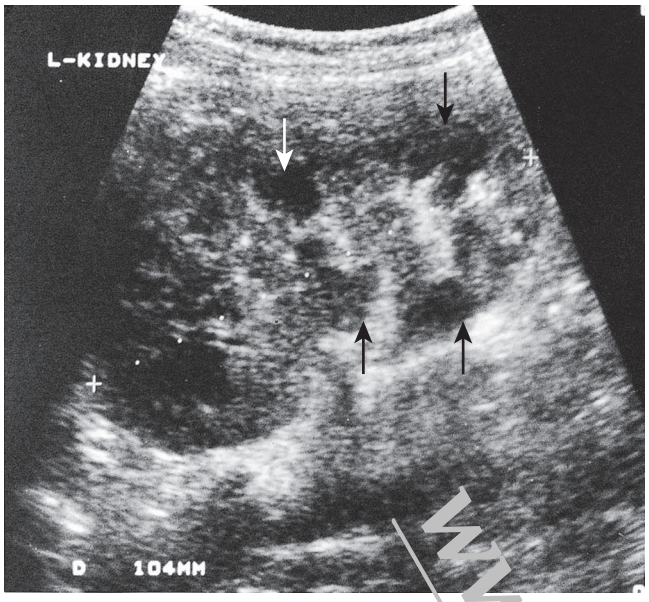
# Contents

## Section B: Genetics in Medicine and Genomic Medicine



## Section C: Clinical Genetics, Counseling, and Ethics




• **Fig. 11.4** Renal ultrasonogram of an asymptomatic person with tuberous sclerosis showing abnormal echogenicity attributed to presumed angiomyolipomata (*arrows*).

well as renal ultrasonography to identify the cysts known as angiomyolipoma(ta) (Fig. 11.4). Use of these relatively non-invasive tests in relatives of persons with TSC can often detect evidence of the condition in asymptomatic persons, especially as sequencing of the *TSC1* and *TSC2* genes is not guaranteed to identify a pathogenic variant.

Similarly, assessment for Marfan syndrome involves ophthalmic examination for evidence of ectopia lentis, echocardiography for measurement of the aortic root diameter, and sometimes magnetic resonance imaging of the lumbar spine to look for evidence of dural ectasia—all of which are important criteria. Their absence does not exclude the diagnosis, and further assessments are necessary if sequencing of the Marfan gene, *FBN1*, reveals a variant of uncertain significance, which is not uncommon for this gene.

## Biochemical Tests

Biochemical tests are very useful in some autosomal dominant disorders. Examples include the use of serum cholesterol levels in those at risk of familial hypercholesterolemia (pp. 147, 277), although genetic testing is increasingly available, as well as assay of the appropriate urinary porphyrins or the specific enzyme deficiency in the various dominantly inherited porphyrias (p. 282).

## Ethical Considerations in Carrier Detection and Predictive Testing

One of the main reasons for determining the carrier status of a person at risk of an autosomal or X-linked recessive disorder is to help couples make an informed choice when having children. For some, however, the knowledge that there is a significant risk of having an affected child may present options and choices that they would rather avoid. Knowledge of the risk and the awareness that prenatal diagnosis is available may create a sense of guilt whichever decision is taken—either to have a child knowing it could be affected, or to have prenatal testing that may lead to termination of pregnancy. The latter option is especially difficult when the prognosis of the disease cannot be stated with certainty because of its variability or reduced penetrance, or if treatment may be developed in time to help the child.

Experience with these potential dilemmas has resulted in normal practice promoting the flow of information within families, rather than from professionals. In general this approach works well, but professional dilemmas can arise if family members refuse to communicate with one another even when the condition carries significant morbidity and the risk is high, particularly with X-linked conditions.

Presymptomatic diagnosis for some late-onset autosomal dominant disorders has clear medical advantages in relation to early intervention and prevention. In familial adenomatous polyposis, for example (p. 197), colonoscopy looking for the presence of colonic polyps can be offered as a regular screening procedure to those who have been shown to be at high risk of developing colonic cancer by molecular studies. Conversely, individuals who have not inherited a pathogenic variant in the *APC* gene do not need to be screened.

