



***Molecular Analysis of
Chronic Granulomatous
Disease (CGD).
Applications for a rapid
method of diagnosis using
DHPLC***

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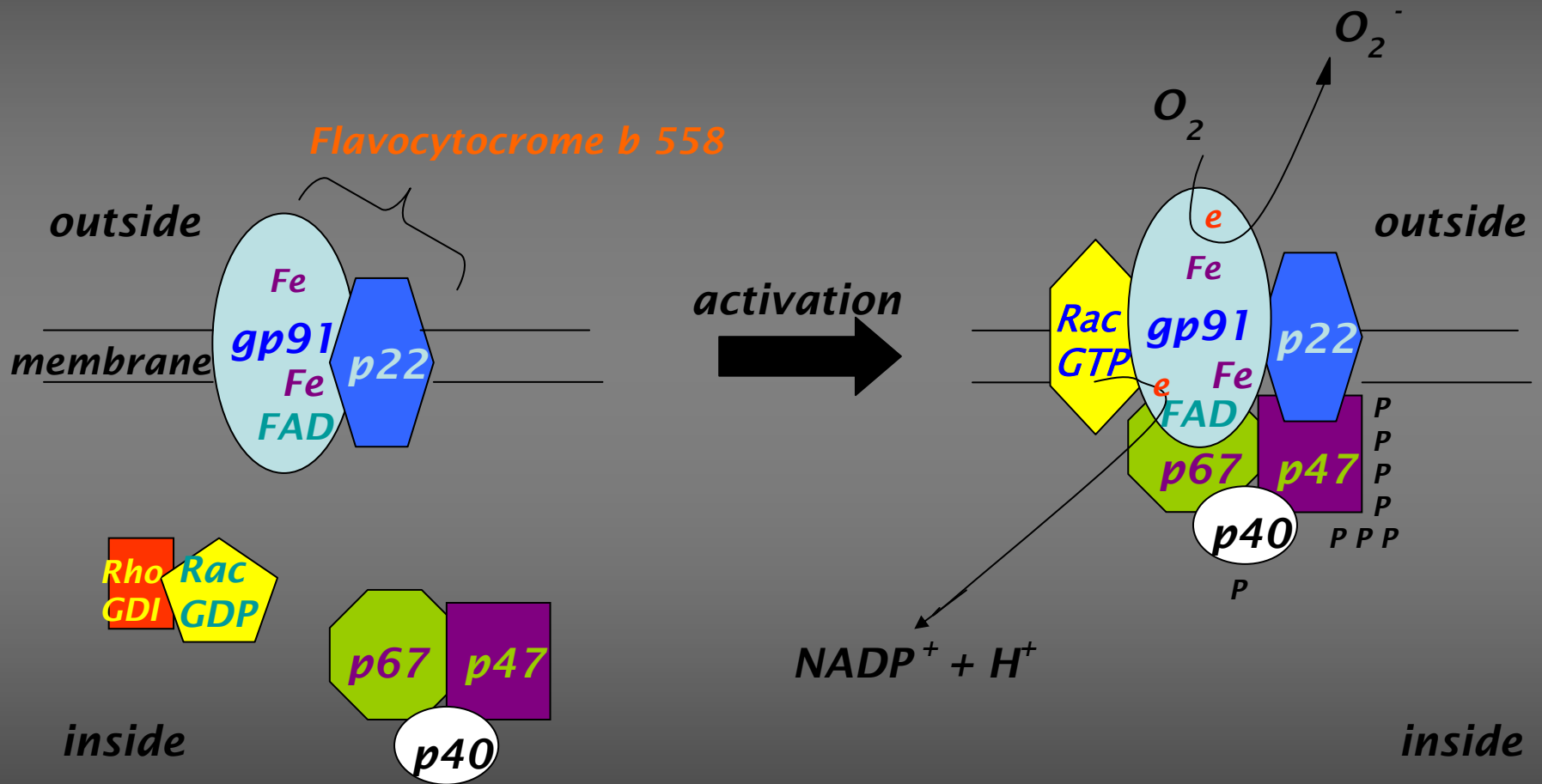
Prof. Paolo Rossi

