which the disease only becomes apparent after a period of time. Non-penetrance or incomplete penetrance occurs when an individual known to be heterozygous for the allele does not manifest the disease.
- The expressivity of the gene is the degree to which the particular disease manifests in affected individuals. In neurofibromatosis a mildly affected parent may have a child who is severely affected.
- New mutation rates vary significantly between AD conditions – 50% of cases of neurofibromatosis I are new mutations.
- A few AD dominant conditions (Huntington disease, myotonic dystrophy) demonstrate anticipation where there is worsening of the disease severity with each generation. This characteristically occurs in triplet repeat disorders when expansion of the triplet repeats occurs in either the maternal or paternal germline.
- In some cases of new dominant mutations there is a significant risk that a second child may be affected, despite both parents being normal. This is usually due to germline or gonadal mosaicism and can carry a high recurrence risk for some conditions (osteogenesis imperfecta type II).

Autosomal recessive (AR) inheritance

- Also encoded on the autosomes but only manifests in homozygotes or compound heterozygotes. Both parents are obligate carriers and will not usually have any manifestations of the disease. AR conditions are much more common in consanguineous families.
- Both males and females can be affected. The risk of having an affected child if both parents are carriers is 25%.
- Affected siblings generally show a similar clinical course, which is more consistent than for many AD conditions.

X-linked dominant (XLD) inheritance

- This is an uncommon form of inheritance and is caused by a dominant disease allele on the X chromosome. XLD disorders manifest very severely in males with spontaneous fetal loss or neonatal death commonly occurring.

HANDBOOK OF FETAL MEDICINE

- Female heterozygotes are less severely affected. The distribution of features in a female is a reflection of the X-inactivation pattern seen in specific tissues.
- Asymmetric involvement of the body is an important feature (e.g. in X-linked chondrodysplasia punctata, asymmetric limb shortening occurs).
- X inactivation in the embryo is a random process with 50% of cells containing the inactive maternal X chromosome and 50% containing the inactive paternal X chromosome.
- Significant deviation away from the normal 50:50 ratio is occasionally seen (skewed X inactivation).
- X-linked semi-dominant disorders manifest severely in males who are hemizygotes and mildly or subclinically in females who have two X chromosomes (normal and mutated copy).
- When an affected male reproduces, all female offspring will inherit the mutation but the male offspring will be unaffected. The family tree shows no male-to-male transmission.
- Females with severe features of an XLD disorder or an X-linked semi-dominant disorder may be affected because of highly unfavorable skewed X-inactivation, Turner syndrome (hemizygote) or X-autosome translocation.
- Males with features of a severe XLD disorder may have Klinefelter syndrome. Karyotyping is indicated in all cases.

X-linked recessive (XLR) inheritance

- XLR disorders manifest in males who are hemizygotes. Females are carriers because they carry two copies of the X chromosome (normal and mutant copy). Unfavorable skewing of X inactivation in key tissues is the main factor that determines whether or not a heterozygote expresses the disease.
- Some XLR conditions are never seen in females, whilst others have only infrequent symptoms (Duchenne and Becker muscular dystrophy). In other conditions (fragile X syndrome) carrier females are frequently symptomatic but never as severely affected as males.
- Duchenne and Becker muscular dystrophy have a significant risk of germline mosaicism.
- An affected male will father carrier daughters, but none of his sons will be affected. The family tree will show no male-to-male transmission.

Mitochondrial inheritance

- Mitochondrial DNA in humans is a double-stranded DNA encoding 13 proteins, 2 ribosomal RNAs and 22 transfer RNAs. Most of the mitochondrial genome is coding sequence.
- Tissues most often affected in mitochondrial disease are energy demanding organs (central nervous system, muscle, liver, and kidney). Preferential accumulation of mutant DNA in affected tissues explains the progressive nature of mitochondrial disorders.

- Mitochondrial DNA is maternally inherited except in very rare circumstances. Paternal mitochondria constitute only 0.1% of total mitochondria at fertilization and are rapidly eliminated. Males do not transmit mitochondrial disorders with very rare exceptions.
- A mitochondrially inherited condition can affect either sex. Human mitochondrial DNA has a much higher mutation rate ($>10\text{--}20\times$) than nuclear DNA.
- When a mutation arises in a cellular mitochondrial DNA, it creates the existence of both mutant and normal DNA. This is defined as heteroplasmy. In homoplasmy only one type of mitochondrial DNA is present (pure mutant or normal).
- If a mother is heteroplasmic for a particular mutation, the proportion of mutant DNA in her children may vary widely.
- The difference in mitochondrial function between normal and defective cells can be very small. This is called threshold expression.
- The available evidence suggests that there is little or no tissue variation in the mutant DNA in affected individuals. A prenatal sample from chorionic villous sampling (CVS)/amniocentesis therefore, can predict the mutant load in most tissues after birth, although trying to predict the phenotype from this is very difficult.

