



Institute of
Biomedical
Science

Bones to Chromosomes

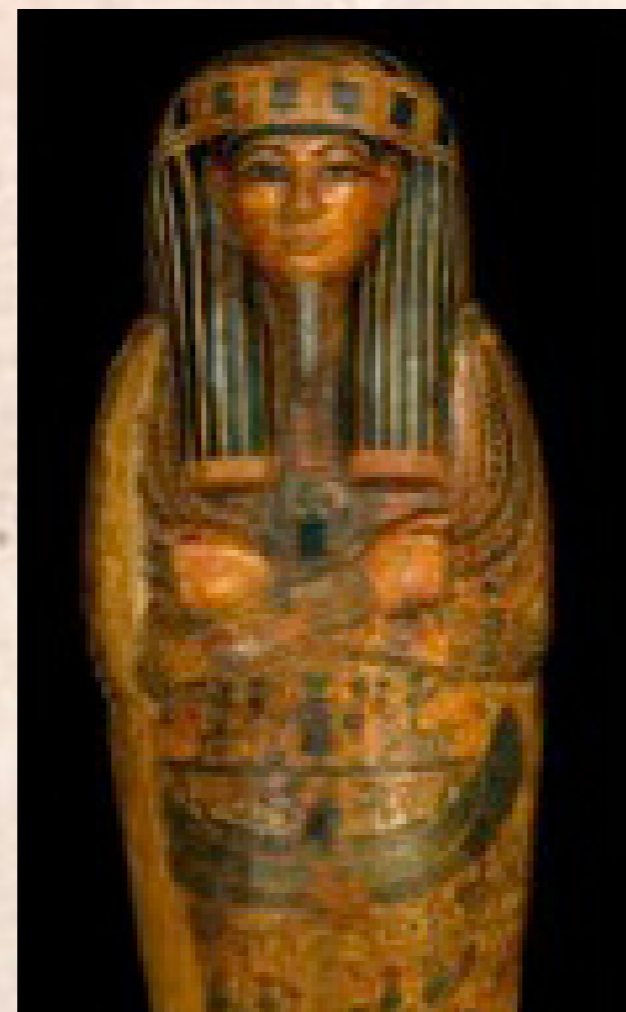
Antonie van Leeuwenhoek
(1632-1732)



Many of the genetic diseases reviewed here are monogenic, resulting from a single error in one gene of human DNA; however, the nature of the disease depends on the functions performed by the modified gene. At least 6000 monogenic disease are known, and the examples selected are based on prevalence and relevance to practical biomedical science.

Ancient Evidence

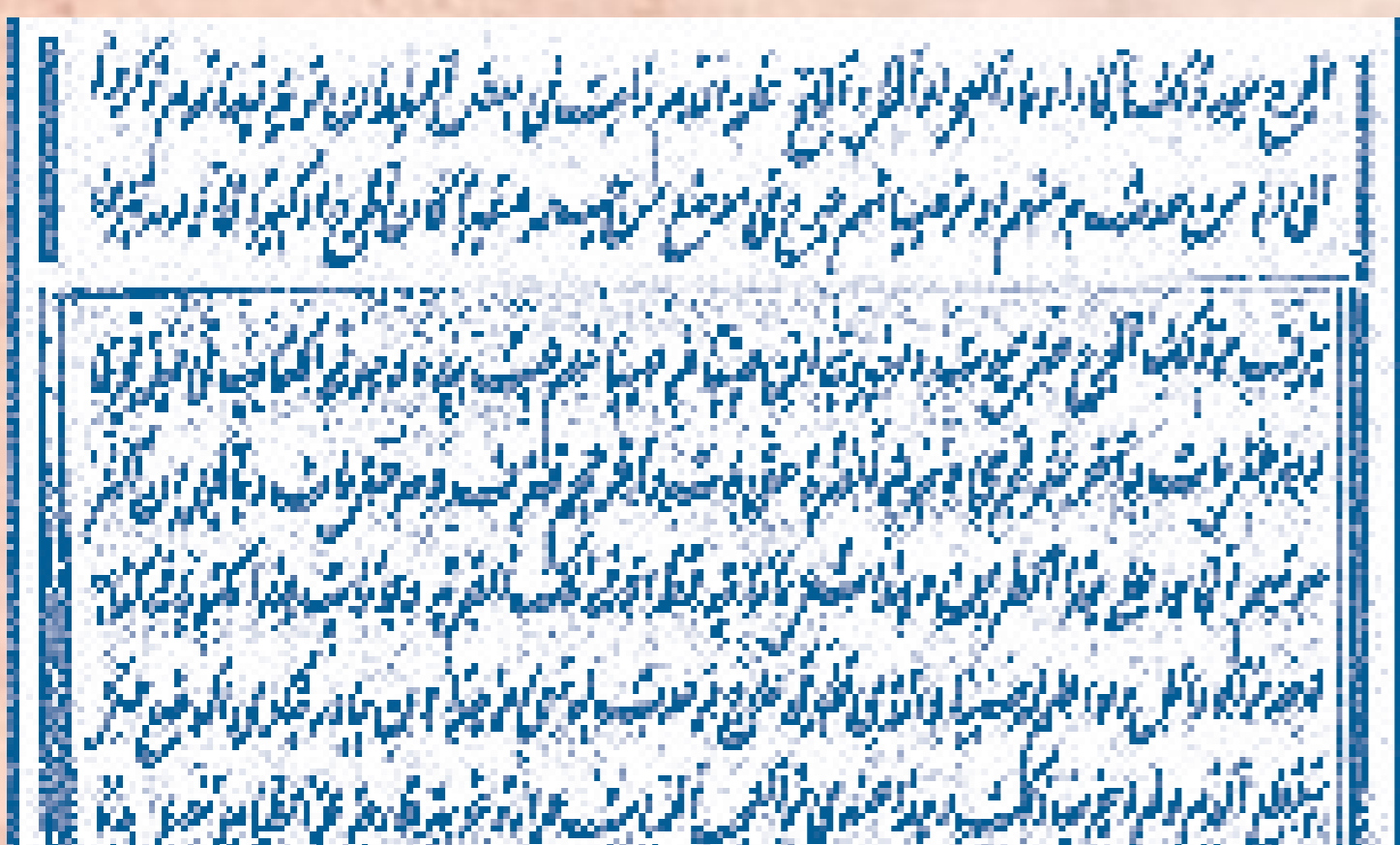
- Some phylogenetic evidence shows that mutations for genetic diseases existed with the first human life on Earth some 200,000 years ago.
- Stone artwork shows an Egyptian Queen with a form of **muscular dystrophy** (1480 BC).
- DNA sequencing of dental bones from Egyptian mummies (ca 3200 BC) show mutations for **sickle cell anaemia**, while chemical analysis of their other bone deposits indicates **alkaptonuria**.
- Some genetic studies date the most common mutation for **cystic fibrosis** to Neolithic times at least 4500 BC.