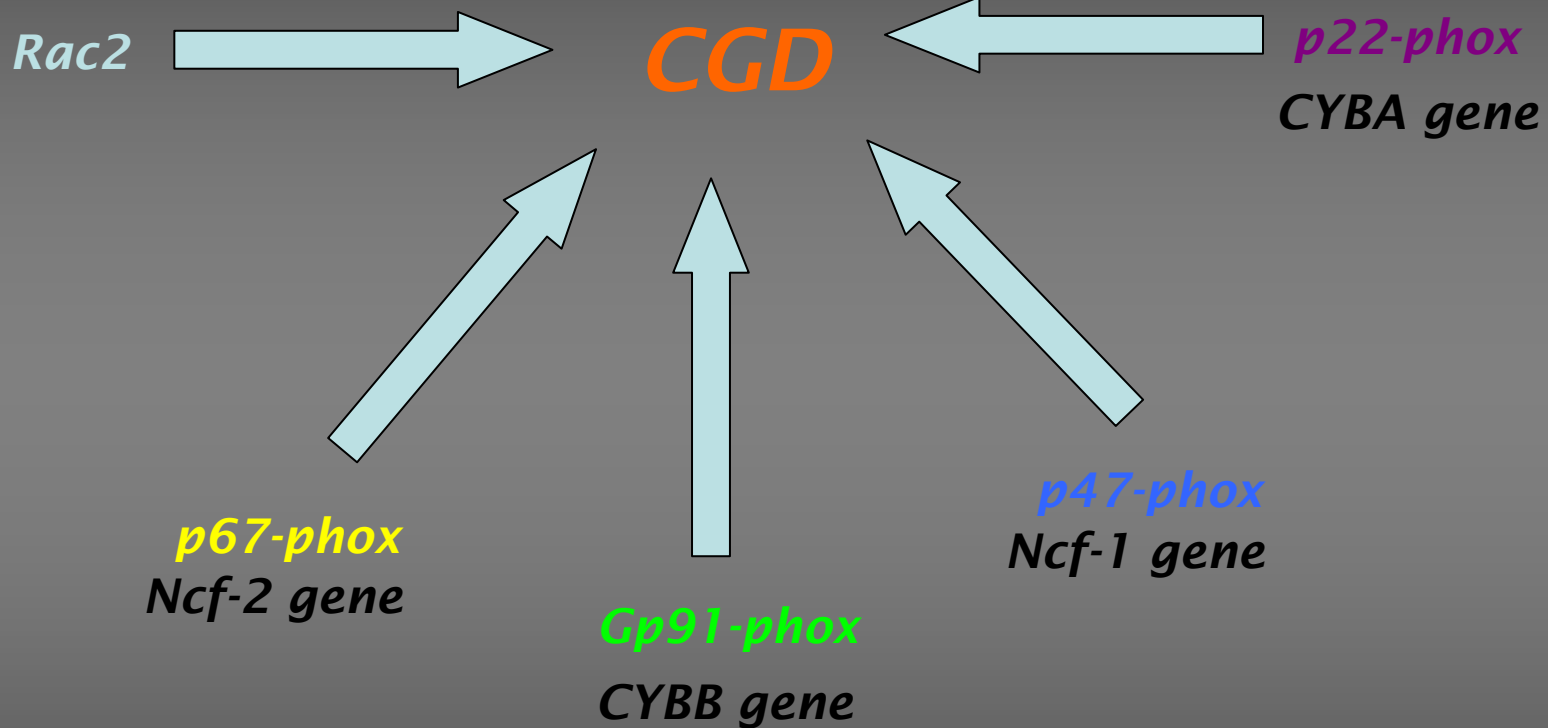
Chronic Granulomatous Disease (CGD) is an immunodeficiency that affects phagocytes of the innate immune system and is characterized by a greatly increased susceptibility to severe bacterial and fungal infections of the subcutaneous tissues, lungs, lymphonodes, liver and bones.

Patients with Chronic Granulomatous Disease are defective in the enzymatic system of the phagocytes known as NADPH oxidase that acts mainly by transferring electrons from NADPH to molecular oxygen in order to form superoxide.

The main goal is to kill ingered microorganisms in particular catalase-positive.

