CHILDREN'S RESEARCH CENTER (CRC)

INVEGEM

Feasibility of Rapid Second-Tier Molecular Testing for CAH-Screening by International Cooperation

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Steroidogenic Pathway

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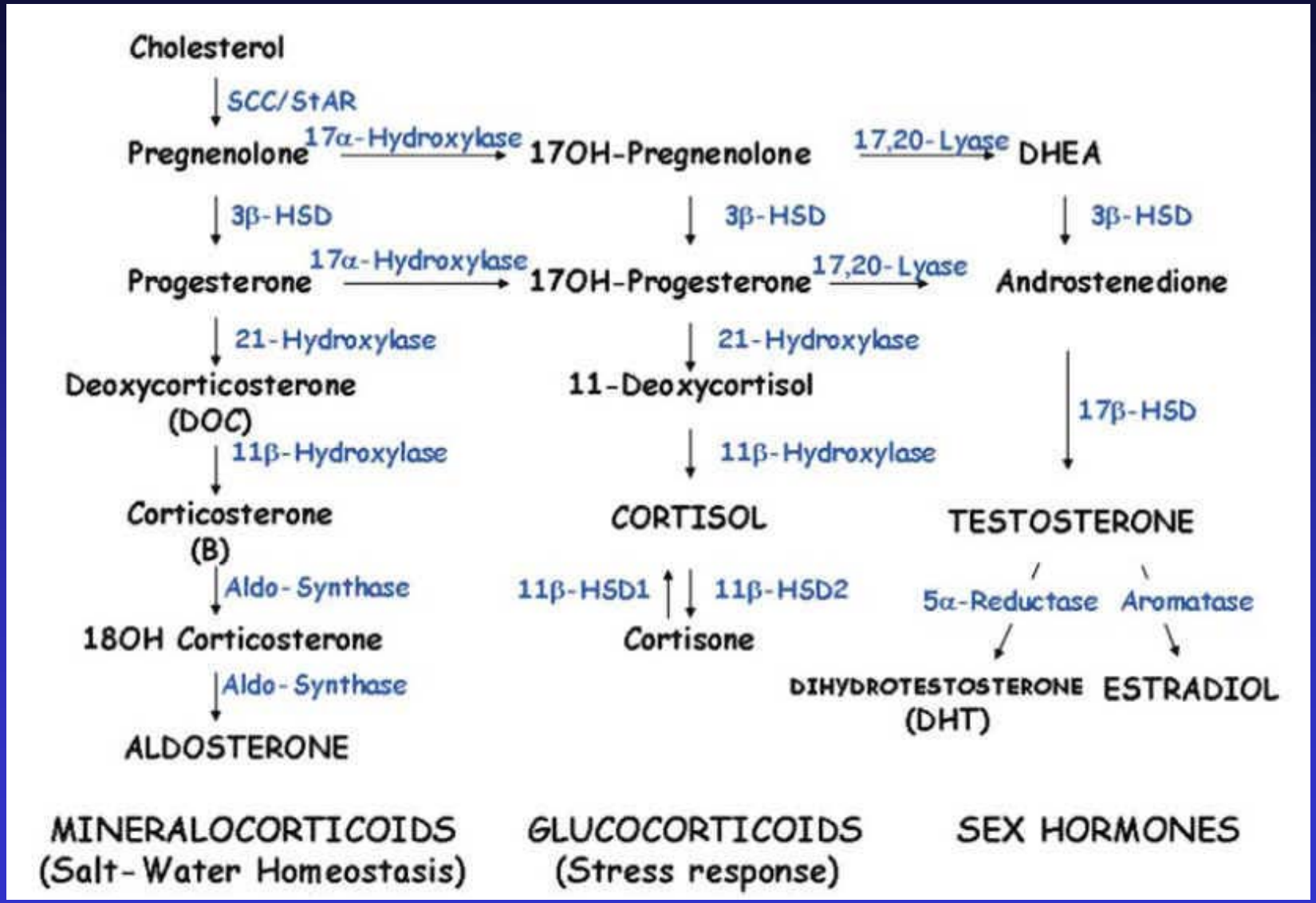

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Case History 1

- L.P., a girl 6 weeks of age
- ambigous genitale (Prader I-II)
- Cortisol, LH, FSH, and Testosterone normal
- TSH 4.513 mU/L (normal 0.490-4.670)
- T4 0.79 mg/dL (normal 0.70-1.85)
- T3 152.70 mg/dL (normal 0.79-149)
- ACTH a.m. 163.00 pg/mL (normal 0.05-46).
- Pelvic USG: Ovaries and womb normal for age
- Normal karyotype (46 XX) for female sex.
- Sodium: 139 mmol/L (normal 135-146),
- Potassium: 4.0 mmol/L (normal 3.6-4.8)

Guthrie cards for selective screening were investigated in Zurich.

