Allele-specific treatments for cystic fibrosis

October 24, 2005
Cystic fibrosis

Most common lethal genetic disease in people of European descent (carriers ~1/28, affected live births ~1/3500)

Autosomal recessive inheritance, single gene on chromosome 7

Positionally cloned in 1989 using chromosome walking (no a priori assumptions about the nature of the protein)

Found to encode an atypical chloride channel: CFTR

1480 amino acids

Expressed in most polarized epithelia on the apical side

Regulated by cAMP via phosphorylation on the R (regulatory) domain

Negative regulator of ENaC…salt balance is disrupted in a complex way
Phenotypic Features Consistent with Diagnosis of Cystic Fibrosis

**Chronic sinopulmonary disease manifested by:**
- Persistent colonisation/infection with typical CF pathogens including *S aureus*, non-typeable *H influenza*, mucoid and non-mucoid *P aeruginosa*, and *B cepacia*
- Chronic cough and sputum production
- Persistent chest radiograph abnormalities (e.g., bronchiectasis, atelectasis, infiltrates, hyperinflation)
- Airway obstruction manifested by wheezing and air trapping
- Nasal polyps; radiographic or computed tomography abnormalities of paranasal sinuses
- Digital clubbing

**Gastrointestinal and nutritional abnormalities, including:**
- Intestinal: meconium ileus; distal intestinal obstruction syndrome; rectal prolapse
- Pancreatic: pancreatic insufficiency; recurrent pancreatitis
- Hepatic: chronic hepatic disease manifested by clinical or histological evidence of focal biliary cirrhosis or multilobular cirrhosis
- Nutritional: failure to thrive (protein-calorie malnutrition); hypoproteinaemia and oedema; complications secondary to fat-soluble vitamin deficiency

**Salt loss syndromes:**
- Acute salt depletion
- Chronic metabolic alkalosis

**Male urogenital abnormalities resulting in obstructive azoospermia**
Improved treatment of symptoms has extended CF lifespan

Mechanical airway clearing
Antibiotics
Nutritional supplements and replacement of pancreatic enzymes

Common comorbidities

Compare metabolic diseases:
Adult patients with a “pediatric disease”
Diagnosis by measuring ionic imbalance

Sweat test: Normal NaCl around 20 mM
CF around 80 mM
Nasal epithelial potential: no response to CFTR agonists

Amiloride blocks ENaC
Isoproterenol increases cellular cAMP
>1200 CF alleles cause disease; a handful account for most cases

90% of CF patients have at least one ΔF508 allele
ΔF508 allele age estimates give an origin ~10,000-40,000 years ago
Prevalence and distribution patterns suggest a possible heterozygote advantage (compare sickle cell disease)

http://www.genet.sickkids.on.ca/cftr/

Most common CFTR mutations in the world

<table>
<thead>
<tr>
<th>Name of Mutation</th>
<th>Frequency</th>
<th>(%)</th>
<th>Population with the highest prevalence</th>
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<td>28,948</td>
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<tr>
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<td>10-30%*</td>
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Cell biological classification of CFTR defects

Evidence that ΔF508 is primarily a trafficking defect:
Amounts of surface CFTR increase when protein folding in the ER is slowed (cold temperature) or aided by chemical chaperones (e.g. glycerol)
Single-channel electrophysiology of ΔF508 channels at the surface shows normal gating and current, no defect in regulation by phosphorylation
Of course it is not so simple…

Additional defects for ΔF508:
- Diminished reponse to beta-adrenergic agonists (stays closed too long)
- Less stable on the cell surface (shorter net half-life)
- Slower or less efficient endosomal recycling

How is misfolded CFTR degraded in the ER?

“Quality control” via the calnexin/calreticulin cycle
Misfolded proteins recognized by UDP-glucosyl glycotransferase (mechanism unknown)
Calcium-dependent ER chaperones assist folding through repeated cycles, retain unfolded substrates in the ER
Stubbornly unfolded substrates are retrotranslocated and fed to cytosolic proteasomes associated with the ER membrane
Compare virally-driven MHCI “dislocation”
Possible intervention points:

1. Disrupt the ability of calcium-dependent chaperones (calnexin and calreticulin) to retain ΔF508 CFTR; perhaps blocking calcium channels? PAPER #1: EGAN et al.

2. Increase the likelihood of readthrough transcription for alleles with nonsense codons PAPER #2: Wilchanski et al.

3. Gene replacement therapy: Various early-stage trials, many using adeno-associated virus delivered directly to lung epithelium…generally unimpressive outcomes

4. Aid folding or stabilize folded form of ΔF508 CFTR with chemical chaperones, especially small molecule ligands

5. Over 15 ER chaperones associated with ΔF508 CFTR by proteomics… RNAi to knock down chaperones?

6. Proteasome inhibitors??? Bortezomib approved for treatment of multiple myeloma
CYSTIC FIBROSIS FOUNDATION THERAPEUTICS PIPELINE

AVAILABLE TO PATIENTS

PHASE 3
(Definitive Trial)

PHASE 2
(Human Safety and Efficacy Trial)

PHASE 1
(Human Safety Trial)

PRE-CLINICAL
(Initial Testing in Laboratory)

RESEARCH
(Finding Potential Drugs)

Gene Therapy
CFTR Protein Rescue
Restore Ion Transport
Anti-Inflammatory
Mucus Regulation
Anti-Infective
Nutrition

Ins37217
SPI-8811
Parion 552-02
Mebi 1901
Inhaled Nacetylcysteine
Oral Nacetylcysteine
HIE-2000
Simvastatin
Lorcanin
Corus 1020 (Aztreonam)
TIP (Tobi Inhaled Powder)
MP-610.2205
Pseudomonas Vaccines
SLT-amilacrin
TheraCFC

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http://www.cff.org
What is the heterozygote advantage?

Postulated improved survival of epidemic diarrheal diseases (especially cholera) due to decrease in salt content of intestinal secretions. BUT…

Alleles are most common in Europe, epidemic cholera is most common elsewhere

Mouse models show no improved survival for diarrheal diseases

Human heterozygotes do not have measurably altered salt balance

Other possibilities:

Heterozygote disadvantage outweighs heterozygote advantage everywhere except Europe

There is a more specific molecular function of CFTR…
Salmonella typhi uses CFTR to enter intestinal epithelial cells


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Bacterial uptake in mouse gut

Heterozygote advantage in typhoid fever?
Why are CF patients particularly prone to lung infections by *Pseudomonas aeruginosa*?

*P. aeruginosa* forms a biofilm; is there a particular defect in clearance because of compromised mucociliary escalator?

OR… are there other bacterial clearance functions in which CFTR participates?

Note contrast with typhoid…

Goldberg and Pier, 2000, Trends in Microbiol. 8: 514
CFTR is a pattern recognition molecule that extracts *Pseudomonas aeruginosa* LPS from the outer membrane into epithelial cells and activates NF-κB translocation

How?
May be indirect

Why?

NFκB induces broad innate immune response

ΔF508 CFTR