In contrast, for persons at risk for Huntington disease (HD), a disorder for which there is currently no effective treatment to delay onset or progression, the benefit of predictive testing is not immediately obvious. The same is true for familial forms of Alzheimer disease, motor neurone disease, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and the spinocerebellar ataxias. Although choice is often considered to be of paramount importance in genetic counseling for those at risk of inherited disorders, remember that those considering presymptomatic or predictive testing should proceed only if they can give truly informed consent and are free from coercion from others. It is possible that employers, life insurance companies, and society in general will put indirect, and on occasion direct, pressure on those at high risk to be tested (p. 350). Indeed, there are examples in which individuals at risk of HD have received prejudicial treatment in relation to employment, and higher than average insurance premiums can be expected on the basis of the family history alone.

Predictive testing for late-onset disorders can, in theory, be used for children and minors, but this can be a contentious issue. Parents sometimes argue that it is their right to know the status of their child(ren). However, this conflicts with upholding the principle of individual autonomy wherever possible. Presymptomatic testing of children is therefore usually discouraged unless an early medical intervention or screening is beneficial for the disorder, which is certainly true for a number of the familial cancer conditions. The issue of genetic testing of children is addressed more fully in Chapter 22 (p. 348).

## Population Screening

One definition of population screening is: "The systematic application of a test or inquiry, to identify individuals at sufficient risk of a specific disorder to warrant further investigation or treatment, amongst persons who have not sought medical attention on account of symptoms of that disorder." Neonatal screening for phenylketonuria is the paradigm of a good screening program and has been available since 1969 in the United Kingdom, with screening for congenital hypothyroidism from 1981. In the United Kingdom, since 1996, population screening has been overseen by the UK National

**• BOX 11.2 Current Nationally-Managed Screening Programs in the United Kingdom (for Conditions With Genetic or Potentially Genetic Causes)**

**Antenatal**
Down syndrome
Sickle-cell disease
Thalassemia
Structural abnormalities (fetal anomaly scanning at 18–20 weeks' gestation)

**Newborn Bloodspot**
Phenylketonuria
Congenital hypothyroidism
Sickle-cell disease
Cystic fibrosis
Medium-chain acyl-CoA dehydrogenase deficiency
Maple syrup urine disease
Isovaleric acidemia
Glutaric aciduria type 1
Homocystinuria

**Newborn and Infant Physical Examination**
Newborn hearing

**Adult**
Breast cancer (women >50 years old)
Bowel cancer (> 60 years old, fecal occult blood)
Sight-threatening diabetic retinopathy
Abdominal aortic aneurysm (men >65 years old)

Screening Committee (NSC) and Public Health England (PHE). The current, nationally managed, screening programs are listed in Box 11.2. The implementation of a screening program is a huge logistical exercise requiring financial and statistical expertise and technology resources, as well as practical mechanisms to introduce the program and monitor outcomes and quality assurance.

## Criteria for a Screening Program

Criteria for a screening program can be considered under the headings of the disease, the test and the practical aspects of the program (Box 11.3). These criteria apply equally to prenatal screening, also addressed in Chapter 20.

**• BOX 11.3 Criteria for a Screening Program**

**Disease**
High incidence in target population
Serious effect on health
Treatable or preventable

**Test**
Non-invasive and easily carried out
Accurate and reliable (high sensitivity and specificity)
Inexpensive

**Program**
Widespread and equitable availability
Voluntary participation
Acceptable to the target population
Full information and counseling provided

### The Disease

To justify the applied effort and resources allocated to screening, the disease should be sufficiently common and have potentially serious effects that are amenable to prevention or amelioration. This may involve early treatment, as for phenylketonuria diagnosed in the neonatal period (p. 271), or the offer of termination of pregnancy for disorders that cannot be treated effectively and are associated with serious morbidity and/or mortality.

### The Test

The test should be accurate and reliable with high **sensitivity** and **specificity**. Sensitivity refers to the proportion of cases that are detected. A measure of sensitivity can be made by determining the proportion of false-negative results (i.e., how many cases are missed). Thus, if a test detects only 70 of 100 cases, it shows a sensitivity of 70%. Specificity refers to the extent to which the test detects only affected individuals. If unaffected people test positive, these are referred to as false positives. Thus, if 10 of 100 unaffected individuals have a false-positive test result, the test shows a **specificity** of 90%. Table 11.2 explains this further. This feeds into the **positive predictive value** of a screening test, which is the proportion of positive tests that are true positives; this is illustrated in Table 11.3.