Egyptian Mummy case
(ca 1070-945 BC)
At Brooklyn Museum

Some Early Observations

- Hippocrates (400 BC) recognised that some diseases are inborn.
- Prolonged bleeding in **haemophilia** during surgery, accident or in warfare must have been evident in ancient times.
- A 200AD ruling in the 'Talmud' by Rabbi Judeus banned circumcision if a previous haemophiliac son died from the procedure.
- In 1000 AD, Arab physician Albucasis described the hereditary nature of **haemophilia**.
- The extreme skin photosensitivity of certain forms of **Porphyria** must have been recognised by early physicians.



Albucasis Manuscript on Haemophilia

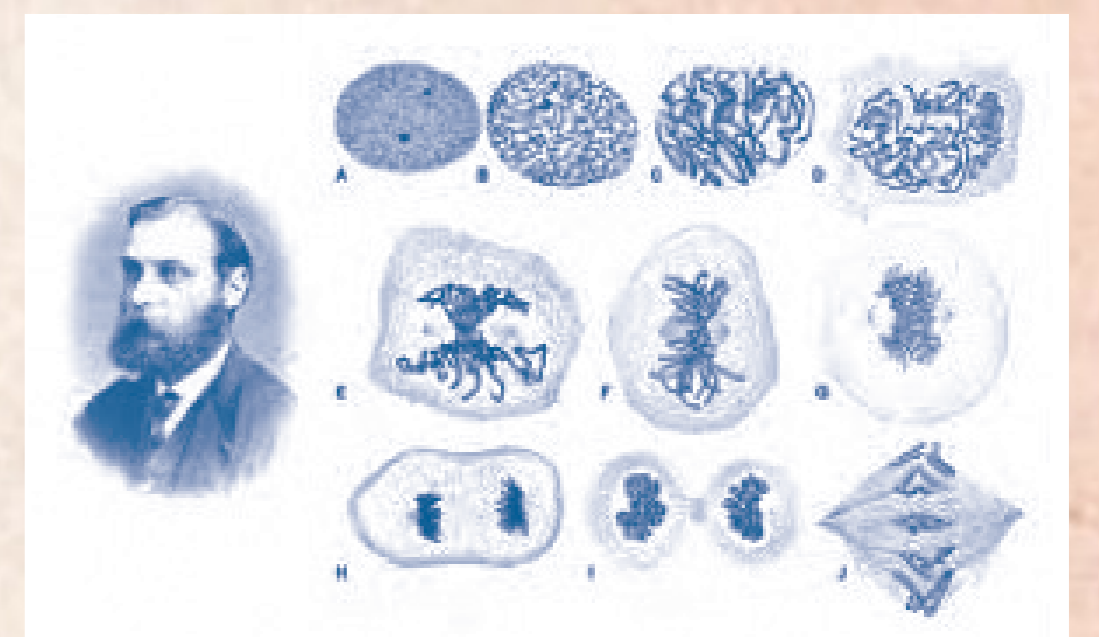
Seeing is believing: discovery of the cell, nucleus and chromosomes

Microscopes developed in late 16th century made study of fine structure of plants, insects and biological materials possible. The English natural philosopher Robert Hooke published his collected results in 1655 and used the term 'cell' in comparing pores of cork sections to cella (Latin: small room). The Dutch microscopist Antonie van Leeuwenhoek (1676-1719) drew images and observed single-celled organisms and the central 'lumen' of nucleated salmon red blood cells. This latter observation was confirmed in 1831 by Scottish botanist Robert Brown, who identified the opaque centres of orchid flower cells and coined the term 'nucleus' (Latin: kernel).



