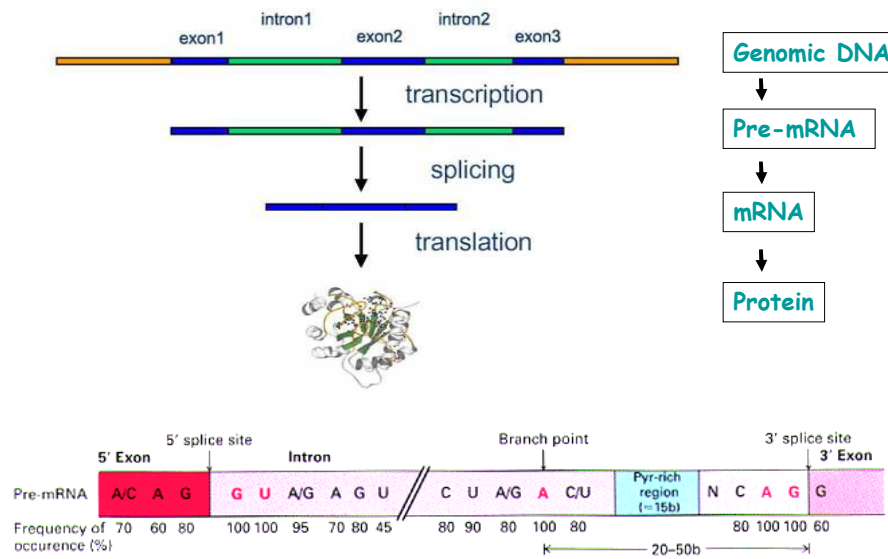


MOLECULAR DIAGNOSTIC APPROACHES FOR IMD'S - GROOTE SCHUUR AND RED CROSS HOSPITAL EXPERIENCE.



Human DNA translation



Molecular diagnostic approaches for IMD's

Cystic fibrosis

Caused by a defect in cAMP-mediated chloride channel that regulates the ion and water balance across epithelial

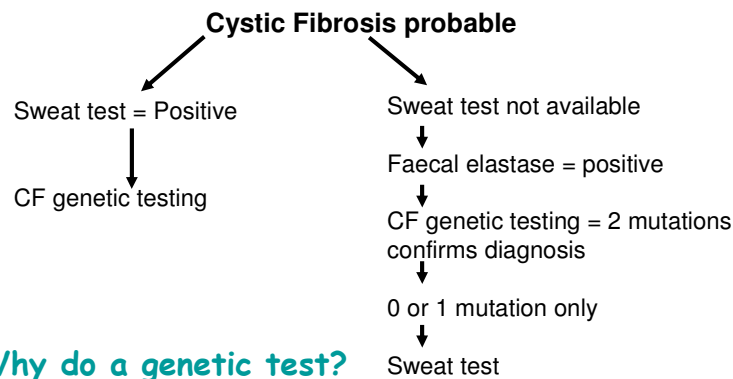
Urea cycle defects – OTC

Caused by a defect in the ornithine transcarbonylase gene in the urea cycle

Glutaric aciduria type 1

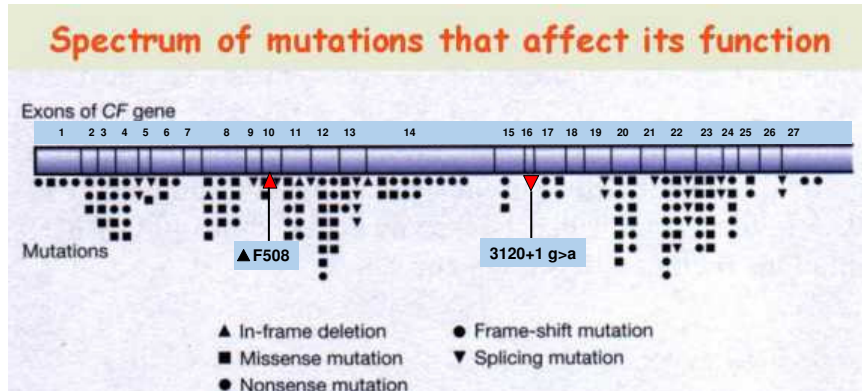
Caused by a defect in the glutaryl-CoA dehydrogenase (GCDH) gene in the pathway for lysine, hydroxyllysine and tryptophane