### The Program

The program should be offered in a fair and equitable manner and should be widely available. It must also be morally acceptable to a substantial proportion of the population to which it is offered.

**TABLE 11.2 Sensitivity and specificity**

| | DISEASE STATUS | |
|---|---|---|
| | **Affected** | **Unaffected** |
| **Screening Test Result** | | |
| Positive | a (true positive) | b (false positive) |
| Negative | c (false negative) | d (true negative) |
| Sensitivity: a/(a + c) = proportion of true positives | | |
| Specificity: d/(d + b) = proportion of true negatives | | |

**TABLE 11.3 In this hypothetical scenario a screening test for congenital adrenal hyperplasia has been implemented, with the following results**

| CONGENITAL ADRENAL HYPERPLASIA PRESENT | | CONGENITAL ADRENAL HYPERPLASIA ABSENT | |
|---|---|---|---|
| **Positive** | **Negative** | **Positive** | **Negative** |
| 96 | 4 | 4980 | 510,100 |

Positive predictive value: 96/(96 + 4980) = 2%

Sensitivity: 96/(96 + 4) = 96%

Specificity: 510,100/(510,100 + 4980) = 99%

## ELEMENTS

1. Targeted or family screening in genetics concerns those who are at relatively high risk because of their family history. Direct gene testing is often possible, but there remains a vital role for detailed clinical examination and specialist clinical investigations, such as biochemical tests and imaging.
2. Consideration should be given to the advantages and disadvantages of presymptomatic or predictive testing from both a practical and an ethical point of view.
3. Population screening involves the offer of genetic testing to all members of a particular population, with the objectives of preventing later ill health and providing informed personal choice. A good screening test has a high sensitivity and specificity.
4. Participation should be voluntary, and each program should be widely available, equitably distributed, acceptable to the target population, and supported by full information and counseling.
5. Prenatal screening based on ultrasound examination at approximately 12 and 20 weeks' gestation is routinely available, as well as combined testing to refine the risk for aneuploidies such as Down syndrome, which may lead to the offer of amniocentesis for genetic testing of the fetus.
6. Newborn screening for phenylketonuria was introduced in the 1960s but has now expanded to incorporate a wide range of metabolic conditions, as well as hearing testing.
7. Population screening programs for carriers of β-thalassemia have resulted in a major fall in the incidence of births of affected homozygotes. This has provided the paradigm for the introduction of screening for other disorders with serious long-term morbidity.
8. Well-organized genetic registers provide an effective means of identifying individuals eligible for testing and screening when new programs or modalities are introduced.

## CLINICAL SCENARIO 1

Consideration is being given to the introduction of a newborn screening program for a metabolic disease not yet covered by the current program. The criteria for early screening, in terms of the medical need, are satisfied.

The data for the new test are as follows:

| | Affected | Unaffected |
|---|---|---|
| Screening Test Result | | |
| | True positives: 115 | False positives: 1312 |
| | False negatives: 22 | True negatives: 460,364 |

Regarding this test, what is the:

a. Sensitivity?
b. Specificity?
c. Positive predictive value?

## CLINICAL SCENARIO 2

With reference to **Clinical Scenario 1**, a technical modification of the screening test has been made, and it has been reevaluated.

The data for this modified new test are as follows:

| | Affected | Unaffected |
|---|---|---|
| Screening Test Result | | |
| | True positives: 83 | False positives: 9529 |
| | False negatives: 2 | True negatives: 348,109 |

Regarding this modified test, what is the:

a. Sensitivity?
b. Specificity?
c. Positive predictive value?

Which is the better test, this one or the earlier test (Clinical Scenario 1), and why?

## Further Reading


Axworthy D, Brock DJH, Bobrow M, Marteau TM. Psychological impact of population-based carrier testing for cystic fibrosis: 3-year follow-up. *Lancet.* 1996;347:1443–1446.

*A review of the impact of carrier testing for cystic fibrosis on over 700 individuals.*

Baily MA, Murray TH, eds. *Ethics and Newborn Genetic Screening: New Technologies, New Challenges*. Baltimore, MD: Johns Hopkins University Press; 2009.

*A multiauthor volume with a focus on the health economics of newborn screening and distributive justice.*

Harper PS. *Practical Genetic Counselling*. 7th ed. London: Hodder Arnold; 2010.

*A very good starting point in almost every aspect of genetic counseling, including carrier testing.*

Marteau T, Richards M, eds. *The Troubled Helix*. Cambridge: Cambridge University Press; 1996.

