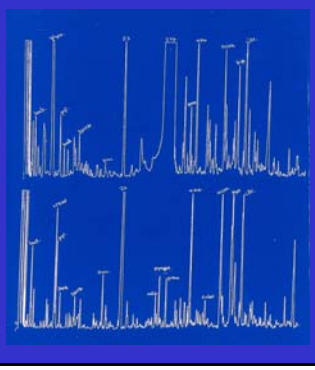
Why is there a need for a new diagnostic tool?



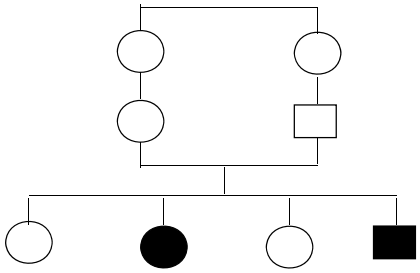
Homozygosity mapping as a primary, new diagnostic tool

ERNDIM meeting, Basel, Oct 22, 2009

Orly Elpeleg

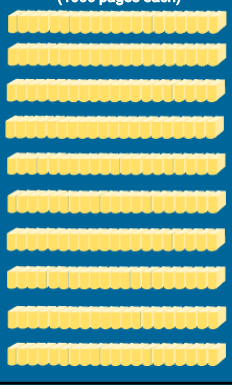
Department of Genetics and Metabolic Diseases
Hadassah, Jerusalem

if the patient originates from a consanguineous family
(and has a similarly affected sib)



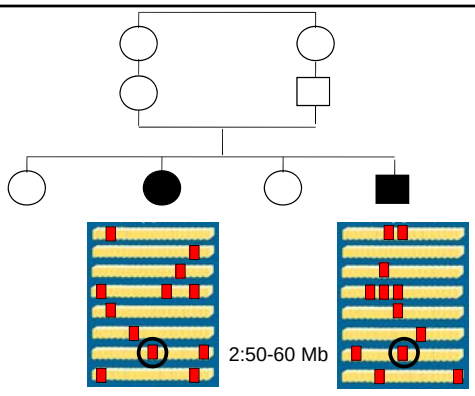
Search for homozygous regions

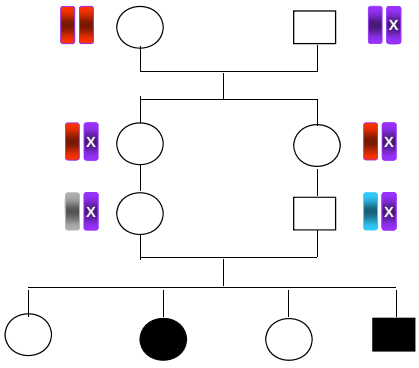
Human genome
30,000 telephone books
(1000 pages each)

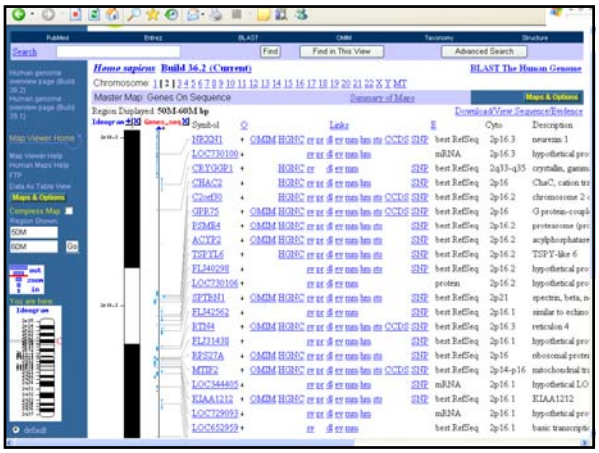


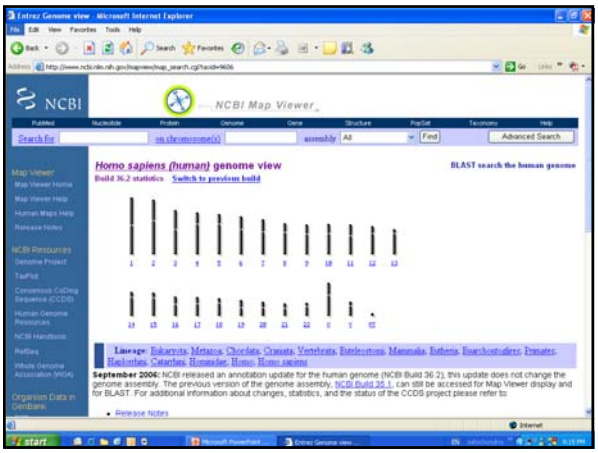
?