- 52 d of life: 1st dried blood sample (DBS) taken
- 67 d of life: 17-OHP 198 nmol/L (strongly elevated)
- 68 d of life: Permission for mutation analysis
- 70 d of life: Mutation analysis of CYP21: (I2G/I2G) or (I2G/del)
- 85 d of life: 2nd DBS from the patient and 1st DBS from the parents
- 97 d of life: final confirmation of the diagnosis 17-OHP of the patient 75 nmol/L (still strongly elevated)

Both parents heterozygous for I2G and normal 17-OHP (0.9 & 1.5 nmol/L)

After thorough genetic counseling, the parents unfortunately moved to another town and could not be contacted anymore.

Case History 2

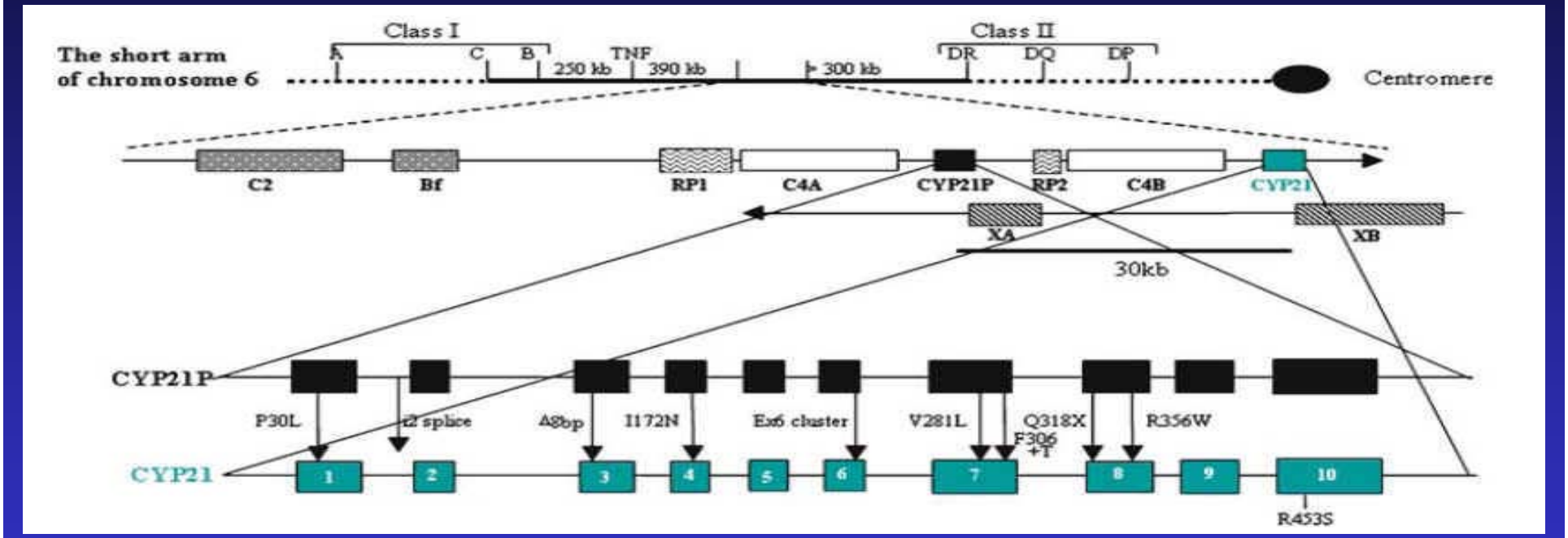
- boy, 4 weeks of age
- GA: 34 weeks
- bw: 3 kg
- at 16 days: vomiting, poor feeding, hiccup
- at 18 days: abnormal neurological features, hypotonic, decreased neurological reflexes
- Routine lab normal
- Sodium: 120 mmol/L (normal 135-146),
- Potassium: 5.5 mmol/L (normal 3.5-4.5)