Multifactorial inheritance

- Many conditions depend on interaction between genetic factors and the environment to manifest. Diseases inherited this way are called complex diseases (diabetes mellitus, schizophrenia, ischemic heart disease) and are transmitted due to multifactorial inheritance.
- The mapping and identification of responsible genes is difficult because the candidate genes occur with similar frequency in affected and normal individuals.

Chromosomal abnormalities

Down syndrome (Trisomy 21)

- 95% of cases are the result of meiotic non-disjunction. 2% result from Robertsonian translocation (especially 14;21) of which 50% are familial. 2% of cases are due to mosaicism and 1% of cases occur from chromosome rearrangements. Trisomy for the 21q22 region results in many of the clinical features.
- The incidence increases with maternal age and there is a significant risk of fetal loss.
- 40%–50% of cases have cardiac abnormalities. Ventricular septal defect is the most common effect followed by patent ductus arteriosus. Atrioventricular septal defect (AVSD) is much more common in Down syndrome than in the general population.
- The risk of recurrence is influenced by maternal age and parental germline mosaicism. Overall, the risk of recurrence is approximately 1% for the common variant of Down syndrome.
- After two affected children, a recurrence risk of 10% may be more appropriate. If a previous child had a *de novo* Robertsonian

HANDBOOK OF FETAL MEDICINE

translocation, the risk of recurrence is low ($<2\%$ if parental chromosomes are normal).

- If the father carries a Robertsonian translocation involving chromosome 21, the recurrence risk is $<1\%$. If the mother carries the translocation, the risk is $10\%–15\%$.
- There are many different screening methods (combined test, integrated test, quadruple test, etc.) in low-risk pregnancies. Prenatal diagnosis is possible from any fetal sample (usually CVS or amniocentesis).

Edward syndrome (Trisomy 18)

- 94% of infants with Edward syndrome have trisomy 18. The remainder have trisomy 18 mosaicism or partial 18q trisomy. The majority of cases are due to meiotic non-disjunction. The risk increases with maternal age.
- Multiple fetal abnormalities are usually evident on antenatal ultrasound. There is an extremely high rate of fetal loss and the majority of live born infants die during the neonatal period.
- Prenatal diagnosis is possible from chorionic villi, amniocytes, or fetal blood.

Patau syndrome (Trisomy 13)

- 90% of cases are due to non-disjunction during meiosis, $5\%–10\%$ are caused by an unbalanced Robertsonian translocation (13;14) and a very small proportion is due to mosaicism. The risk increases with maternal age but the overall risk is still very low.
- The average survival is 7–10 days with the majority of pregnancies ending with fetal demise.
- Multiple fetal abnormalities are present on antenatal ultrasound and should prompt karyotyping.
- The overall recurrence risk is low (0.5%).
- Prenatal diagnosis is possible from any fetal sample.

22q11 deletion syndrome

- Also known as DiGeorge syndrome or velocardiofacial syndrome.
- 96% of cases have a defined microdeletion (1.5–3 Mb) of 22q11 which includes 24–30 genes.
- Cardiac defects, thymic hypoplasia, distinctive facial appearance, parathyroid insufficiency, and pharyngeal problems are part of the phenotypic spectrum.
- The phenotype arises from the failure of development of the third and fourth branchial arches. Haploinsufficiency of the *TBX1* gene is the major contributor to the phenotype.
- The diagnosis should be considered in any fetus with a cardiac abnormality particularly involving the outflow tracts. 75% of cases have congenital heart anomalies (20% tetralogy of Fallot). Cleft palate is present in 9% of cases and 36% have structural urinary tract abnormalities. Karyotyping is indicated and the microdeletion easily confirmed using fluorescent in-situ hybridization (FISH).

- If the diagnosis is made antenatally, fetal echocardiography and a detailed anomaly scan should be performed to look for common associated anomalies. Neonatal calcium levels should be monitored. Feeding problems are common and referral to a plastic surgeon and feeding specialist necessary.
- If one parent carries the 22q11 deletion, the recurrence risk is 50%. If neither parent carries the deletion, the risk of recurrence is very small (<1%).