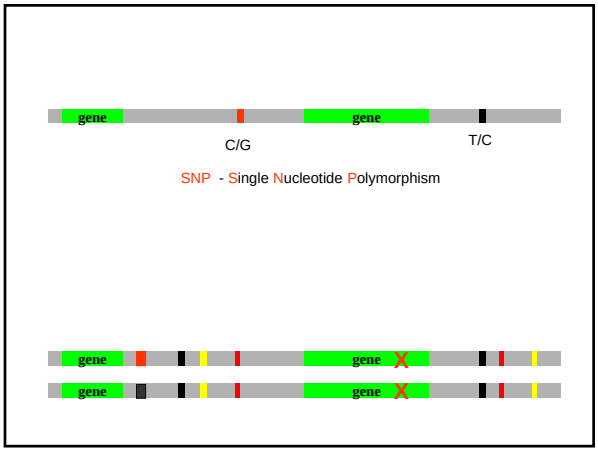
NGS



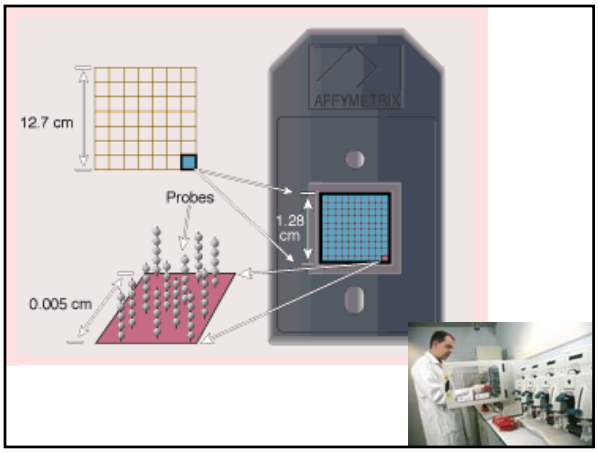


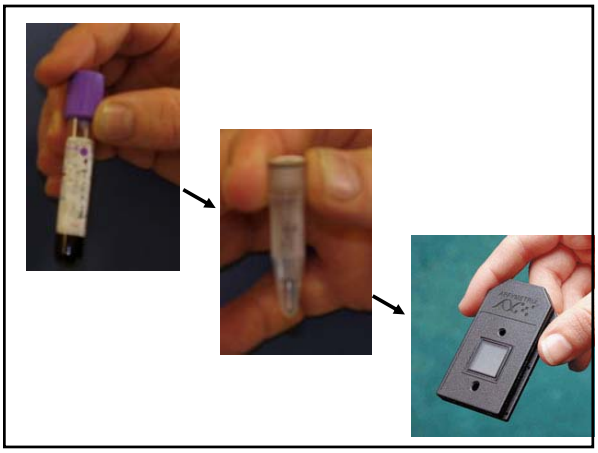


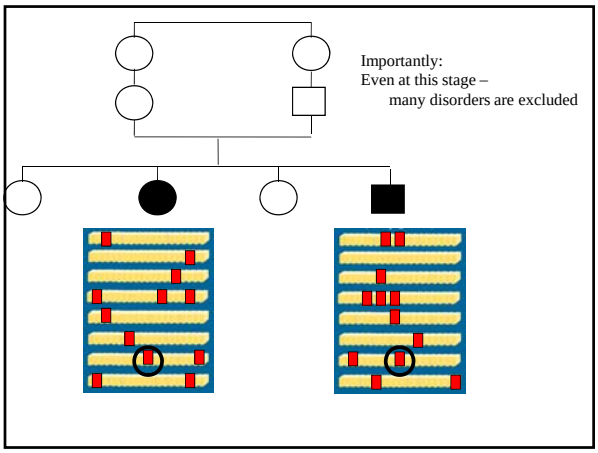


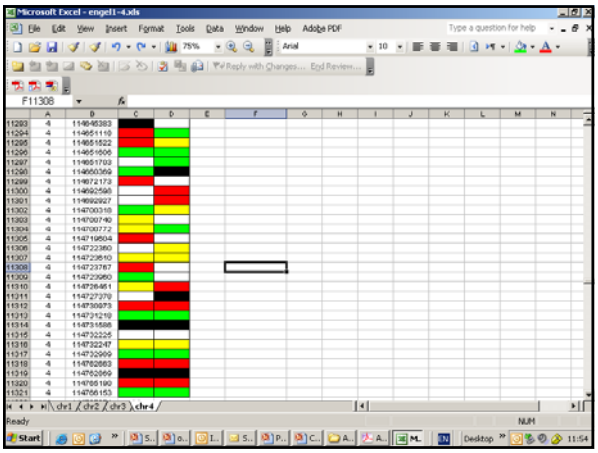


How do we find a homozygous region?



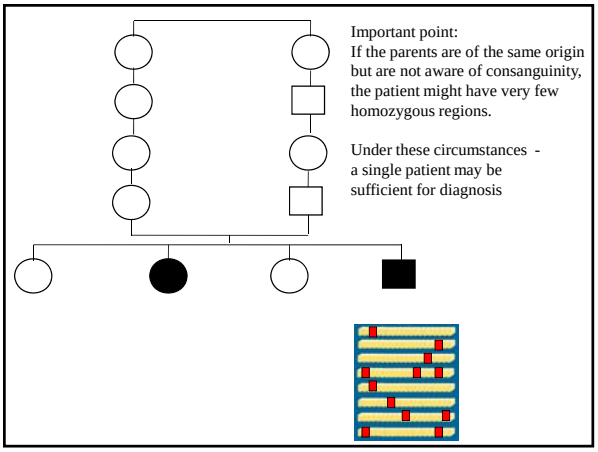


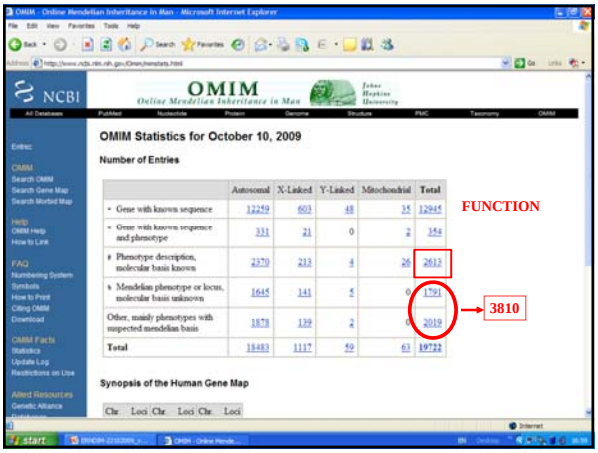




Homozygosity mapping – advantages

- Non-invasive
- Non-expensive
- Enables genetic counseling
- Enables diagnosis for families of mixed origin
- Elucidation of the normal gene function





Normal results

- CBC, electrolytes, alk. phos., PT, PTT, albumin and total proteins, cholesterol, TG, urea, creatinine, uric acid and blood gasses.
- Plasma **lactate**, total and free carnitine, **acylcarnitine**, amino acids.
- Fatty acid oxidation and **CPT2** in lymphocytes
- Urinary organic acids.
- EMG, brain CT scan, abdominal US and echo.
- **OXPHOS activities** in muscle biopsy.
- The muscle contained no excess of fat or glycogen, the **cyto-architecture** seemed normal.