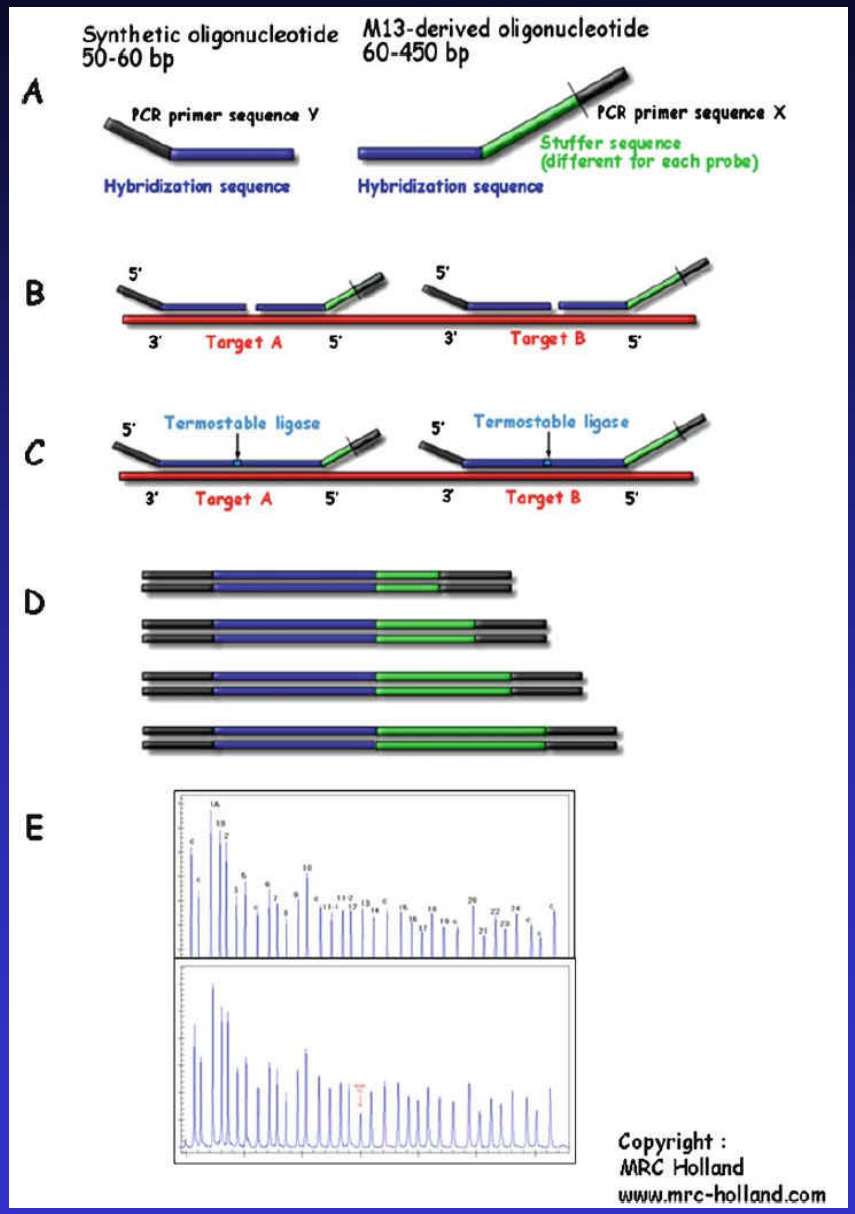
Guthrie cards for selective screening were investigated in Zurich.

- 18 d of life: 1st dried blood sample (DBS) taken
  start of treatment
- 29 d of life: 17-OHP 9.2 nmol/L
  (slightly elevated)
- 43 d of life: 2nd DBS from the patient
  17-OHP 13 nmol/L
  and 1st DBS from the mother
  father was not available
  Permission for mutation analysis
- 50 d of life: Mutation analysis of CYP21:
  (I2G/Q318X)
  final confirmation of the diagnosis

Mother heterozygous for Q318X

Up to now:
Normal neurological development
Normal growth


The short arm of chromosome 6
Class I
A
C
B
TNF
250 kb
390 kb
300 kb
Class II
DR
DQ
DP
Centromere
C2
Bf
RP1
C4A
CYP21P
RP2
C4B
CYP21
XA
XB
30kb
CYP21P
P30L
i2 splice
8bp
I172N
Ex6 cluster
V281L
Q318X
F306 +T
R356W
CYP21
1
2
3
4
5
6
7
8
9
10
R453S



Multiple ligase-dependent amplification: general scheme of method (copyright MRC Holland, from Schouten et al. 2002; www.mrc-holland.com).

(A) MLPA probes.