*Perspectives on the social and psychological implications of genetic testing and screening.*

Nuffield Council on Bioethics. Genetic Screening: Ethical Issues. London: Nuffield Council on Bioethics; 1993.

*This report remains a very helpful read even though technologies have progressed.*


## Websites


Centers for Disease Control and Prevention. Newborn Screening. (http://www.cdc.gov/newbornscreening/).

*A source of information on newborn screening in the United States.*

Burton H, Moorthie S. *Expanded Newborn Screening. A Review of the Evidence*. Cambridge: PHG Foundation; 2010 (https://www.phgfoundation.org/documents/229_1275040921.pdf).

National Health Service websites offering information on screening programs. (https://www.nhs.uk/conditions/nhs-screening/; https://phescreening.blog.gov.uk/about/)

UK National Screening Committee (https://www.gov.uk/government/groups/uk-national-screening-committee-uk-nsc).

*A source of up-to-date information on screening in the United Kingdom.*

# 12

# Hemoglobin and the Hemoglobinopathies

*Blood is a very special juice.*

***Johann Wolfgang von Goethe, in Faust I (1808)***

Over a quarter of a million people are born in the world each year with one of the disorders of the structure or synthesis of hemoglobin (Hb)—the hemoglobinopathies. Originally conditions of the tropics and subtropics, migration has seen these conditions have worldwide impact. In fact, the impact on morbidity and mortality is the greatest of any single group of disorders following mendelian inheritance and, as such, has served as a paradigm for our understanding of the pathology of inherited disease at the clinical, protein and DNA levels. The mobility of modern society means that new communities with a high frequency of Hemoglobinopathies have become established in countries whose indigenous populations have a low frequency. Because they are a major public health concern, many countries have introduced screening programs. In England and Wales, there are an estimated 600,000 healthy carriers of Hb variants.

To understand the various Hemoglobinopathies and their clinical consequences, it is first necessary to consider the structure, function, and synthesis of Hb.

## Structure of Hemoglobin

Hb is the protein present in red blood cells that is responsible for oxygen transport. There are approximately 15 g of Hb in every 100 mL of blood, making it amenable to analysis.

### Protein Analysis

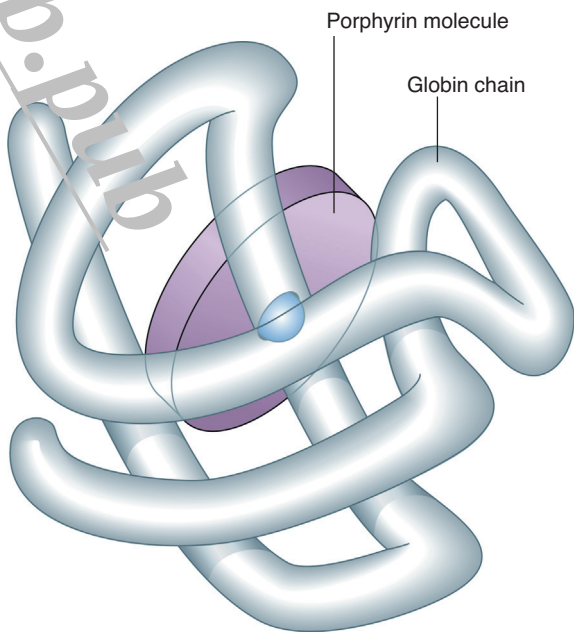
In 1956, by fractionating the peptide products of digestion of human Hb with the proteolytic enzyme trypsin, Ingram found 30 discrete peptide fragments. Trypsin cuts polypeptide chains at the amino acids arginine and lysine. Analysis of the 580 amino acids of human Hb had previously revealed a total of 60 arginine and lysine residues, suggesting that Hb was made up of two identical peptide chains with 30 arginine and lysine residues on each chain.

At about the same time, a family was reported in which two Hb variants, HbS and Hb Hopkins II, were both present in some family members. Several members of the family who possessed both variants had children with normal Hb—offspring who were heterozygous for only one Hb variant, as well as offspring who, like their parents, were doubly heterozygous for the two Hb variants. These observations provided further evidence that at least two different genes were involved in the production of human Hb.