MODEL OF PHAGOCYTE NADPH OXIDASE (RESPIRATORY BURST) ACTIVATION



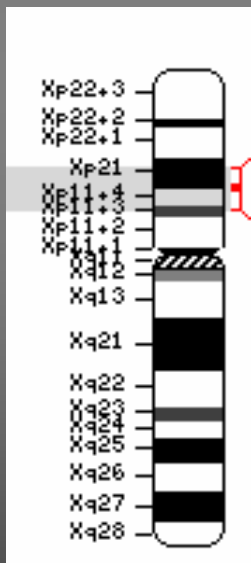


Classification

| <i>Type</i> | <i>GENE</i> | <i>PROTEIN</i> | <i>INHERITANCE</i> | <i>FREQUENCY</i> |
|-----------------|-------------|------------------|--------------------|------------------|
| <i>X91° CGD</i> | <i>CYBB</i> | <i>gp91-phox</i> | <i>XR</i> | <i>55-60%</i> |
| <i>X91- CGD</i> | | | | <i>5%</i> |
| <i>X91+ CGD</i> | | | | <i>1-3%</i> |
| <i>A22° CGD</i> | <i>CYBA</i> | <i>p22-phox</i> | <i>AR</i> | <i>~ 5%</i> |
| <i>A22+ CGD</i> | | | | <i>~ 1%</i> |
| <i>A47° CGD</i> | <i>NCF1</i> | <i>p47-phox</i> | <i>AR</i> | <i>25%</i> |
| <i>A67° CGD</i> | <i>NCF2</i> | <i>P67-phox</i> | <i>AR</i> | <i>~ 5%</i> |

95% OF CGD MUTATIONS RESULT IN A COMPLETE ABSENCE OR GREATLY DIMINISHED LEVEL OF PROTEIN

CYBB GENE and gp91 protein



MGNWAVNEGLSIFVILVWLGLNVFLFVWYRVDIPPKFFYTRKLLGSALA
 LARAPAACLNFCMLILLPVCRNLLSFLRGSSACCSTRVRRQLDRNLTFH KM NTERM
 VAWMIALHSAIHTIAHLFNVWCVNARVNNSDPYSVAL SELGDRQNESYL
 NFARKRIKNPEGGLYLAVTLLAGITGVVITLCLILIITSSTKTIRRSYFEVFWYT
 HHLFVIFFIGLAIHGAERIVRGQ TAESLAVHNITVCEQKISEW GKIKECPIQ F
 AGNPPMTWKWIVGPMFLYLCERLVRFWRSQKVVITKVVTHPFKTIELQMK FADBD
 KKGPKMEVGGQYIFVKCPKYSKLEWHPFTLTSAPEDFFSIHRIVGDWTEGLFN
 ACGCDKQFPQDAWKLPKIAVDGPFGTASEDVESYEVMMLVGAGIGVTPFASIL NADPHBD
 KSVWYKYCNNATNLKLLKKIYFYWLCRDTHAFEFWADLLQLLESQMQRNNA
 GFLSYNIYLTGWDESQANHFAVHHDEEKDVITGLKQKTLYGRPNWDNEFKTI L
 ASQHPNTRIGVFLCGPEALAEATLSKQSI SNSESGPRGVHFIENKENE NADPHBC