Sketches of cells
Leeuwenhoek (1674)

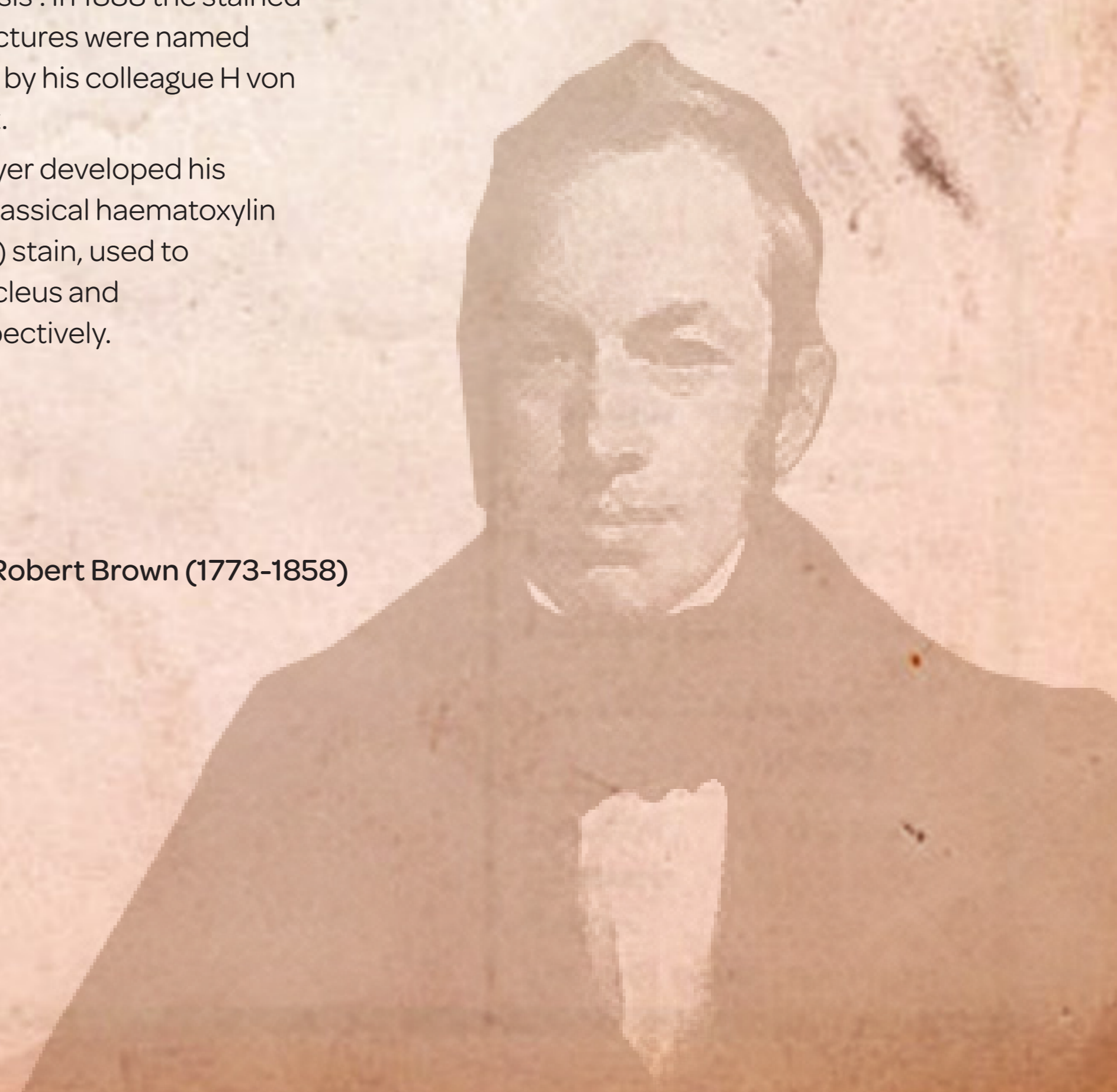
Theories for the function of cell were proposed by German scientists Matthias Schleiden and Theodor Swann (1839) and by Rudolf Virchow (1858). The collective theory identifies the cell as the basic unit of life, formed from pre existing cells by division. Animal fertilisation studies by August Weissmann (1873) and Oscar Hertwig (1876) confirmed the role of the nucleus in inheritance and meiosis. In the same year, Walther Flemming used aniline dyes to stain material he termed 'chromatin' and also studied cell division and the separation of thread-like structures in a process he named 'mitosis'. In 1888 the stained thread-like structures were named 'chromosomes' by his colleague H von Waldeyer-Hartz.



Stained cells and mitosis
Walther Flemming (1876)

In 1896 Paul Mayer developed his version of the classical haematoxylin and eosin (H&E) stain, used to highlight the nucleus and cytoplasm, respectively.

Robert Brown (1773-1858)



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Mendel, mutations and molecular mechanisms

Gregor Mendel (1822-1884)

In 1866, meticulous studies undertaken by Gregor Mendel of the physical characteristics of pea plants set rules of inheritance to predict expression of traits in future generations. Integrated with later chromosome theory of heredity, Mendel's findings became founding principles of classical genetics.

Cytological approaches to genetics

- 1900: Mendel's work was re-evaluated by Dutch botanist Hugo de Vries and found to apply to other plants, resulting in the concept of **genes** and **mutations** for spontaneous variants.
- 1903: US geneticist Walter Sutton studied grasshopper chromosomes and showed that **nuclein** filaments occur in pairs, separated in meiosis for parents to pass on genetic material. With German biologist Theodor Boveri, he proposed that genes were located on chromosomes.
- 1911: Term **gene** used by Danish botanist Wilhelm Johannsen.
- 1913: Alfred Sturtevant produced the first genetic map for *Drosophila*.
- 1915: US geneticist Thomas Morgan undertook breeding studies on *Drosophila* and provided evidence that genes carried genetic information.