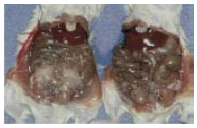
K/O mice are not always helpful

Recurrent myoglobinuria

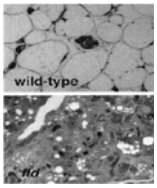
- Recurrent episodes of myoglobinuria, **precipitated by a febrile illness,** lasted 7-10 days.
- Onset ~ 2 years of age
  - myalgia, generalized weakness
  - refusal to lift the legs against gravitation
  - areflexia at the patella and Achilles
  - Normal muscle strength, tone and reflexes at the upper limbs.
- plasma **CK peak level 180,000-450,000 U/L**

letter

Lipodystrophy in the *fld* mouse results from mutation of a new gene encoding a nuclear protein, lipin



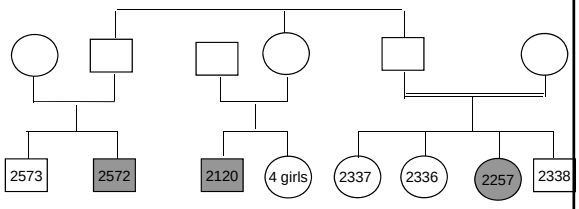
Reduced epididymal adipose tissue in the *fld* mouse (right)



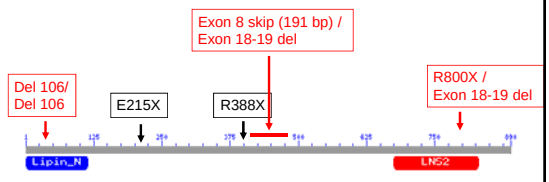
adipocytes in epididymal pad of fat (H&E x330).

chr 2 6.14-12.99 Mb

39 genes
Only one is predominantly expressed in muscle – LPIN1



LPIN1 sequenced in 10 French patients with rec. myoglobinuria



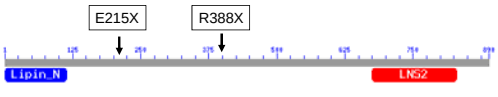
Mauritaniens, Consang.
DOB 2003
1st episode – 27 m
3 episodes
Peak CK – 200,000

French non-consng.
DOB 2000
1st episode – 18 m
4 episodes
Peak CK – 80,000

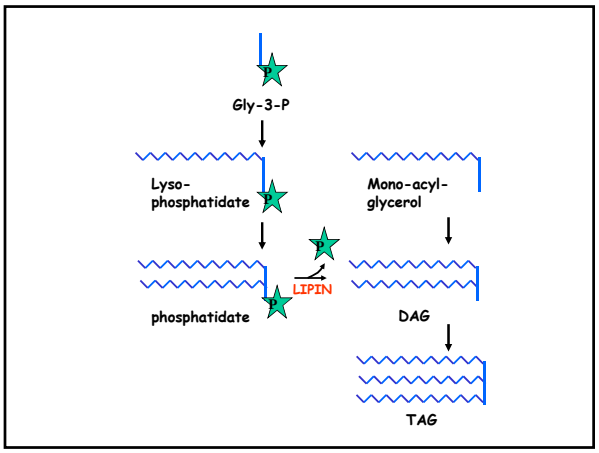
(brother died 16m of myoglobinuria)

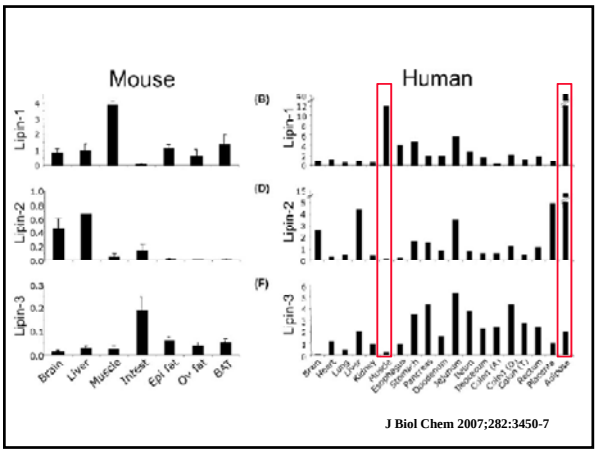
French non-consng.
DOB 2003
1st episode – 15 m
6 episodes
Peak CK – 100,000

LPIN1 sequenced in 12 patients with rec. myoglobinuria



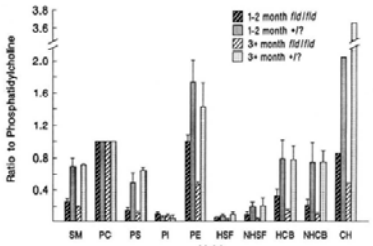
D.O.B. 13.2.2002
2/4 to consang. parents
31.12.05 and 20.11.06 :
CPK – 15-20,000
K – 6.4,
Creatinine – 2.4 mg%,
GFR – 11 ml/m2/min.