Cystic Fibrosis



Genetic counselling
Confirm diagnosis

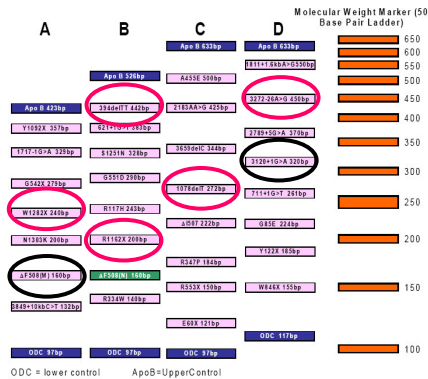
Cystic Fibrosis



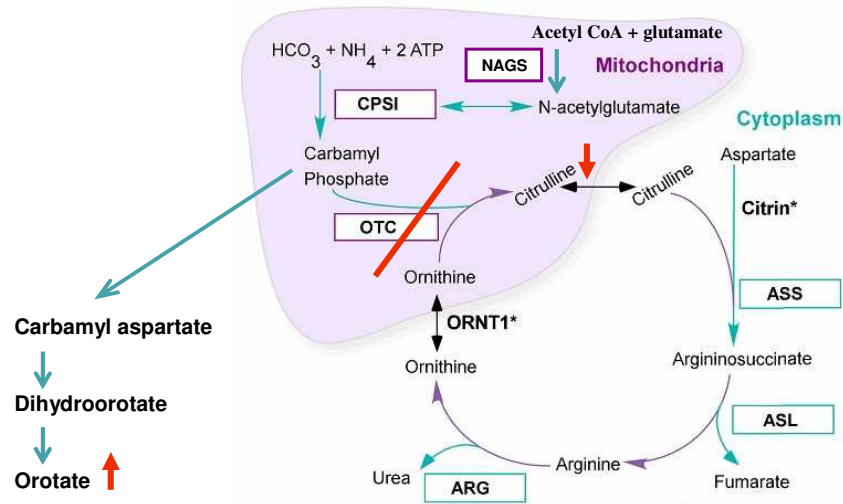
Delta F508 was identified 20 years ago and since then more than 1500 mutations are now known

Cystic Fibrosis

- Initially tested for the two common mutations
 - Δ F508 in Caucasian (76%)
 - 3120+1g->a in African race (46%)
 - Δ F508 and 3120+1g->a in Mixed race (70%)
- Also possible to sequence the gene but expensive
- Offer a 30 mutation screen (most frequent in SA)
- Only few of African mutations in panel



OTC defects



OTC gene found on X chromosome

OTC defects

Why do a genetic test?

Provide prenatal testing

Genetic counselling.

Confirm OTC deficiency especially in girls and late onset boys.

Out of the 341 mutations (Hum Mutation 2006 27 626-32)

45% neonatal onset of hyperammonemia.

20% male patients with later onset of hyperammonemia,

35% are affected girls (skewing of X chromosome liver)

20% of patients **NO** defect be seen ---- **Why?**

OTC defects

- No orotate test, defect may be CPS or NAGS.
- Late onset diagnoses may be boarder line
- Technically large deletions in girls difficult to detect.
- Mutations in introns / controlling regions.
- Liver Biopsy for OTC activity and mRNA analysis

Table 1
Clinical history and laboratory data of the patients described in this study

Patient	Sex	Ethnic background	Age at first symptoms/ at follow-up	Clinical course	Liver biopsy	OTC activity in mIU/ mg prot (normal > 160)
1	Male	Turkish	6 months/4½ years	Very mild movement disorder	Needle	84/52
2	Male	Saudi Arabian	1 week/2 years	Severely retarded	Open	23
3	Male	German	2 days	Died on day 4	Needle	n.a.
4	Female	German	2½ years/3½ years	Well after liver transplantation	Needle	70
5	Male	German	11 months	Died at age 11 months	Open (post-mortem)	39

Since the liver biopsy was repeated in patient 1, two levels of OTC activity are given for this patient. n.a., not available.