Thomas Morgan (1866-1945)

Biochemical approach to genetics

- 1908: Archibald Garrod's concept of IEM suggested that certain lifelong diseases were due to a reduced activity of an enzyme regulating a single metabolic step in **alkaptonuria**, **cystinuria**, **essential pentosuria** and **albinism**.
- 1929: US biochemist Phoebus Levene proposed nucleic acid were polymers of nucleotides, each composed of a five carbon sugar, phosphate group and purine or pyrimidine bases.
- 1933: Belgian biochemist Jean Brachet showed that chromosomes contain DNA, and RNA produced in the nucleus moves to cytoplasm during cell protein synthesis.

Advances in protein, enzyme biochemistry and metabolic pathways elucidated, notably by German-born British biochemist Hans Krebs, the urea cycle (1932) and citric acid cycle (1937). Carl and Gerty Cori described the lactic acid cycle (1937). This resulted in the greater understanding of the key role of enzymes in regulating metabolic pathways.

Hans Krebs (1900-1981)



One gene-one enzyme hypothesis

- 1935: US geneticist George Beadle and Boris Ephrussi induced eye pigment mutation in *Drosophila* to demonstrate genetic control of eye pigment synthesis. Beadle, with Edward Tatum, induced mutations in *Neurospora*, studied requirements for specific vitamin metabolites in culture medium, traced to losses of single chemical reactions, each dependent on a different enzyme. By 1941 they formulated their hypothesis, which fitted Garrod's concept of IEM.
- 1948: Quentin Gibson described recessive **methaemoglobinaemia**, the first enzyme defect in human disease. In 1952, Cori described glucose 6 phosphatase deficiency in **type 1 glycogen storage disease**.



George Beadle (1903-1989)



Edward Tatum (1909-1975)

Molecular aspects of genetic disease

- 1953: US zoologist James Watson and British physicist Francis Crick constructed a detailed 3D molecular model of DNA as the perfect fit with data from X-ray crystallography images created by biophysicists Rosalind Franklin, Raymond Gosling and Maurice Wilkins. This was a double helix, with two long complementary chains of nucleotides, going in reverse directions, with matching base pairs (A-T, G-C) interlocked at the centre of the double helix.



DNA double helix
(Nature Education 2013)

- 1955: Severo Ochoa developed methods to synthesise single-stranded mRNA used by Marshall Nirenberg in a cell-free system for protein synthesis to solve in 1961 the genetic code, with H Gobind Khorana's group, as triplet base combinations coding for specific amino acids.
- 1961: Sydney Brenner, Francois Jacob, Matthew Meselson found infection of bacteria by T4 phage takes over protein synthesis confirmed that mRNA carries information from nucleus to cytoplasm following transcription.
- 1964: Robert Holley discovered tRNA, which brings specific amino acids to the ribosome for assembly into polypeptides and proteins.

Transcription

RNA polymerase builds a complementary copy of one strand of DNA; this mRNA carries the coding sequence for specific proteins, moving to cytoplasmic ribosomes (the site of synthesis).

Translation

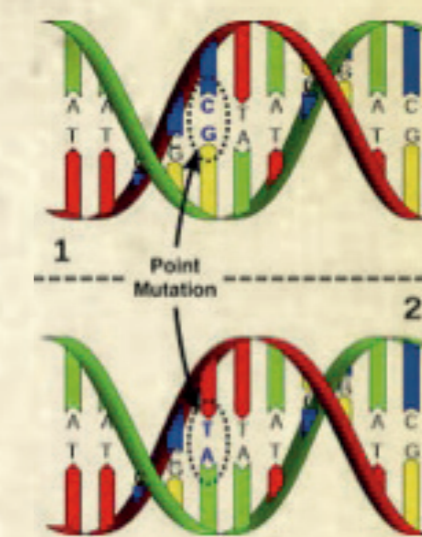
tRNA binds one end to specific amino acids and the other end to mRNA, with direction from rRNA. The ribosome moves along the mRNA from the start codon to elongate the chain of amino acids, completing the protein at the stop codon. These processes may be observed using electron microscopy.

Genome sequencing

- 1995: With improved methods of DNA sequencing, US biochemist John Venter's group sequenced the whole genome of *Haemophilus influenzae* using 'shotgun' sequencing to reduce segments for analysis.
- 2001: Venter working with Celera Genomics published the whole human genome using automated sequencing of three billion base pairs and 25,000 genes.