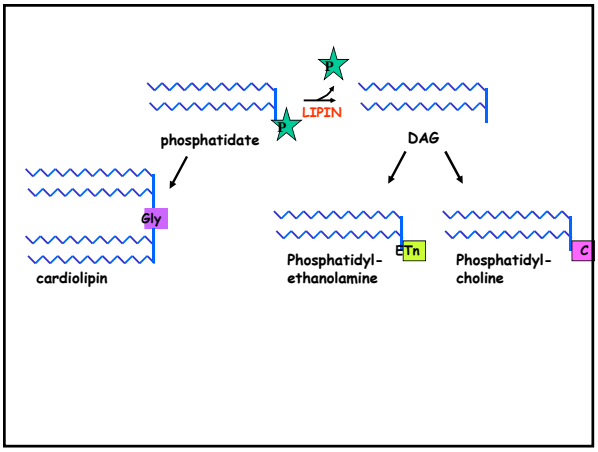


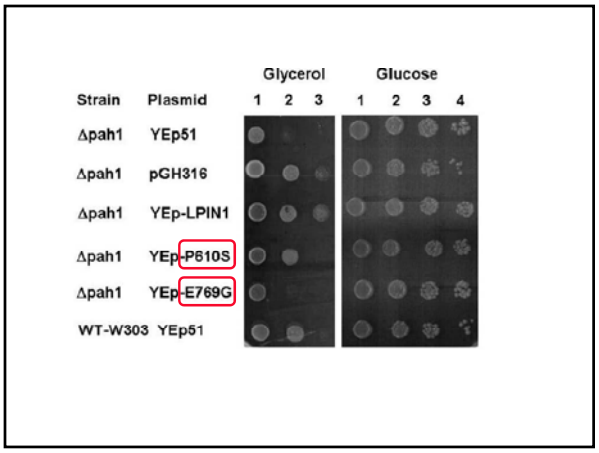
J Biol Chem 2007;282:3450-7

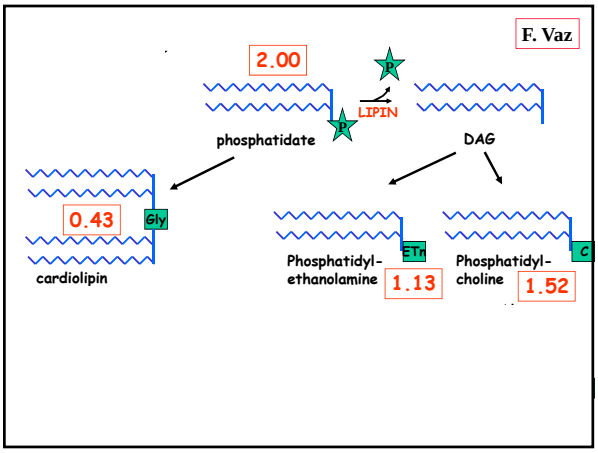
Quantitation of phospholipids in sciatic nerve of mutant mice