METHODS

➤ *DHPLC (Denaturing High Performance Liquid Chromatography)*

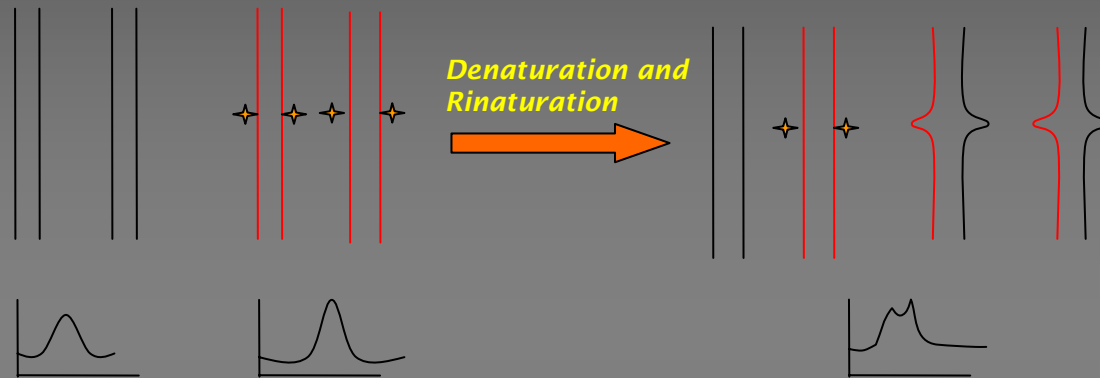
➤ *SEQUENCING*

DHPLC **(Denaturing High Performance Liquid Chromatography)**

DNA is allowed to bind a hydrophobic column in a buffer of triethyl ammonium acetate and is eluted with an increasing gradient of acetonitrile, under certain key parameters of temperature and buffer concentration, partial denaturation of the double stranded DNA occurs.

IF THE SAMPLE CONTAINS HETERODUPLEX MOLECULES (PRESENCE OF MUTATIONS OR POLYMORPHISMS), THESE WILL BE VISUALIZED AS A PEAK OR PEAKS WITH SHORTER RETENTION TIMES THAN HOMODUPLEXES.

DETECTION OF MUTATION BY DHPLC



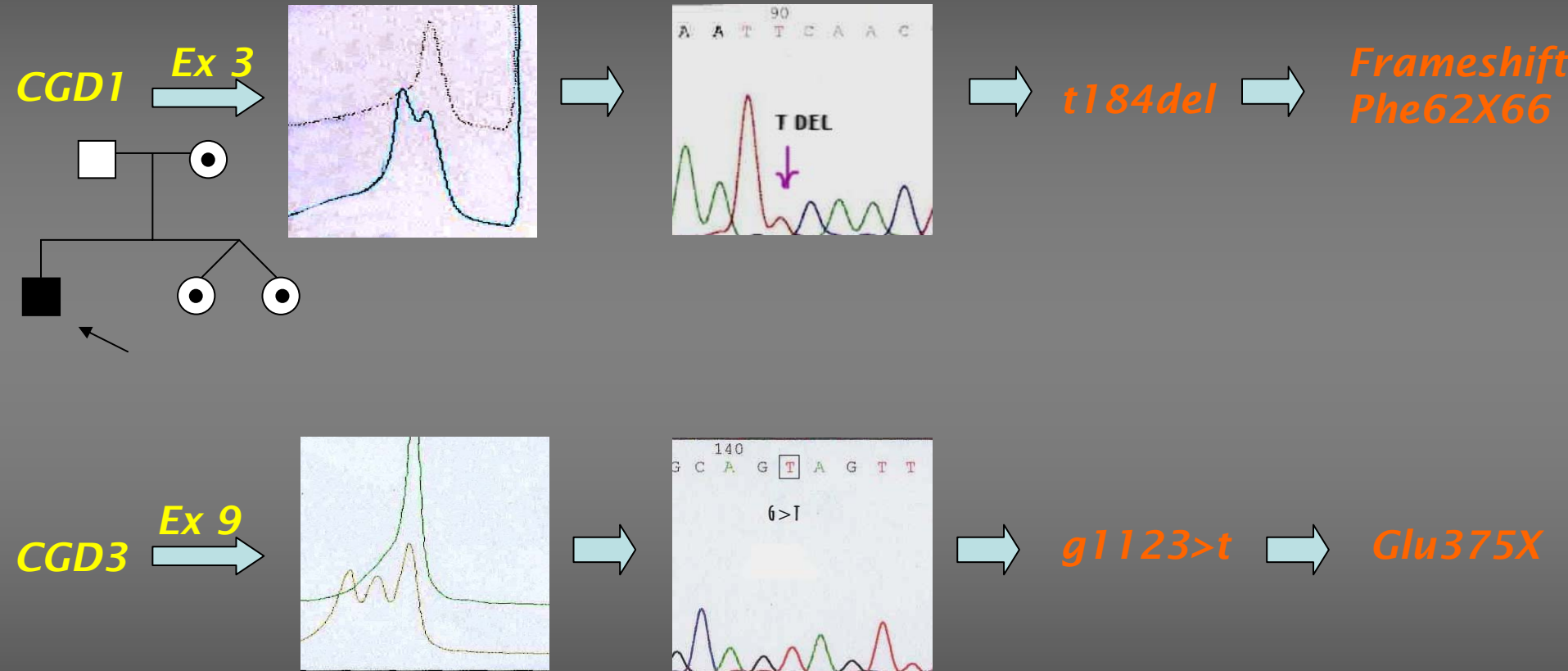
PROTOCOL:

- ◆ **AMPLIFICATION OF SAMPLES AND CONTROLS.**
- ◆ **DENATURATION AND RENATURATION OF PCR PRODUCTS MIXED IN EQUIMOLAR QUANTITY OF SAMPLE AND CONTROL (FORMATION OF HETERODUPLEX AND HOMODUPLEX MOLECULES)**
- ◆ **CHROMATOGRAPHIC ANALYSIS AT DIFFERENT CONDITIONS OF TEMPERATURE AND BUFFER CONCENTRATION.**

CGD PATIENTS

| <i>PATIENT</i> | <i>BIRTH</i> | <i>AGE OF DIAGNOSIS</i> | <i>MOLECULAR DIAGNOSIS</i> |
|----------------|--------------|-------------------------|----------------------------|
| <i>CGD1</i> | <i>1970</i> | <i>5</i> | <i>2001</i> |
| <i>CGD2</i> | <i>1998</i> | <i>2</i> | <i>IN PROGRESS</i> |
| <i>CGD3</i> | <i>1999</i> | <i>3</i> | <i>2002</i> |
| <i>CGD4</i> | <i>1966</i> | <i>38</i> | <i>2002</i> |
| <i>CGD5</i> | <i>1979</i> | <i>10</i> | <i>2003</i> |
| <i>CGD6</i> | <i>1977</i> | <i>?</i> | <i>2004</i> |
| <i>CGD7</i> | <i>2001</i> | <i>3</i> | <i>2004</i> |
| <i>CGD8</i> | <i>1998</i> | <i>6</i> | <i>2004</i> |
| <i>CGD9</i> | <i>1996</i> | <i>2</i> | <i>2004</i> |
| <i>CGD10</i> | <i>1956</i> | <i>49</i> | <i>2005</i> |

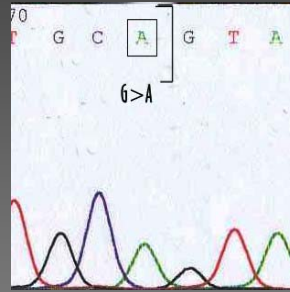
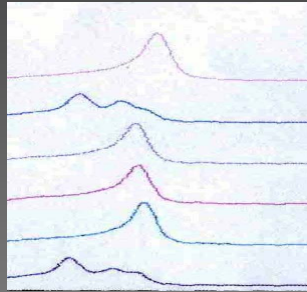
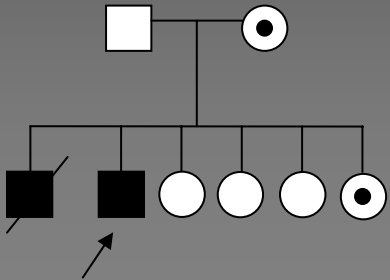
MOLECULAR ANALYSIS



CGD10

CGD4

Ex 3



g252>a

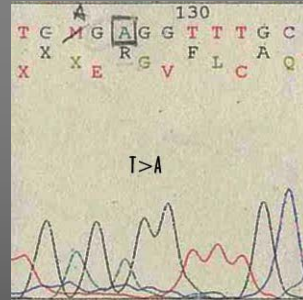
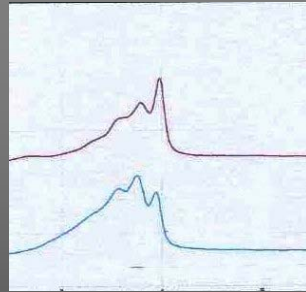
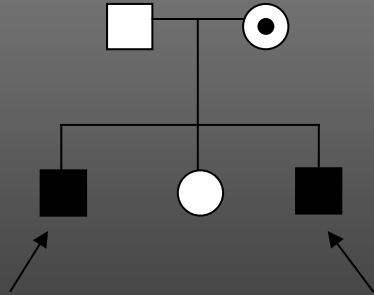