Types of mutation

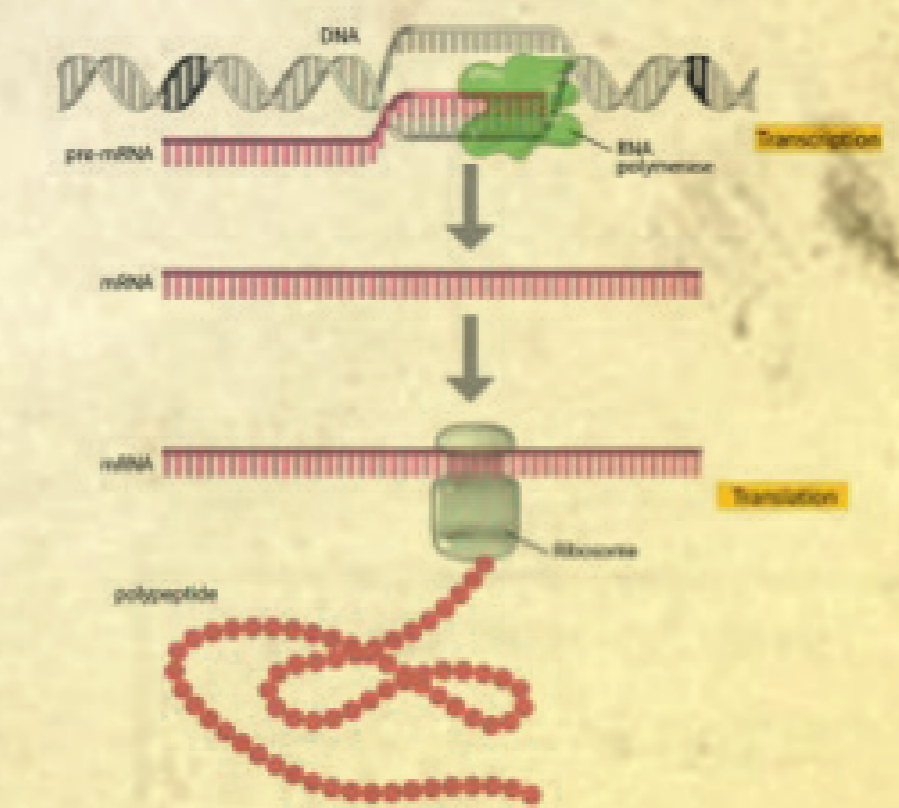
Many mutations occur at more complex translational stages, with single base change (point mutation) resulting in altered protein composition, or the substitution may introduce a stop codon to reduce the length of polypeptide chain. Other mutations may add or lose more bases to cause a ribosomal coding frame-shift error.



Point mutation in
sickle cell anaemia

Genetic resource (OMIM)

Victor McKusick (1921-2004), a US medical geneticist, published the first of 12 volumes of detailed genetic database information in 1966; online in 1985 and updated to hold records of over 26,000 human genetic diseases.



DNA Transcription &
translation (Nature 2013)

**Lost in Translation:
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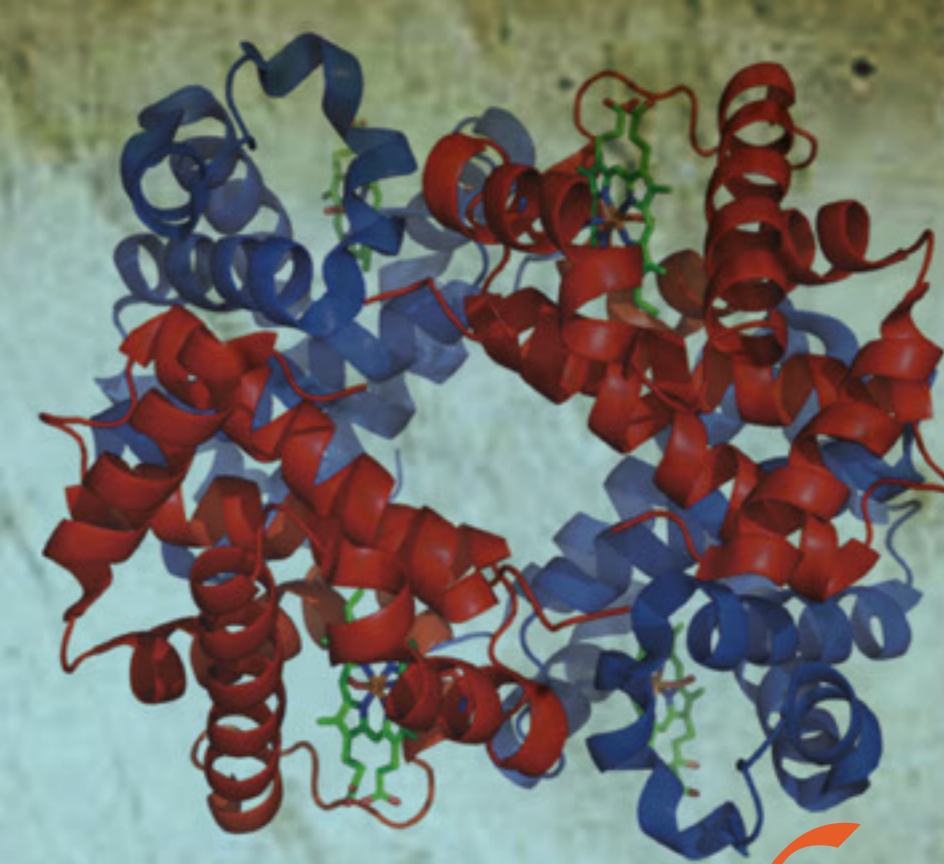
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Heritage – haemophilia, haem and haemoglobin disorders

Ribbon model of haemoglobin



Haemophilia & von Willebrand disease (vWD) Haemoglobinopathies

Haemophilia A & B are X-linked recessive traits with prolonged bleeding due to deficiencies in factor VIII and factor IX, respectively. Queen Victoria passed mutation for Haemophilia B to son and, through daughters, to many royal families of Europe.



Queen Victoria
(1819-1901)

Haemophilia reported by US physician John Otto (1803) with family studies

- 1936: Warner, Patek and Stetson identified factor VIII, anti-haemophilic factor.
- 1952: Factor IX, also known as Christmas factor described by Biggs and MacFarlane.
- 1953: Langdale developed activated partial thromboplastin time, modified to measure factor activity.
- Fresh-frozen plasma (FFP) and recombinant factors (1997) main treatment.

VWD described by Erik von Willebrand (1926), in Finland in child and family studies.