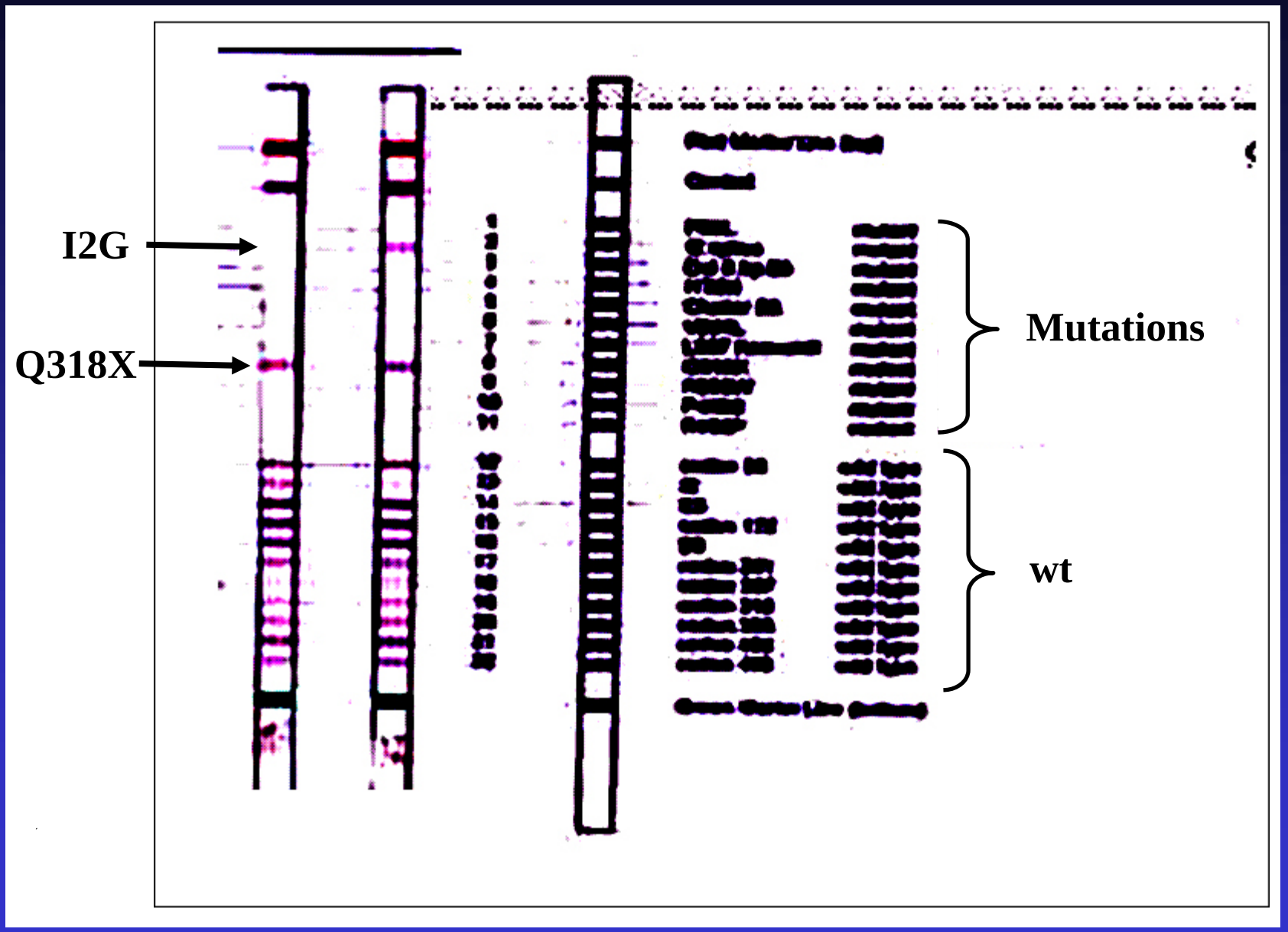
(B) The MLPA probe mix is added to denatured genomic DNA. The two parts of each probe hybridize to adjacent target sequences.

(C) Probes are ligated by a thermostable ligase.

(D) A universal primer pair is used to amplify all ligated probes. The amplification product of each probe has a unique length.

(E) Separation and quantification by capillary electrophoresis. Each peak is the amplification product of a specific probe. Samples are compared with a control sample. A difference in relative peak height or peak area indicates a copy number change of the probe target sequence (1).

Lit.: Torresani T, Lauber A. *Congenital adrenal hyperplasia: diagnostic advances.* J Inherit Metab Dis. 2007;30:563-75.


I2G
Q318X
Mutations
wt

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Conclusion

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- Confirmation of positive screening results for CAH is possible, even if samples have to shiped by courier

- If samples are sent by express courier, results are available within 3-4 d

- The success of the introduction of Newborn Screening in developing countries, is mainly depending on the ability of the screening program, and the treating pediatricians, to keep track of the patients and their families, and the compliance of the patients`parents.