Splicing defect

Ishibashi et al. Blood 2001

CGD7-8

Ex 11



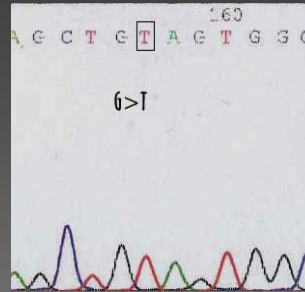
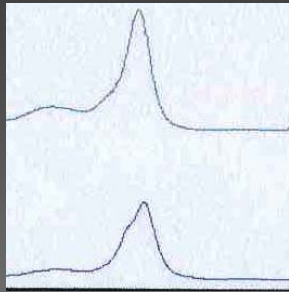
t1357>a



Trp453>Arg

CGD5

Ex 9



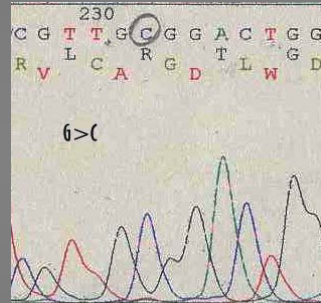
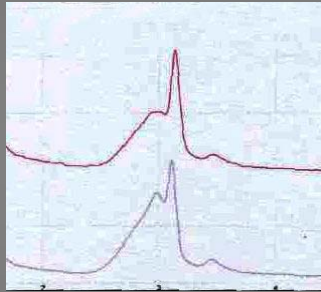
g1006>t