Analysis of mRNA transcripts improves the success rate of molecular testing in OTC deficiency - Molecular Genetics and Metabolism 2008 94 291-297

- Insertions of intronic sequence in boys.
- Deletion of exons in the girl.

OTC defects

Patients from RXH and GSH over the last 10 years referred for confirmation and future antenatal analysis, all neonatal boys.

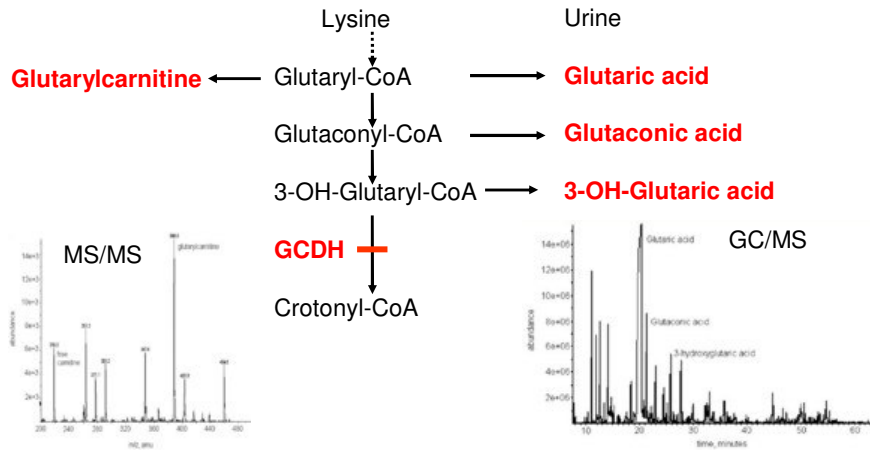
- Family 1 – aa 91 Lysine -> Serine
- Family 2 – aa 104 Leucine -> phenylalanine
- Family 3 – aa 141 Arginine -> glycine
- Family 4 – aa 195 Glycine -> arginine
- Family 5 – deletion “g” in exon 4
- Family 6 – deletion exons 6-10 (4 previous deceased sons)
- Family 7 – deletion whole gene

No girls or late onset boys referred for genetic analysis - why?

The incidence of UCDs at least 1:30,000 births.

GA1

Glutaric aciduria type I (GA-I) is a progressive neurodegenerative disease caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH)



GA1

Frequency is 1:100,000 infants.

Easily treatable disease with carnitine and amino acid restriction

Early diagnosis in newborns can reduce risk of acute dystonia in 80%.

Why do a genetic test?

Counselling

Understanding mutation spectrum in South Africa

New test 2008

GA1

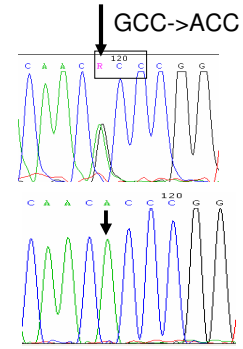


Size of gene - Sequencing

A293T in exon 8, homozygous.

Another 5 African families same mutation.

Gene frequency? NBS?



Interaction

Comments

Clinician/patient



Communication within a hospital good

Across the country limited

Routine laboratory



Refer for metabolic tests

Small metabolites

Enzyme activity

Genetic analysis

Limited genetic tests for IMD in SA



Other centres

Few patients for each disease

New tests setup regularly

Comparison to UK

UK (NHS) - 300 genetic tests – (70,000 tests in 2003)

SA (NHLS) - 80 genetic tests

Problems

Resources limited on all sides.

No NBS

No one site where all tests are listed.