- 1950s: caused by deficiency of a glycoprotein, vW factor involved in platelet adhesion, activation and protective carrier of factor VIII.
- 1985: vW gene mapped to chromosome 12 encoding for multimeric protein of 2813 amino acids.
- Tests devised for vW glycoprotein last few decades are antigen or activity based, include immunoassays, binding tests or platelet binding assays.
- 2006: Sadler proposed 3 main types with type 1 autosomal dominant the most common.
- Most common inherited bleeding disease, prevalence 1%, mainly asymptomatic, severe cases require desmopressin to increase VIII/VWF after trauma or pre-surgery.

Porphyrias

Porphyrins -metabolites in biosynthesis of haem, acquired and inherited partial enzyme. Variation in symptoms and severity (many extremely rare). Porphyrins may accumulate in red cells, bone marrow, liver or skin and may produce light sensitive dermatitis, neuropathies and liver disease.

Landmarks in the investigation of porphyria

- 1844: Chemical and spectral studies by Mulder and Ernst Hoppe-Seyler (1871).
- 1889: Hallmark red urine in acute porphyria reported by Stokvis
- Hans Fischer studied porphyrin structure, synthesis and classification-identified uroporphyrin in urine (1913) and coproporphyrin in faecal extracts (1915).



Hans Fischer
(1881-1945)

- 1937: Urine screening tests using Ehrlich's aldehyde reagent devised by Jan Waldenstrom, modified for urine and faeces by Cecil Watson and Samuel Schwartz (1949).
- 1946: Haem synthesis pathway elucidated by Shemin and Rittenberg.
- Analytical techniques based on spectrofluorimetry or chromatography in red blood cells, plasma, urine and faeces, assays of red cell-deficient enzymes and DNA mutation analysis for diagnostic confirmation.

Two most common porphyrias are autosomal dominant with a prevalence of ~1/10000 -Porphyria cutanea tarda with extreme photosensitive dermatitis or also acquired by excessive iron or alcohol, Hepatitis C or HIV and Acute intermittent porphyria with acute episodes of gastrointestinal & neuropathy or triggered by hormones, drugs or dietary restrictions.



Porphyria cutanea tarda

Large group of diverse genetic disorders affecting chemical structure of one polypeptide chain of the globin component of haemoglobin, which may alter its physiological function. Most common monogenic inherited diseases with over 300 haemoglobin variants, many without clinical symptoms (HbC, HbD, HbE may show mild haemolytic anaemia). Heterozygous HbS trait usually symptomless but homozygous HbSS has most serious clinical consequences. Compound heterozygotes (HbSC or HbS^{Thal}) show variable anaemia, require close management during infection.

Sickle cell anaemia

- 1910: First reported case by James Herrick/Ernest Irons, with hallmark sickle-shaped red cells.
- 1930: Scriver and Waugh showed sickling correlated with oxygen tension, and later that Hb polymerisation with RBC rigidity leads to vaso-occlusion, vascular disease and fragility haemolytic anaemia.
- 1949: Linus Pauling, using moving boundary electrophoresis, demonstrated HbS and proposed autosomal recessive inheritance.
- 1957: US biologist Vernon Ingram identified single amino acid substitution in polypeptide chain using combination of electrophoresis and chromatography.
- 1965: Various forms of electrophoresis (eg paper) used in diagnosis.
- 1968: Sickling tube test with dithionite and saponin devised with electrophoresis confirmation.
- 1980s: HBB gene mapped to chromosome 11.
- 1990s: Prophylactic penicillin in childhood, self-care measures and hydroxycarbamide to stimulate production of compensating HbF to reduce 'crises'. Bone marrow transplantation (2007).
- 2005: Automated HPLC used in newborn screening with confirmation by isoelectric focusing or capillary electrophoresis.



Vernon Ingram
(1924-2006)



Sickle cells in
blood film

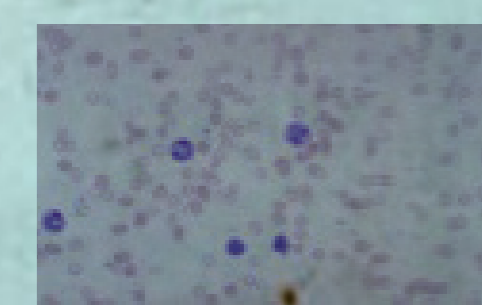
Thalassaemias

Thalassaemias are a diverse group of globally prevalent autosomal recessive conditions with reduction or absence of synthesis of one of two types of polypeptide chains of the globin component of haemoglobin. This imbalance leads to disordered erythropoiesis and varying degrees of anaemia. Thalassaemia classified as alpha type (AT), with deficiency of alpha chain synthesis or beta type (BT), with reduced beta chains both result from various chromosomal mutations. Compound heterozygotes occur with other abnormal haemoglobins, notably HbS and HbE.

- 1925: First clinical report of BT in small group of children of Italian descent, by Thomas Cooley, with severe anaemia and failure to thrive.
- 1925: F Rietti showed similar findings with jaundice, splenomegaly and decreased RBC fragility.
- 1957-9: Studies by Pauling with Hb electrophoresis, and haemoglobin structure studies of Ingram and Perutz, established defect in globin polypeptide synthesis.
- 1959: Ingram with Stretton classified two forms, AT and BT.
- 1977: Deisseroth mapped alpha gene to chromosome 16.
- 1978: Flavell, using endonuclease restriction and recombinant DNA techniques, mapped the beta globin gene and locus to chromosome 11.
- 1979-81: Many types of mutation (eg deletion, point, frame shift) identified. Hypochromia and microcytic anaemia are consistent findings in blood films.
- Blood transfusion therapy with chelators to reduce iron overload; bone marrow transplantation first undertaken by Ley (1982).



