Clinical and Molecular Genetic Spectrum of Slovenian Patients with CGD

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Slovenia

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Chronic Granulomatous Disease

Heyworth PG et al. *Curr Opin Immunol* 2003;15:578-84

- Primary immunodeficiency due to absent or decreased NADPH oxidase activity in phagocytic cells
- The majority of CGD patients suffer from severe recurrent infections
  - Staphylococcus aureus
  - Aspergillus spp.
  - G-negative enteric bacteria
  - Serratia marcescens
- Diffuse granulomas (presumably caused by microbes)
  - obstructive or painful lesions

Chronic Granulomatous Disease

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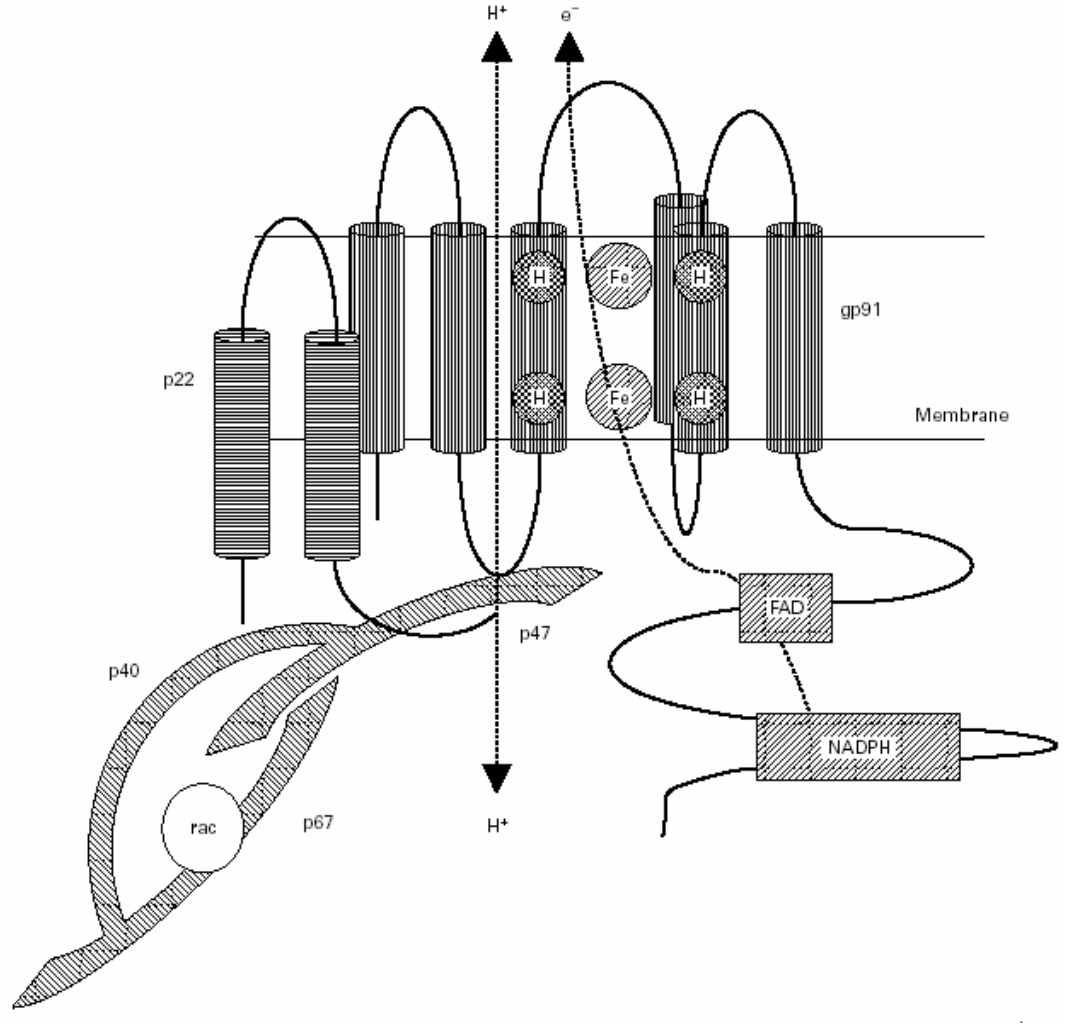
Mutation analysis

