Cystic Fibrosis

CF Clinical Characterisation

- single gene disease identified through positional cloning
- fat and protein malabsorption, poor infant growth, lung disease and abnormal electrolyte composition in sweat
- plugs in the pancreas ducts → early symptom of CF
- estimated to affect ~180000 people worldwide
- median life expectancy is increasing - used to be a few months, now 40-55 years and rapidly improving (approaching normal life expectancy of general population)
    - evidence that we are understanding the molecular basis of CF and developing targeted/specialised therapies based on what is happening in CF

Worldwide Prevalence of CF

- prevalence: total no. of people with the condition
- >100,000 diagnosed, many remain undiagnosed
- birth prevalence varies with ethnic background
    - ~1 in 2500 European descent - most people with CF come from Northern European descent
    - ~1 in 4000 Latin American
    - ~1 in 15,000-20,000 African American
    - ~1 in 350,000 Japanese; also uncommon in Africa and Asia
    - least common in people with Japanese decent, also in Africa and Asia
- however, because it is thought of as a 'white disease', people in other ethnic groups were not tested for CF until fairly recently
    - so many people who are undiagnosed are thought to live in countries with few white people
        - estimated total of patients with CF (diagnosed + undiagnosed) = 180,000
- CF has ethnic-specific disease-associated variants
    - variants in the CFTR protein that are specific to a particular ethnic group
    - this means there are rarer mutations in other ethnic groups
        - there may not be available treatments for these variants
- Estimates of incidence and prevalence vary widely
    - incidence - number of newly diagnosed within a specific timeframe (eg incidence per year)
    - prevalence - total number of people with a condition
    - can be expressed as a total number or relative to population

Diagnosed Prevalence of CF

- note - graph shows worldwide prevalence expressed as a total number (not relative to population)
- Australia has a relatively high prevalence, because there are a lot of Caucasians
- countries with lots of people have higher numbers of CF cases
- largest number of CF cases come from countries that have a lot of people descended from Europe

Undiagnosed Prevalence of CF

- the undiagnosed prevalence of CF is higher in Europe but also India

- India is a large country - but CF is largely undiagnosed

Correlation between resources available and survival age

- the survival age of patients with CF has gone up rapidly
  - this is true in high income countries
  - CF is a chronic and rare disease that requires lots of management and resources
    - it is also hard to get targeted therapies for these diseases because there is not a lot of incentive to develop them - so usually only really rich countries have access to them
- so in lower and middle income countries (LMICs), there is a lower survival age for CF patients

Trends in CF

- There is increasing recognition that CF exists in other populations
- in **countries with good/enough resourcing**, there has been widespread implementation of **newborn screening**
  - heel prick - drop of blood is put onto a Guthrie card and used to test for markers of many diseases including CF
  - getting diagnosed early means that people can live for longer because they start treatment early
    - because people are getting diagnosed earlier, there is now an **increased number of people living with CF** due to improved therapies
- also in higher income countries:
  - there has also been an **increase in the number of adults living with CF**
    - people are not dying as early anymore
  - however, the **number of children born with CF has not increased** due to the increase in technologies
    - due to preconception carrier screening, prenatal testing
  - CFTR modulator therapy is now used by most CF patients
  - rise in pregnancy rates in CF patients

Predicted survival age of CF

- increasing expected median life expectancy with improved therapy (where it is available)
  - people who fall under this trend tend to have the best access to supportive care and earlier treatment
- the earlier the availability of treatment, the better they can manage their symptoms and reduce their exacerbations, the better their life expectancy
  - triple therapy → Trikafta treatment
    - modulator therapies that target the specific mutation that the person has in their disease

CFTR Gene

- cystic fibrosis transmembrane conductance regulator
  - large gene - 190kb
  - has 27 exons (coding regions)
  - has many introns - require splicing when generating mRNA
  - on chromosome 7 (long arm)
- CFTR was the first disease-causing gene to be discovered through positional cloning
  - scientists looked at families with CF and did restriction analysis of their genomes and compared these to families without CF
  - work out what part of the genome is altered in people with CF but not in people without CF

- CF is an **autosomal recessive** disease - pathogenic/disease-causing variants (mutations) must be present on **both copies of the CFTR gene**

CFTR Protein

- CFTR codes for an ion channel protein
  - it is a large membrane spanning protein
    - spans the plasma membrane of epithelial cells in many organs
- belongs to the ABC (ATP-binding cassette) superfamily of membrane transporters with **4 canonical (conserved) domains** and a **regulatory domain**
  - different from other ABC transporters - other ABC transporters use ATP as energy to drive ions against their concentration gradient (active transport using ATP)
  - CFTR allows ions to diffuse down its concentration gradient
- unlike other ABC transporters, the CFTR channel uses ATP as a gating mechanism to open and close the channel
  - CFTR is gated and transports both Cl^- and HCO_3^-
    - indirectly regulates sodium transport (regulates the epithelial sodium channel ENaC)
- expressed on the apical (lumen-facing) side of epithelial cells in many tissues, affecting many organs
  - lumen facing means that affects ion flow in the lumen - causes most symptoms and the pathology of CF

CFTR Structure

- the membrane spanning domains (MSD1 and MSD2) form a channel for the passage of ions
  - forms the ion pore facing the lumen
- the nucleotide binding domains (NBD1 and NBD2) come together when they bind ATP
  - has 2 ATP binding sites - bottom hydrolyses ATP, top does not
- regulatory domain (R domain)
  - has several sites that are phosphorylated by cAMP dependent protein kinase (PKA)
- note - the CFTR gene codes for **one long polypeptide** that folds up together and forms the 4 canonical domains and regulatory domain

CFTR Molecular Mechanism

- When the CFTR channel is closed, the regulatory domain is in the middle of the NBDs - physically blocks the NBDs from forming a dimer
  - but it can also move in and out of this position
- NBDs as a dimer have 2 ATP binding sites
  - a non-catalytic upper binding site - cannot hydrolyse ATP
  - a catalytic lower binding site - can hydrolyse ATP
- both ATPs must bind before the NBD domains can dimerise
- dimerisation of the NBDs allow the channel to open

So what are the steps involved in CFTR gating?