Glu336X

CGD9

Ex 9

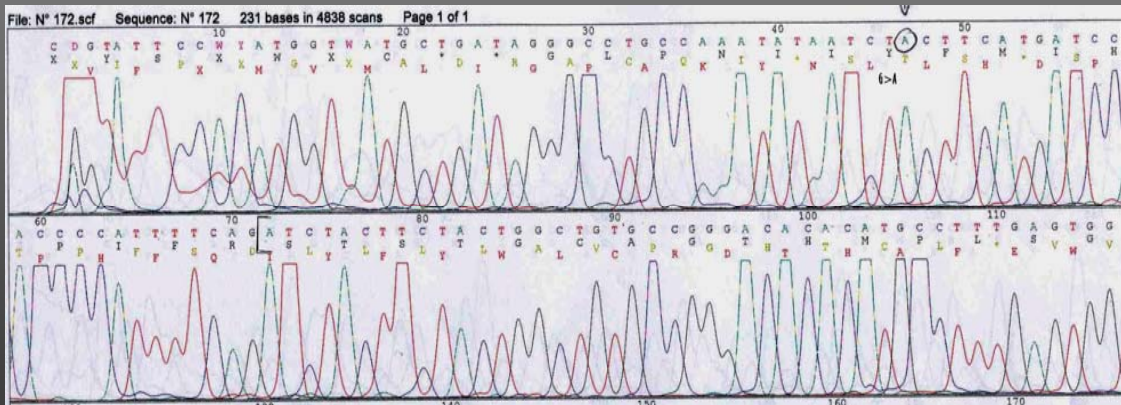


g1076>c



Gly359Ala

Ex 11



MGNWAVNEGLSIFVILVWLGLNVFLFVWYYRVYDIPPKFFYTRKLLGSALA

LARAPAACLN**F**NCMLILLPVCRNLLSFLRGSS**A**CCSTRVRRQLDRNLTFH KM

VAWMIALHSAIHTIAHLFNVEWCVNARVNNSDPYSVAL SELGDRQNESYL

NTERM

NFARKRIKNPEGGLYLA VTLLAGITGVVITLCLILIITSSTKTIRRSYFEVFWYT

HHLFVIFFIGLAIHGAERIVRGQ TAESLAVHNITVCEQKISEW GKIKECPIPQ F

AGNPPMTWKWIVGPM**F**LYLCERLVRFWRSQKVVITKVVTHPFKTIELQMK

FADBD

KKGFKMEVGQYIFVKCPKVS**K**LEWHPFTLTSAP**E**EDFFSIHIRIV**G**DWTEGLFN

ACGCDKQ**F**QDAWKLPKIAVDGPF**G**TASED**V**FSYEVVMLVGAGIGVTPFASIL

NADPHBD

KSVWYKYCNNATNLK**L**KKIYFYWLCRDTHAF**E**WFADLLQLLESQM**Q**ERNNA

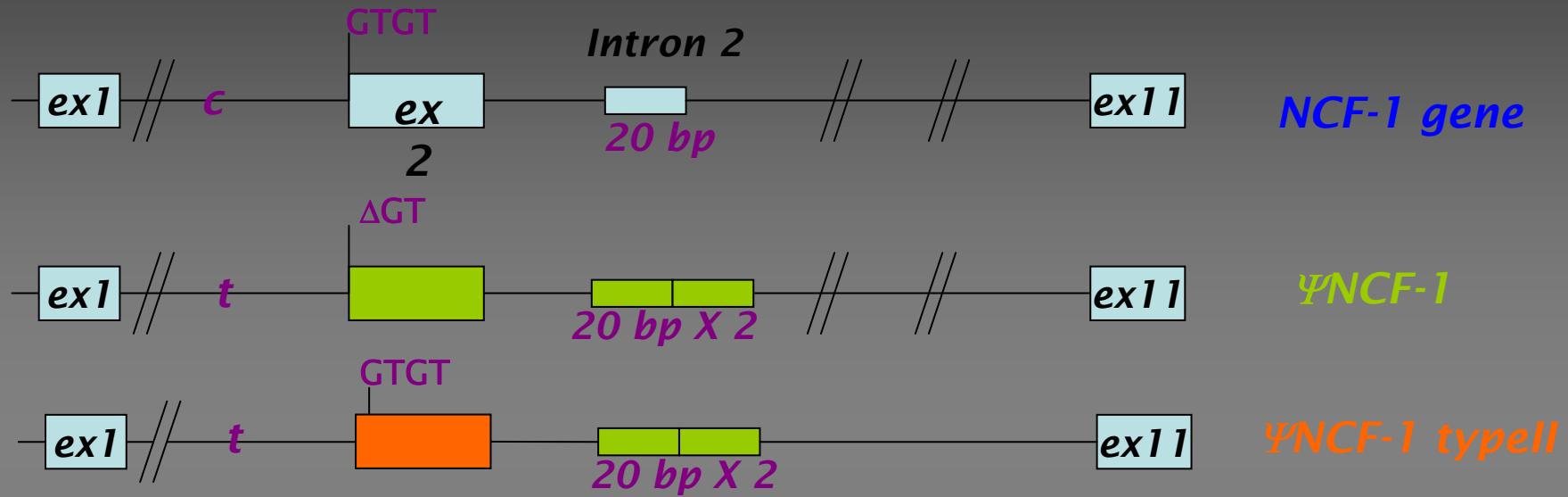
GFLSYNIYLTGW**D**ESQANHF**A**VHHDEEKDVIT**G**LKQKTLYGRPNWDNEFKTI

L

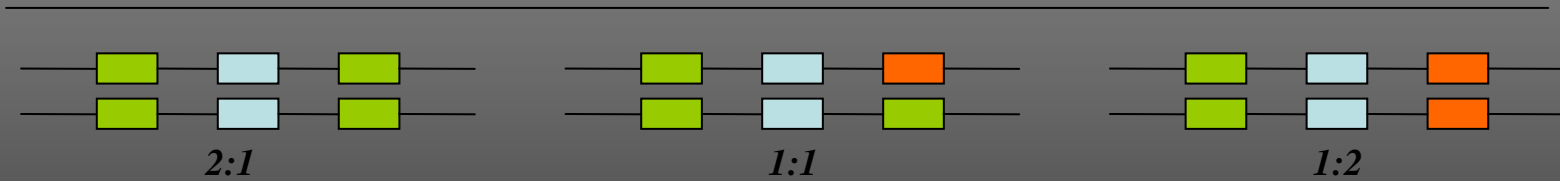
ASQHPNTRIGVFLCGPEAL**A**ETLSKQSI**S**NSESGPRGVHFIFNK**E**NF

NADPHBC

NCF-1 GENE (CHROMOSOME 7q11.23)