Soon after, the amino-terminal amino acid sequence of human Hb was determined and showed valine–leucine and valine–histidine sequences in equimolar proportions, with two moles of each of these sequences per mole of Hb. This was consistent with human Hb being made up of a tetramer consisting of two pairs of different polypeptides, referred to as the α- and β-globin chains.

Analysis of the iron content of human Hb revealed that iron constituted 0.35% of its weight, from which it was calculated that human Hb should have a minimum molecular weight of 16,000 Daltons (Da). In contrast, determination of the molecular weight of human Hb by physical methods gave values of the order of 64,000 Da, consistent with the suggested tetrameric structure, $\alpha_2\beta_2$, with each of the globin chains having its own iron-containing group—heme (Fig. 12.1).

Subsequent investigators demonstrated that Hb from normal adults also contained a minor fraction, constituting 2% to 3% of the total Hb, with an electrophoretic mobility different from the majority of human Hb. The main component was called HbA, whereas the minority component was called $HbA_2$. Subsequent




• **Fig. 12.1** Diagrammatic representation of one of the globin chains and associated porphyrin molecule of human hemoglobin.

**• BOX 16.2 Isolated (Non-syndromal) Malformations That Show Multifactorial Inheritance**

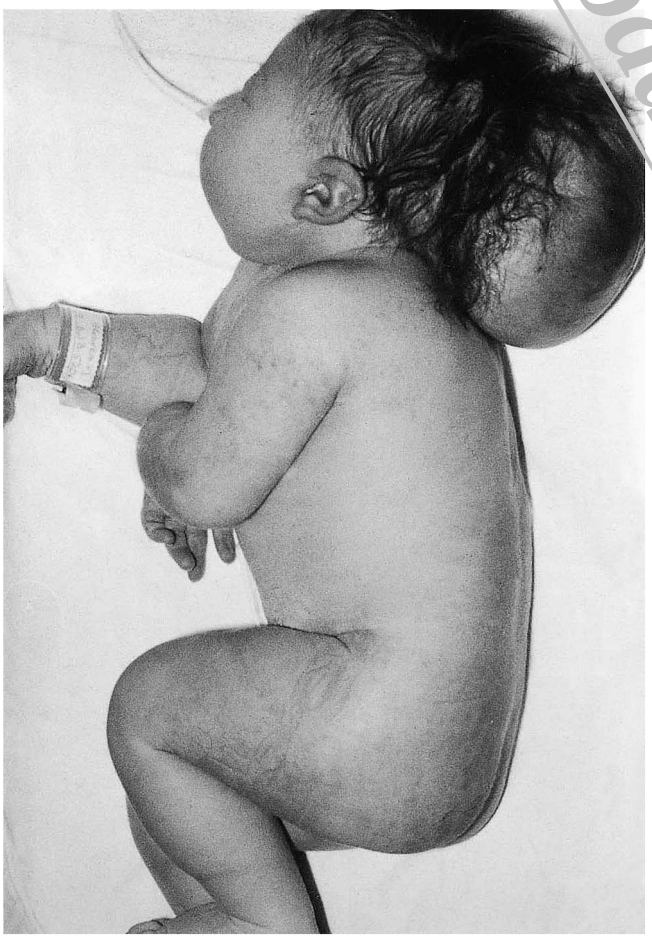
**Cardiac**
Atrial septal defect
Tetralogy of Fallot
Patent ductus arteriosus
Ventricular septal defect

**Central Nervous System**
Anencephaly
Encephalocele
Spina bifida

**Genitourinary**
Hypospadias
Renal agenesis
Renal dysgenesis

**Other**
Cleft lip/palate
Congenital dislocation of hips
Talipes

declines but remains higher than among the indigenous population. These observations suggest the presence of susceptibility genes in Celtic populations.



• **Fig. 16.17** A baby with a large occipital encephalocele.