Klinefelter syndrome (47 XXY)

- Klinefelter syndrome is the most common sex chromosome disorder affecting 1 in 660 males. Increasing maternal age is a risk factor.
- The diagnosis is usually made incidentally following a prenatal diagnostic procedure. There are no reliable ultrasound findings to make/suggest the diagnosis non-invasively.
- Males enter puberty normally but soon develop hypergonadotrophic hypogonadism with low testosterone levels. The testis involutes and infertility results. The IQ can vary widely, but many affected individuals have some degree of intellectual impairment.
- The recurrence risk is low (<1%). There is an increased risk of sex chromosome aneuploidy and trisomy 21 to the offspring of affected males (in the rare event of successful conception) and prenatal diagnosis is advisable.

Genetic syndromes

Beckwith–Widemann syndrome (BWS)

- BWS is a somatic overgrowth and cancer predisposition syndrome with an incidence of 1 in 13 700 individuals. Males and females are equally affected. The phenotypic spectrum of this disorder also includes certain types of hemihypertrophy.
- 85% of cases are sporadic. 10%–15% of cases of BWS are part of autosomal dominant pedigrees demonstrating preferential maternal transmission. The overall risk of cancer in children is 7.5% with the majority being embryonal (hepatoblastoma, Wilms). Most tumors present by the age of 8 years.
- BWS is a complex, multigenic disorder caused by alterations in growth regulatory genes on chromosome 11p15. A number of imprinted genes (*IGF2*, *H19*, *CDKN1C*, *LIT1*) involved in growth may be affected.
- Approximately 60% of patients carry an epigenetic error at one of the two imprinting centers (*DMR1* and *DMR2*) on 11p15. The next largest category is paternal uniparental disomy (20%). Chromosomal abnormalities are relatively rare and include paternal duplications (<1%) and inversions/translocations (<1%). Mutations in the gene *CDKN1C* are responsible for about 10% of cases.
- In 10%–15% of individuals with BWS, the etiology is unknown.
- There is a significant correlation between uniparental disomy (UPD) and hemihypertrophy.

HANDBOOK OF FETAL MEDICINE

1

GENETICS

- The recurrence risk due to paternal UPD is very low. If BWS is caused by 11p15 chromosome translocations/inversions/duplications, the recurrence risks may be as high as 50%. Recurrence risk may be up to 50% if either parent is affected.
- If karyotype and UPD analysis are normal and, in the absence of any family history, the risk of recurrence is approximately 5%.

Duchenne and Becker muscular dystrophy

- Duchenne muscular dystrophy (DMD) affects 1 in 3500 male births. It is the most common and severe form of childhood muscular dystrophy. The mean age of loss of mobility is 9 years followed by death in the late teens or early 20s.
- Becker muscular dystrophy (BMD) is clinically similar to Duchenne but with milder symptoms. The average age of onset is 11 years with late (40–50 years) loss of the ability to walk.
- Both are X-linked recessive disorders caused by mutations in the dystrophin gene. 60%–65% of DMD is caused by large out-of-frame mutations. 5% of cases are caused by exon duplication with the remaining cases caused by nonsense or frameshift mutations that result in chain termination.
- 30% of affected individuals will have mild developmental delay that is usually not progressive. The cause of death is usually respiratory insufficiency and cardiomyopathy. There is also a high risk of developing scoliosis.
- BMD is caused by in-frame mutations in the dystrophin gene that cause reduced amounts of dystrophin being produced.
- 20% of female carriers of DMD have evidence of cardiac involvement and may also have proximal muscle weakness. BMD carriers are much less frequently and severely affected.
- A carrier female is at 25% risk of producing an affected son and a carrier daughter, respectively. Penetrance is complete in affected males.
- Prenatal diagnosis is available to carriers of a known dystrophin mutation. The method of choice is a chorionic villous sample at 11–13 weeks' gestation.
- Even if the precise mutation has not been detected, linkage analysis may identify a "high risk X," which can be used in prenatal diagnosis.
- Prenatal diagnosis is offered to carrier women who are carrying male fetuses. Fetal sexing using free fetal DNA in maternal blood is now available.

Fragile X syndrome

- Fragile sites are gaps, constrictions, or breaks on metaphase chromosomes that arise when cells are exposed to a perturbation of the DNA replication process. Fragile sites are seen on all human chromosomes and are named according to the chromosome band they are observed in.
- Fragile X syndrome is the most common form of inherited mental retardation with an incidence of 1 in 4000–6000 males. It is characterized

by a constellation of clinical manifestations, including mild to severe mental retardation, hyperactivity, and autism.