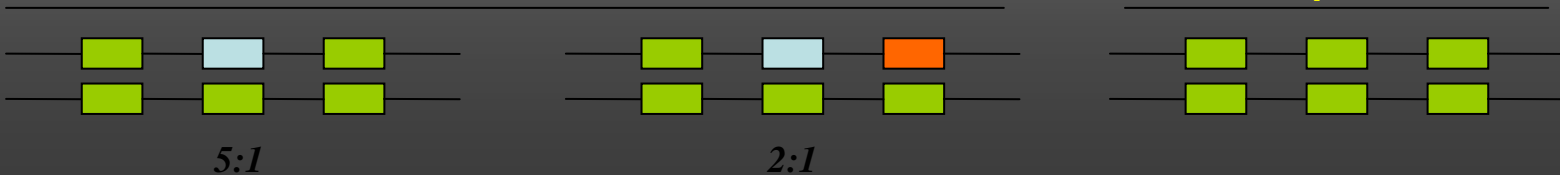


Unaffected controls (non-CGD)



A47° CGD carriers

A47° patient

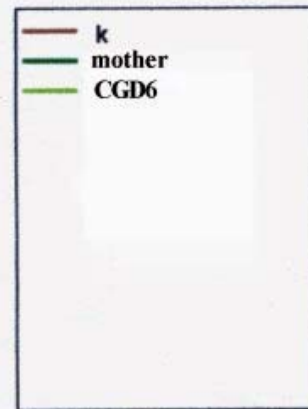
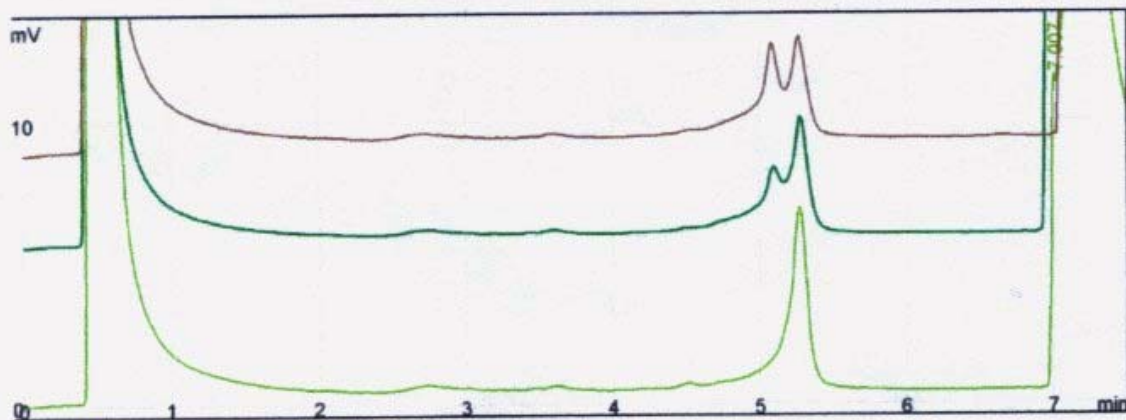


CGD6 PATIENT

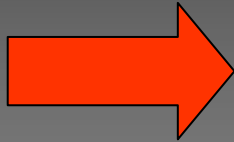
ncf-1 ex2 60.6

CONSOLIDATED ANALYSIS REPORT

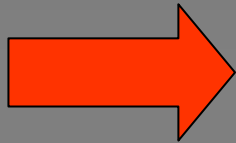
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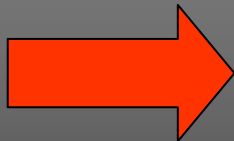
CONCLUSIONS



*ADVANCES IN PRENATAL
DIAGNOSIS AND GENETIC
COUNSELLING*



*ANALYSIS OF THE OTHER
COMPONENTS OF THE NADPH
OXIDASE SYSTEM*



*STUDIES OF GENOTYPE-PHENOTYPE
CORRELATION*