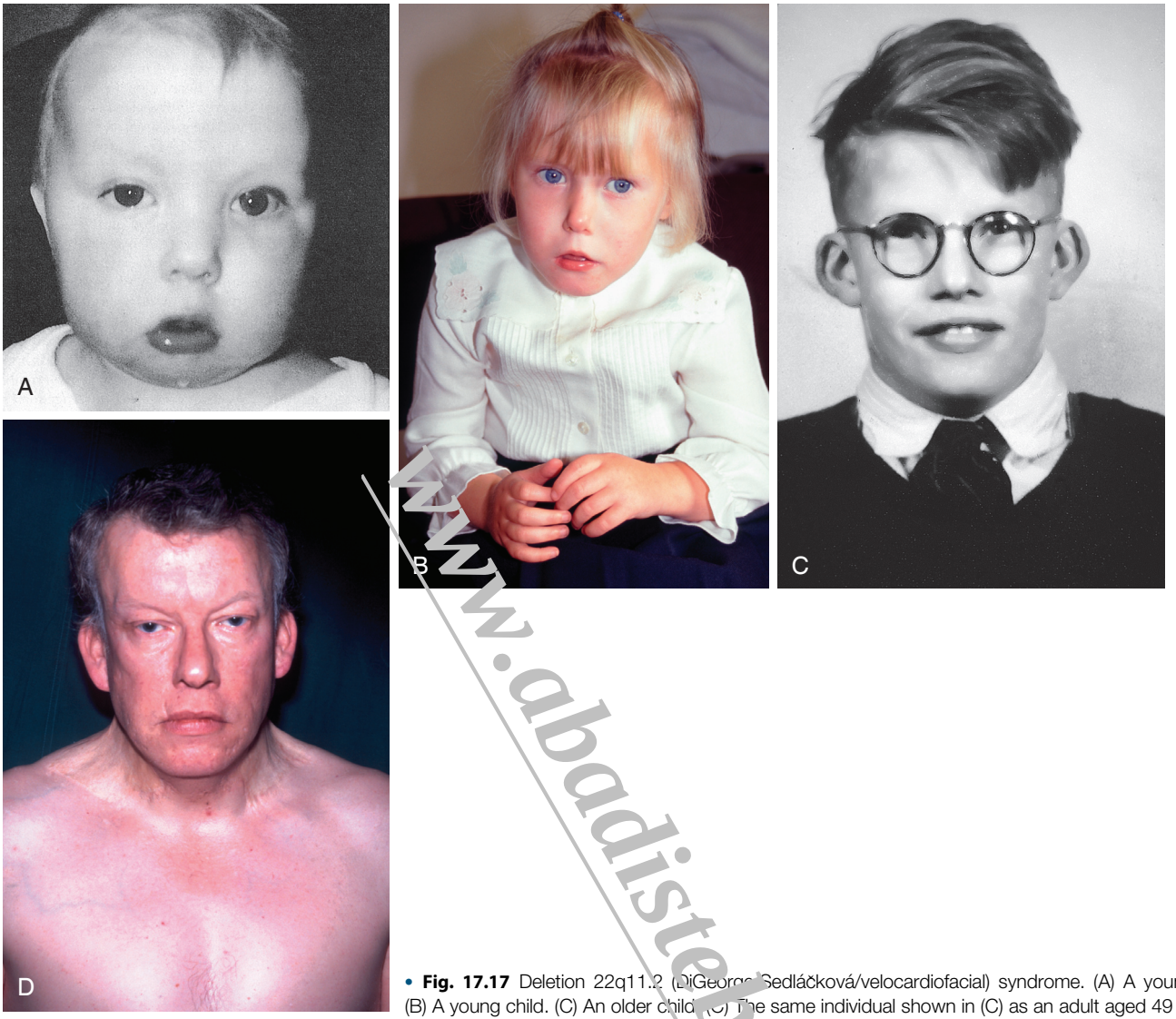
No single NTD susceptibility gene has been identified in humans, although there is some evidence that the common 677C > T polymorphism in the *methylenetetrahydrofolate reductase* (*MTHFR*) gene can be a susceptibility factor in some populations. Reduction in MTHFR activity results in decreased plasma folate levels, which are known to be causally associated with NTDs (see the following section). Research efforts have also focused on developmental genes, such as genes belonging to the *PAX* family (p. 113), which are expressed in the embryonic neural tube and vertebral column. In mouse models, approximately 80 genes have been linked to exencephaly, approximately 20 genes to lumbosacral myelomeningocele, and approximately five genes to craniorachischisis. One example is an interaction between mutations of *PAX1* and the *platelet-derived growth factor* α gene (*PDGFRA*) that results in severe NTDs in 100% of double-mutant embryos. This rare example of digenic inheritance (p. 76) serves as a useful illustration of the difficulties posed by a search for susceptibility genes in a multifactorial disorder. However, to date there have been no equivalent breakthroughs in understanding the processes in human NTDs.