- The fragile site FRAXA is expressed in fragile X syndrome. The causative mutation is a (CGG) expansion of the 5'-untranslated region of the X-linked *FMR1* gene (Xq27.3). The expansion mutation leads to the hypermethylation of the promoter region resulting in silencing of the gene, causing reduced expression of the Fragile X Mental Retardation Protein (FMRP).
- The *FMR1* CGG repeat is polymorphic in the general population, with a normal range of 6–53 repeats. Alleles having between 55 and 200 CGG repeats are called premutations and generate *FMR1* mRNA and FMRP protein. Repeats in the premutation range are unstable in females and can expand further during oogenesis and postzygotic mitosis. The premutation frequency in the general population is approximately 1 in 259 in females and 1 in 813 in males.
- Affected individuals and full mutation carrier females have in excess of 200 CGG repeats.
- The fragile site FRAXE is less common with an incidence of 1 in 23 000 males. It is caused by a different repeat (GCC) in the *FMR2* gene on Xq28. Affected males have less severe disease.
- In females with a premutation, expansion of the repeat may not occur in every pregnancy although a massive expansion into the affected range is always a possibility. Males with the premutation will pass it on to all their daughters but none of their sons. The repeat size tends to remain stable.
- Prenatal diagnosis is possible using chorionic villi. A larger sample is usually required.

Myotonic dystrophy

- This is an autosomal dominant neuromuscular condition with a prevalence of 1:8000.
- Two genetic loci (DM1 on chromosome 19 and DM2 on chromosome 3) are associated with the myotonic dystrophy phenotype. The mutation responsible for DM1 is a CTG expansion located in the 3'-untranslated region of the dystrophin myotonic-protein kinase gene (*DMPK*), whereas DM2 is linked to CCTG expansion in intron 1 of the zinc finger protein gene *ZNF9*.
- Affected individuals have expansion repeats of >50. Affected females (>1000 repeats) who are symptomatic are at risk of a congenitally affected infant.
- Polyhydramnios is a common finding and the affected baby is frequently very floppy with diaphragmatic hypoplasia. Neonatal mortality may be as high as 20%. Preterm labor and postpartum hemorrhage are additional risks.
- The risk of having a child with features of myotonic dystrophy depends on the sex of the transmitting parent and degree of clinical severity. Mothers who are asymptomatic with a small repeat (<80) tend not to have affected babies. The mutation is transmitted in a relatively stable fashion.

HANDBOOK OF FETAL MEDICINE

- If the mother has neuromuscular involvement and a moderate expansion (200–500 repeats) with no previous affected child, the risk of congenital disease is 20%–30%. If there has been a previously affected child, the risk increases to 40%–50%.
- The risk of congenital myotonic dystrophy due to transmission from an affected father is very small.
- Affected families are at risk of anticipation with a tendency of increasing severity in subsequent generations.
- Prenatal diagnosis by CVS is possible although predicting the prognosis is more difficult. Most affected babies will have >1000 repeats.

Noonan syndrome

- This is an autosomal dominant condition with an incidence of 1 in 2500. It is caused by a mutation in the *PTPN11* gene on chromosome 12q. A family history is present in half of the cases. Some cases may occur as a result of new mutations.
- Affected fetuses can manifest nuchal edema and lymphatic abnormalities. Pulmonary stenosis may be present. Polyhydramnios may also be a feature of the pregnancy.
- Prenatal diagnosis for the *PTPN11* gene mutation is possible.

Tuberous sclerosis

- Tuberous sclerosis is a dominantly inherited disease of high penetrance, characterized by the presence of hamartomata in multiple organ systems, developmental delay, skin lesions, and seizures. Renal angiomyolipomata, cysts, and carcinoma are also features of the disease.
- The condition is highly penetrant but variable in its clinical manifestation.
- 60% of cases arise as new mutations. The condition is caused by mutations in the *TSC1* gene on chromosome 9q and *TSC2* gene on chromosome 16p.
- Cardiac rhabdomyomas identified on prenatal ultrasound are a well-recognized feature of congenital tuberous sclerosis. The risk of the baby being affected is >50%.
- An affected parent has a 50% risk of transmitting the mutant gene. If neither parent is affected, the risk is 2% due to germline mosaicism.
- Prenatal diagnosis is possible if the mutation is known.