- Phosphorylation + ATP binding
- when the R domain moves away from the NBDs, it is accessible to PKA
- PKA phosphorylates the R domain, locking it in its place away from the NBD dimerisation site
- at this point, the 2 molecules of ATP need to bind

- then, the NBDs can dimerise, which leads to a conformational change in the membrane spanning domains that form the ion pore, allowing ion movement
- when the bottom ATP is hydrolysed, it causes dissociation of the NBD dimer
- there are 2 theories as to what happens after this (unsure):
  - both ATPs completely dissociates and we have to wait for another round of both ATPs binding
  - bottom ATP is recycled through cycles of hydrolysis and binding (causing short term bursts of opening and closing); but once the bottom ATP dissociates, there is long closing

Main events needed to CFTR opening: phosphorylation of R domain + 2 ATP binding

- R domain must be phosphorylated by PKA - locks it outside of dimerisation area
- 2 molecules of ATP need to bind
- NBD dimerise
- Channel open
- bottom ATP at the catalytic site is hydrolysed to ADP → causing dissociation of NBDs

CFTR Function in the Sweat Duct

- when sweat is secreted into the sweat ducts, it is isotonic (conc of salt is the same in blood and sweat)
- cells along the sweat duct try to take up salt to reabsorb it back into the bloodstream
- in a normal sweat duct, CFTR allows Cl^- to flow from the sweat duct into cells lining the ducts
- ENaC is also on the apical membrane of the sweat duct cells
- in the sweat duct, **CFTR activates ENaC** to allow Na^+ reabsorption into the blood (uptake of salt)
- this makes salt hypotonic
- people with CF have dysfunctional CFTR, Cl^- cannot flow back into cells, so it stays in the sweat
  - additionally, CFTR cannot activate ENaC, so ENaC does not work → Na^+ stays in sweat duct → sweat becomes salty

CFTR Function in the Airways

- normally, mucus lines the lungs and is swept up by cilia to the throat to be swallowed (protection against inhaled pathogens)
- in order for mucus to be thin enough to be swept up by the cilia on epithelial cells, the airway surface liquid layer (consisting of 2 layers) must be hydrated
  - water must out of epithelial cells into the airway lumen to maintain hydration
- CFTR allows net movement of Cl^- down its concentration gradient out of the cell, and water follows via osmosis
- in the lung, **CFTR inhibits ENaC**, so there is little Na^+ reabsorption into epithelial cells
- in CF, the faulty CFTR means that ENaC is no longer inhibited, so Na^+ is reabsorbed into the epithelial cells
- due to osmosis, water follows this salt into the epithelial cells
  - this causes dehydrated mucus seen in CF

CFTR Gene Variant Types

- there are different types of variants that can be found in a CFTR gene
  - does not necessarily cause disease
- Most common - Missense (40%)
- CFTR variants are also classified according to pathogenicity

- pathogenic
- likely pathogenic
- uncertain significance
- likely benign
- benign

Geneticists have mapped CFTR gene variant location and severity

- allow them to determine which areas of the CFTR gene are more likely to cause disease if there are variants/mutations in them
  - gives researchers important info about the most important parts of the gene that code for the function of the protein - identify "hot spots" that affect critical functional domains
  - map out likely pathogenic or pathogenic variants - common in exon 4, 8, 14 and 20

CFTR Gene Variant Classes

- made to help clinicians and people developing therapies for CF
- take all pathogenic variants and look how that particular variant in the gene affects the protein (grouped according to their consequence on protein production and/or function)
  - determine which process in making the CFTR - from transcription to turnover - is affected
- Class I - no production of complete protein (eg nonsense mutation - premature stop codon)
- Class II: Defective processing (premature degradation) - most common
  - protein gets made but does not fold properly and never makes it to the cell surface (degraded in ER)
- Class III: Defective channel regulation or gating (e.g. decreased ATP binding and hydrolysis)
- Class IV: Defective or reduced ion conductance
- Class V: Reduced protein production (eg abnormal splicing)
- Class VI: Accelerated turnover from cell surface (protein gets to the surface, but it is unstable and gets turned over prematurely - gets brought away from the membrane before it normally would)
- Understanding the molecular defect can help us with developing targeted therapies

2 things that can go wrong with CFTR in CF

- Protein production
  - CFTR must be made in normal quantities
- Protein function
  - CFTR must work

CF Genotype and Phenotype Correlation

- CFTR class **does not always correlate with phenotype** (can't say that just because someone has this class of CFTR mutation, that they'll experience CF in this particular way)
- the strongest correlation is with exocrine pancreatic function
  - people with very severe mutations - Class I, II and III, tend to not be able to make enzymes in their pancreas - causes malnutrition
- Classes I, II and III and some class VI associated with more severe lung disease
- classes IV and V associated with almost normal exocrine function and milder lung disease
  - because some protein is still present