Thomas Cooley
(1871-1945)



Beta thalassaemia
blood film

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Biochemical genetics and neonatal screening

Archibald Garrod
(1857-1936)

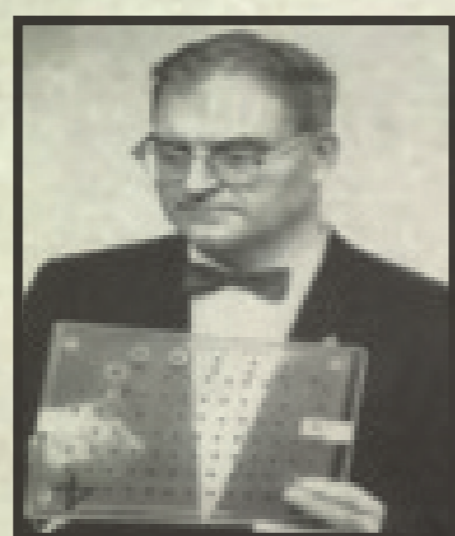
Biochemical methods of separation and analysis, notably qualitative and quantitative chromatography, enhanced progress in biochemical investigation of genetic diseases, notably amino acid disorders. Advances in DNA analysis and applications of the polymerase chain reaction allow specific identification of genes, gene products and disease-related mutations.

Phenylketonuria (PKU)

- 1934: Ivar Folling, a Norwegian physician, investigated two children with mental and physical disability found excessive urine phenylketones and raised blood phenylalanine.
- 1953: George Jervis demonstrated deficiency of phenylalanine hydroxylase leads to accumulation of phenylalanine and metabolites neurotoxic to brain development.
- 1954: German physician Horst Bickel used low phenylalanine diet to improve clinical outcome.
- 1961: US microbiologist Robert Guthrie devised microbiological inhibition assay for phenylalanine using capillary

blood spots for screening neonates in Massachusetts (1963).

- 1984: Locus for PKU gene detected chromosome 12, 1986 first mutation, now over 50 mutations.
- 1998: Tandem mass spectrometry applied for neonatal PKU screening.



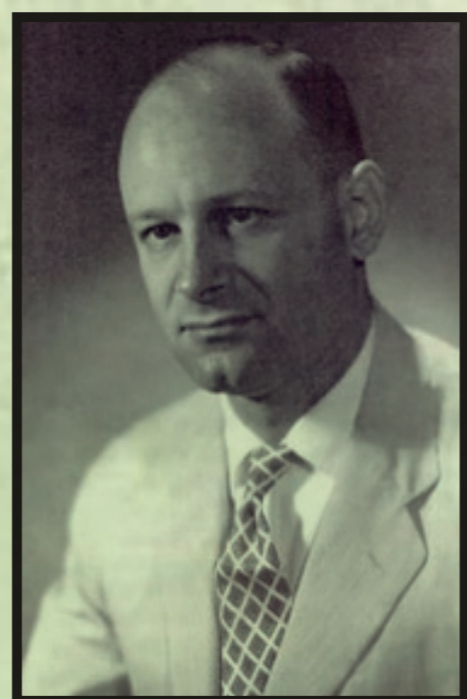
Robert Guthrie
(1916-1995)

Cystic fibrosis (CF)

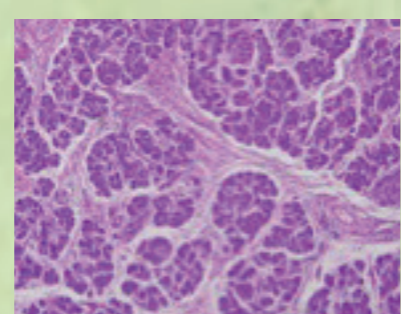
17thC malabsorption, increased skin salt in infants, fibrotic pancreatic changes on autopsy suggest CF.

- 1938: Intestinal and respiratory abnormalities, cystic and fibrotic changes in pancreas described by New York pathologist Dorothy Anderson, who developed assays to measure duodenal enzymes.
- 1948: Prognosis improved with development and use of broad-spectrum antibiotics.
- 1949: Screening test by reduced faecal trypsin digestion of gelatine-coated film in CF.
- 1953: Diagnostic raised sweat sodium and chloride reported by US physician Paul di Sant'Agnesse.
- 1959: Gold standard 'sweat test' devised, using pilocarpine iontophoresis, by US paediatricians Gibson and Cooke.
- 1968: Increased faecal albumin by Albustix, 1975 BM meconium protein strips.
- 1979: Jeanette Crossley demonstrated raised serum and blood spot immunoreactive trypsin (IRT). Referral UK screening service introduced by Anthony Heeley in East Anglia (1981).

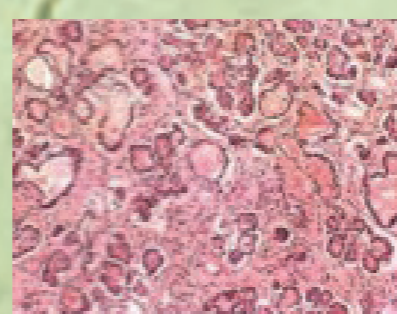
- 1983: Cellular mechanism in CF-defects in transport of electrolytes in sweat gland and lung epithelium, latter producing sticky mucus vulnerable to bacterial infection.
- 1991: Defect identified as mutations in CF transmembrane regulator gene located on chromosome 7, notably a deletion mutation at position 508 of gene channel protein.