Progress

Department of Health “Diagnostic Genetic Tests South Africa 2007”

Web page www.madlab.uct.ac.za (Molecular and Diagnostic labs)

The screenshot shows the website for the Molecular & Diagnostic Lab at Groote Schuur Hospital / Red Cross Children's Hospital. The page is titled "MOLECULAR & DIAGNOSTIC LAB" and includes a search bar and navigation links for "All tests", "Biochemical analyses", "Genetics", and "Metabolic diagnostic assays".

The "GENETIC ASSAYS" section is expanded to show a list of tests:

- A
- B
- C
- D
- E
- F
- G
- H
- I
- J
- K
- L
- M
- N
- O
- P
- Q
- R
- S
- T
- U
- V
- W
- X
- Y
- Z

The "Cystic Fibrosis (CF) mutation panel for > 30 most prevalent" test is selected, showing the following details:

- Clinical details:**
- Methodology:**
- Sample requirements:** 2 ml EDTA blood, dried blood spots or buccal swabs
- Pre-requirements:** CF mutation carrier test
- Stability of sample:**
- turnaround time:** 2 month
- Contact person:** Howard Mendenhall, Tel: 021 4068117, h.mendenhall@uct.ac.za, 0161 461 4614, Suite 1400, Tel: 021 4068117
- Delivery address for samples:** 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614, 0161 4614614

Tests list available if required by email

How to fish the easy way



March 2009 – UCT Upper campus





```

I
T
L
V
MLFNLRIILLN NAAFNGHNF MVRNFRGQP LQNKVQLKGR DLLTLKNFTG
1
D
GS
KT DTQ L P R SF EN R KN G GATS K D
EEIKYHLWLS ADLKFRIKQK GEYLPLLQGK SLGHIFEKRS TRTRLSTETG
51
V
E
E VR L R MG P SP EVT SP GE S
FALLOGHPCF LTTQDINLGV NESLTDARV LSSHADAVLA RVYKQSDLDL
101
E T K Q
P N SE RA H VC H C V R G YEE
RV S TS DR PT PL F PCH MPP GLD R FRK RRRID
LAKEASIPII NGLSDLYHPI QILADYLTIQ EHYSSLKGLT LSWIGDGHNI
151
P IR L R D
PYC VRNTVQ KYFEE A N T V V I Q K
LHSINBSAAK FGHHLQAATP KQYEPDASVT KLAEQYAKEN GTKLLLTNDP
201
I L
P IGHL N
T P S KHAR RTEP Q N S
LEAARGGHVL ITDTWISHGQ EEEKKKRLQA FQGYQVTKMT AKVAASDMTF
251
Q
LY G C
RG H D D
FRFR GH TSFL E G R ISL LP SKHT
LHCLPRKPEE VDDEVFYSR SLVFPFAEHR KWTIMAVNVS LLDYSPQLQ
301
C
KPKF
351

```

- 219 aminoacid changes
- Neonatal presentation are in red
- Late onset presentation are in green
- Females presentation are in purple
- Not shown 130 deletions, insertions and exon/intron boundary mutations

Human DNA mutations

Coding region mutations – disease causing

- Missense mutations = point mutation that changes the amino acid.
- Nonsense mutation = point mutation that results in a premature stop codon.
- Deletions/Insertions = Possible frame shift = incorrect translation.
- Intron/exon boundary point mutation = loss of an exon or inclusion of intron sequence
= frameshift

Coding region mutations – Normal protein function

- Neutral mutation = point mutation that changes the amino acid = normal function of the protein.
- Polymorphism = point mutation no change of amino acid.

Coding/Intron region mutations – disease causing

- Large or small deletions or inversions of whole or part of the gene.
- Intronic mutations can affect control and transcription regions of the gene.

New Born Screening

Problems tandem MS/MS

- Equipment expensive
- Difficult to locate “positive” babies for confirmation in SA.

Founder mutations = **DNA approach** to NBS attractive

- Screen NB babies
- Screen mums (dads) at antenatal clinic -> heterozygotes
- Screen high risk babies.

Problems

- Only detect babies with “common mutation”.
- Reduce test cost.
- Multiplex - many mutations/diseases in one test.
- Automated using readily available equipment.