ANEUPLOIDY SCREENING

- Chromosome abnormalities occur in 0.1%–0.2% of all live births with Down syndrome being the most clinically significant, due to the high incidence of mental handicap and associated structural malformations.
- Screening is the identification of a subset of the screened population, whose increased risk is high enough to warrant a diagnostic test. Screening is generally used for conditions that are clinically significant and prevalent in the population.
- Maternal age is a poor screening criterion, since the majority of children with Down syndrome are born to women younger than 35 years of age.
- The optimal screening test has a low false-positive rate, which will vary according to the maternal age of the population being screened. A false-positive rate of <5% is considered desirable. An ideal screening test should have a high detection rate (sensitivity) for the condition. However, as the detection rate increases, the false-positive rate will also rise, thereby increasing the number of women who will screen positive and require a diagnostic test.
- Screening for aneuploidy or fetal structural anomalies allows parents several choices after a definitive diagnosis is made. This may include preparation for the birth of a child with special needs as well as a management plan for the diagnosis and possible treatment of the condition. In some cases termination of pregnancy may be an option.
- Down syndrome is the most common genetic cause of mental retardation. It is associated with a number of structural malformations (usually cardiac) of varying severity and universal developmental delay. The risk increases with advancing maternal age.
- Down syndrome pregnancies are associated with decreased levels of maternal serum alphafetoprotein (AFP) and unconjugated estriol (uE3) and elevated levels of human chorionic gonadotropin (hCG) (either intact human chorionic gonadotropin or the free beta subunit).
- The median levels of the various hormones in Down syndrome pregnancies in the second trimester are as follows: hCG 2.06 multiples of the median (MoM), AFP 0.72 MoM, Inhibin A 1.79 MoM and unconjugated estriol 0.64 MoM.

First trimester screening

- Nuchal translucency (NT) by itself has an approximately 60% detection rate for Down syndrome with a 5% false-positive rate. However, this method has been superseded by the combined test which incorporates the use of pregnancy associated plasma protein A (PAPP-A) and hCG levels.
- Increased NT is also associated with other chromosomal abnormalities (including trisomy 18 and trisomy 13, Turner's syndrome, and triploidy), genetic disorders and fetal structural anomalies, particularly congenital heart defects.

HANDBOOK OF FETAL MEDICINE

- Two large, first and second trimester comparison studies: Serum, Urine, Ultrasound Study (SURUSS) in the UK, with more than 48 000 pregnancies, and First and Second Trimester Evaluation of Risk (FASTER) in the USA, with more than 36 000 pregnancies, had an 86% and 85% Down syndrome detection rate. The false-positive rate was approximately 5%. Together with two other large studies (BUN) (Blood, Ultrasound and Nuchal) study (USA) and the OSCAR (One stop clinic to assess risk) study (UK), the average detection rate for first trimester screening using the combined test is approximately 85% with a 5% false-positive rate.
- Median first trimester levels of PAPP-A and free beta hCG in Down syndrome pregnancies are 0.45 MoM and 1.79 MoM, respectively.

Second trimester screening**Serum screening**

- This involves calculating a risk based on the maternal age and a blood sample taken between 15 and 22 weeks' gestation. Various screening tests are available: double test (AFP and hCG), triple test (AFP, hCG, and uE3) and quadruple test (AFP, hCG, uE3, and inhibin-A).
- The detection rates vary from 66% for the double test, 77% for the triple test to >80% for the quadruple test.
- Currently, only the combined, serum integrated, full integrated and the sequential integrated incorporate first trimester nuchal translucency measurement as a component of the test (Table 2.1).
- Integrated screening is used to calculate a single adjusted risk of Down syndrome after both the first and second trimester tests have been completed and is associated with a higher rate of detection of trisomy 21 (94%–96% in the FASTER trial and 94% in the SURUSS trial, with rates varying according to gestational age) than either first or second trimester screening alone.
- A major criticism of the integrated (i.e. first and second trimester testing) approach is the withholding of results from the first test until the second blood test is performed. Non-compliance rates of up to 25% for the second sample have been reported in some studies.
- To minimize non-compliance rates, and improve patient choice, two sequential screening strategies: stepwise and contingent screening have been proposed.
- Stepwise testing involves releasing the results of the first trimester combined screening to the patient and, if the risk of aneuploidy is greater than a predetermined cut-off level, the patient is then offered the option of proceeding with a diagnostic test. If the risk is not raised, the patient proceeds with the second trimester test and receives a revised and final risk assessment based on the first and second trimester measurements.
- Contingent sequential screening stratifies women according to the initial adjusted risk of Down syndrome. Only women with an intermediate risk would undergo the second trimester screening.