Paul di'Sant Agnese
(1914-2005)



Normal pancreas



Cystic fibrosis
pancreas

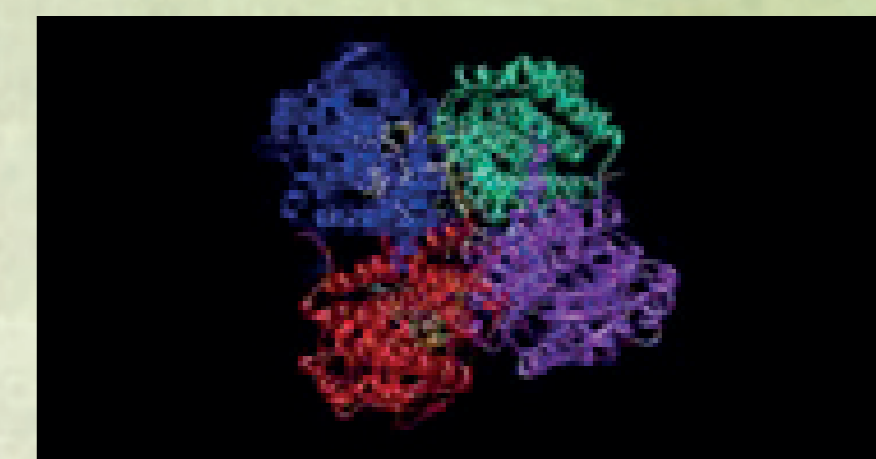
Medium chain acyl-CoA dehydrogenase deficiency (MCADD)

Medium chain acyl-CoA dehydrogenase (MCAD) catalyses first step in mitochondrial beta oxidation of medium chain fatty acids. A 90% genetic point mutation of the MCAD gene on chromosome 1 reduces response to fasting, resulting in severe illness and potentially fatal neonatal hypoglycaemia.

- 1904: Pioneer studies by Knoop and Dakin (1908) characterised basic steps in beta oxidation pathway, Kennedy (1948) identified mitochondrial location. Studies by Fritz (1955) described 'transport' role of carnitine in this process.
- 1976: First clinical description by Gregerson with episodes of lethargy, unexplained unconsciousness with C6-C10 dicarboxylic aciduria.
- 1995: Studies in the USA and UK (1998) using tandem mass spectrometry on neonatal bloodspots showed

acylcarnitine profiles sensitive and specific MCADD markers.

- Avoiding prolonged fasting, carbohydrate supplementation during illness.



Medium chain acyl-CoA
dehydrogenase enzyme

Familial hypercholesterolaemia (FH)

Xanthomata, premature death, hallmarks of FH, reported at end of 18th century.

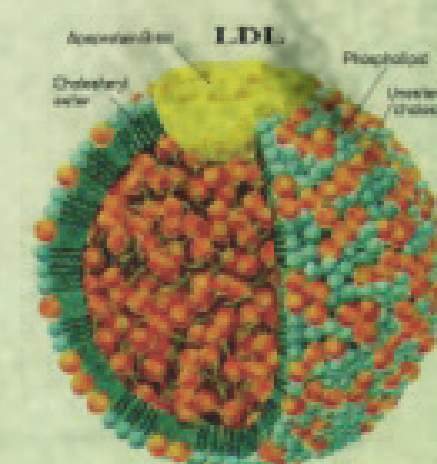
- 1925: Studies by Norwegian pathologist Francis Harbitz and physician Carl Muller (1938) included role of raised blood cholesterol, proposed autosomal dominant inheritance.
- 1955: Studies using ultracentrifugation and chromatography led to characterisation and classification of lipoproteins (Fredrickson, 1967), including low-density lipoprotein (LDL), which transports triglycerides and cholesterol to tissues, notably liver and adipose tissue.
- 1972: Landmark research studies by US researchers Joseph Goldstein and Michael Brown showed by cell culture a deficiency of LDL receptors to bind LDL and mediate endocytosis to release free cholesterol and recycle receptors. In FH, plasma cholesterol and LDL significantly increased from birth, high risk of atherosclerosis and coronary heart disease, severe in the homozygous condition. Statins stimulate production of LDL receptors.
- 1972: With technical difficulties, plasma LDL calculated by equation using total and HDL cholesterol and triglycerides results (Friedewald, 1972).
- 1984: Mutation of LDL receptor on chromosome 19 identified by Southern blotting.
- 1989: Mutation of apoprotein B-100 identified on chromosome 2.
- 2003: PCSK9 mutation identified on chromosome 1.



Joseph Goldstein
(b1940)



Michael Brown
(b1941)



LDL Structure

UK National Newborn Screening Programme

Pioneering work by Robert Guthrie in 1963 using modified filter paper to collect capillary blood, stable for postal transport to screening laboratories for PKU became a model to screen for other disorders selected on WHO Wilson-Jungner criteria (1968), notably severity, early accurate detection and effective treatment.

- Following PKU, screening for sickle cell anaemia was added in 2005, followed by cystic fibrosis (2006) and MCADD (2008).
- 2015: Programme expanded to include homocystinuria, isovaleric acidaemia, maple syrup urine disease and glutaric aciduria type 1 using tandem mass spectrometry.
- Administration of screening services set up at Great Ormond Street Hospital (2002) with a programme centre for QA, performance and strategy, in 2013 transferred to Public Health England for England only.