Environmental factors include poor socioeconomic status, multiparity, and valproic acid (VPA) exposure (p. 240, Fig. 16.20). Firm evidence has also emerged that periconceptional multivitamin supplementation reduces the risk of recurrence by a factor of 70% to 75% when a woman has had one affected child. Studies have shown that folic acid is likely to be the effective constituent in multivitamin preparations, and the World Health Organization recommends periconceptional folate supplementation of 400 μg/day, which is adopted in some form by most nations. In some countries, including the United States, bread is fortified with folic acid. Many nations officially recommend that all women who have previously had a child with an NTD should take 4 to 5 mg of folic acid daily, both before conceiving and throughout the first trimester.

## Environmental Agents (Teratogens)

An agent that can cause a birth defect by interfering with normal embryonic or fetal development is known as a teratogen. Many teratogens have been identified, and exhaustive tests are now undertaken before any new drug is approved for use by pregnant women. The potential effects of any particular teratogen usually depend on the dosage and timing of administration during pregnancy, along with the susceptibility of both the mother and fetus.

An agent that conveys a high risk of teratogenesis, such as the rubella virus or thalidomide, can usually be identified relatively quickly. Unfortunately, it is much more difficult to detect a low-grade teratogen that causes an abnormality in only a small proportion of cases. This is because of the relatively high background incidence of congenital abnormalities, and also because many pregnant women take medication at some time in pregnancy, often for a poorly-defined "flu-like" illness. Despite extensive study, controversy still surrounds the use of a number of drugs in pregnancy. The antinausea drug Debendox was the subject of successful litigation in the United States despite a lack of firm evidence to support a definite teratogenic effect. A group of drugs under scrutiny more recently is the selective serotonin reuptake inhibitors. These are commonly prescribed antidepressants, and in Europe some 3% of pregnant women take antidepressants, rising to approximately 8% in the United States. Despite concerns about a teratogenic potential, particularly congenital heart disease, several large studies have failed to demonstrate a significant difference in the frequency of birth defects.




• **Fig. 17.17** Deletion 22q11.2 (DiGeorge/Sedláčková/velocardiofacial) syndrome. (A) A young infant. (B) A young child. (C) An older child. (D) The same individual shown in (C) as an adult aged 49 years.



### *Deletion 1p36 Syndrome*

This microdeletion syndrome emerged through improved cytogenetic techniques and the use of FISH in the 1990s. In keeping with the modern approach to nomenclature, deletion 1p36 syndrome has no eponym. The features are hypotonia, microcephaly, growth delay, severe learning difficulties, epilepsy (including infantile spasms), characteristically straight eyebrows with slightly deep-set eyes, and midface hypoplasia (Fig. 17.21). Some cases develop dilated cardiomyopathy.

### *Deletion 9q34 (Kleefstra) Syndrome*

Another of the relatively new microdeletion syndromes, this was first reported as a condition featuring significant ID, hypotonia, obesity, brachycephaly, arched eyebrows, synophrys, anteverted nostrils, prognathism, sleep disturbances, and behavioral problems. Many patients have severe speech delay, and not all manifest obesity. The case pictured in Fig. 17.22 bears a passing resemblance to Angelman syndrome. As with some of the other microdeletion syndromes (e.g., Smith-Magenis), some patients with the phenotypic features but no microdeletion have been shown to have mutations in the euchromatin histone methyl transferase 1 *(EHMT1)* gene, which lies within the region. The syndrome might therefore be mainly caused by haploinsufficiency for this gene.

### *Deletion 17q21.31 (Koolen-de Vries) Syndrome*

This was one of the first new deletion syndromes delineated through CMA, has a prevalence of approximately 1:16,000 and is probably significantly underdiagnosed. The main features are severe ID, hypotonia and characteristic facial dysmorphisms including a long face with a high forehead and tubular or pear-shaped nose, a bulbous nasal tip, large ears, and everted lower lip (Fig. 17.23). Individuals tend to be friendly. Other clinically important features include epilepsy, heart defects, kidney anomalies, and long, slender fingers. The *KANSL1* gene is key, and there are patients with pathogenic variants who do not have CMA-detectable deletions.

### *Deletion 22q13 (Phelan-McDermid) Syndrome*

Understanding of this condition, which is distinct from the better-known deletion 22q11.2 (DiGeorge) syndrome, predated the CMA era as cases with cytogenetically visible deletions were