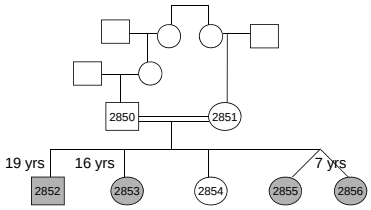
J Biol Chem 1991;266:11955-64







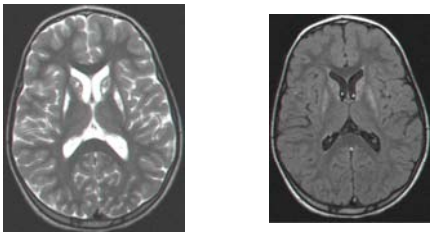
Human K/O is not always helpful



Normal early development
Starting at 3-6 yrs, on the 2nd-3rd day of febrile illness:
rapidly progressive flaccid paralysis (within hours), areflexia, NCV ↓↓
swallowing difficulties, aphasia, encephalopathy,
Mild respiratory involvement.
Tube feeding. Spontaneous, slow resolution of symptoms (~3 wks)
1-3 episodes throughout life.
Residual distal weakness in lower limbs
Progressive motor-sensory polyneuropathy, axonal AND demyelinating .

T.H.M.

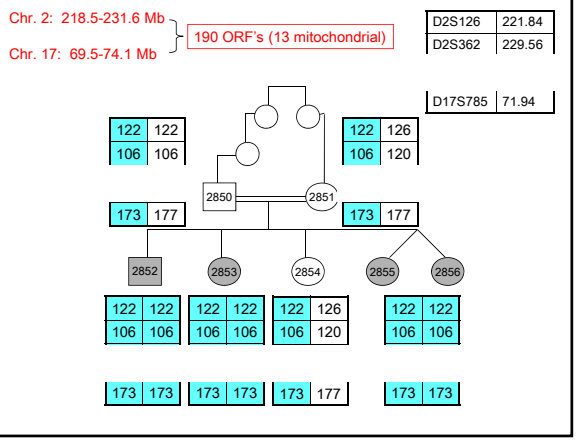
- Deleterious LPIN1 mutations cause recurrent myoglobinuria in childhood
- The mechanism may involve aberrant ratio of membrane phospholipid/lyso-phospholipids
- Heterozygosity for LPIN1 mutations may be associated with statin-induced myopathy

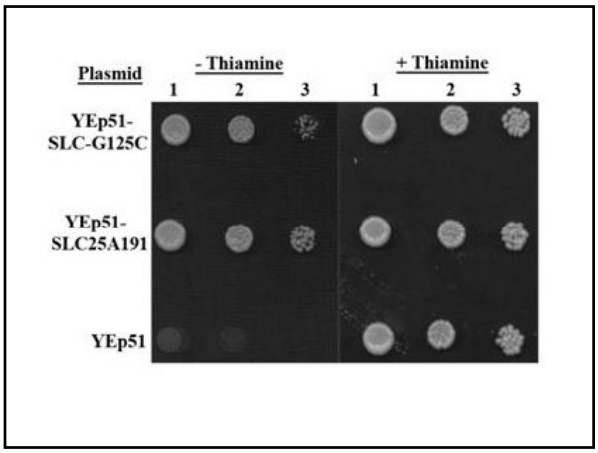


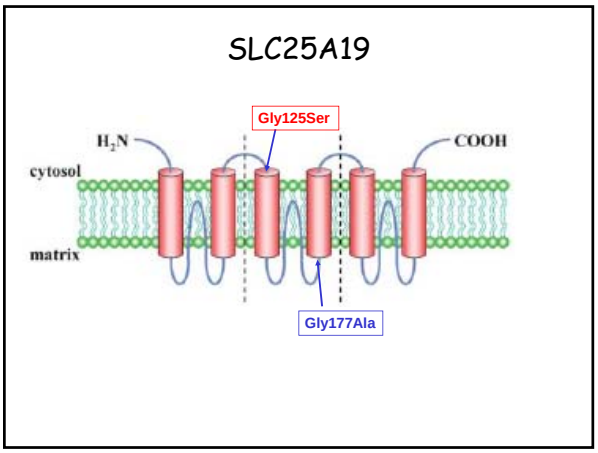
CSF lactate during episodes: 2.9-4.2 mM

Older child
Second daughter
twins

✓PNKD
✓BCS1L
✓FDXR
✓ATP5H
✓SLC19A3
SLC25A19




Plasmid
- Thiamine
+ Thiamine
1
2
3
1
2
3
YEp51-
SLC-G125C
YEp51-
SLC25A191
YEp51


SLC25A19
Gly125Ser
H2N
COOH
cytosol
matrix
Gly177Ala