- X-linked recessive CGD (65%)
  - CYBB gene on chromosome Xp21.1 ($gp91^{phox}$)
- Autosomal recessive CGD (35%)
  - NCF1 gene on chromosome 7q11.23 ($p47^{phox}$)
  - NCF2 gene on chromosome 1q25, coding for $p67^{phox}$)
  - CYBA gene on chromosome 16q24, coding for $p22^{phox}$)

Active NADPH-oxidase complex

Goldblatt D et al. *Clin Exp Immunol* 2000;122:1-9.

- **Multisubunit enzyme system:**
  - **gp91^{phox}**
  - **p22^{phox}**
  - **p47^{phox}**
  - **p67^{phox}**
  - **Rac2**
- **Catalyzes electron transport from NADPH to molecular oxygen and generation of O_2^-**



Study objectives

- To describe clinical and molecular characteristics of Slovenian patients with CGD
- To examine the relationship of clinical presentation with the genotype

Patients

- Clinical data and laboratory values retrospectively collected from the medical records at the University Children's Hospital Ljubljana between Oct. 1986 – Dec. 2005

- 9 male patients from 7 unrelated families identified
  - mean age at analysis 17.4 yrs (range 4.9 to 27 yrs)
  - 2 patients died at the age of 9.2 and 18.9 yrs, respectively
  - 2 patients underwent BMT

Methods

- Genomic DNA isolated from whole blood stored in EDTA
- PCR amplification of all exons and the exon-intron boundaries of the CYBB gene
- Direct sequencing using the Big Dye terminator cycle sequencing kit and ABI PRISM 310 automated sequencer (PE Applied Biosystems, Norwalk, USA)
- Novel mutations identified and named starting numbering from AUG codon (Gene Bank Access No. AF469757)

Clinical manifestations of Slovenian patients with CGD

Chronic conditions and infections	% of cases
Pneumonia	88
Lymphadenitis	75
Cutaneous infections	75
Osteomyelitis	38
Septicemia	38
Hepatic abscess	25
Pylorostenosis	25
Splenic abscess	12
Uretral stenosis	12
Pericarditis	12
Ileocolitis	12

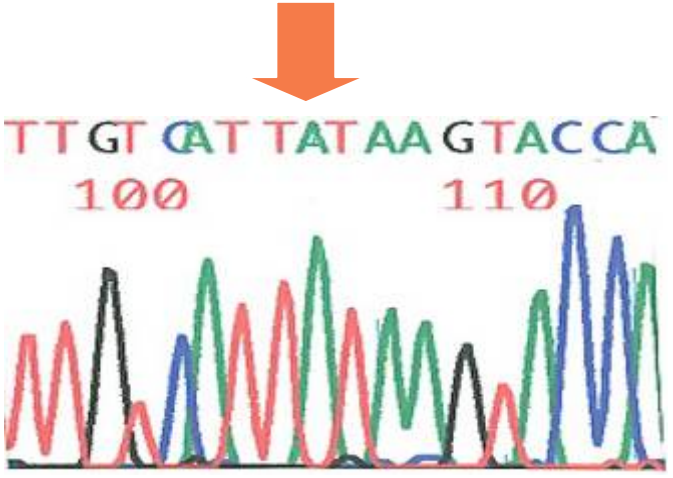
The relative frequencies of infecting organisms

Infecting organisms	Relative frequency (%)
Aspergillus sp.	86
Staphylococcus aureus	57
Salmonella sp.	57
Candida albicans	43
Streptococcus sp.	43
Serratia marcescens	14
Burkholderia cepacia	14
Proteus sp.	14
Pneumocystis carinii	14
Haemophilus influenzae	14

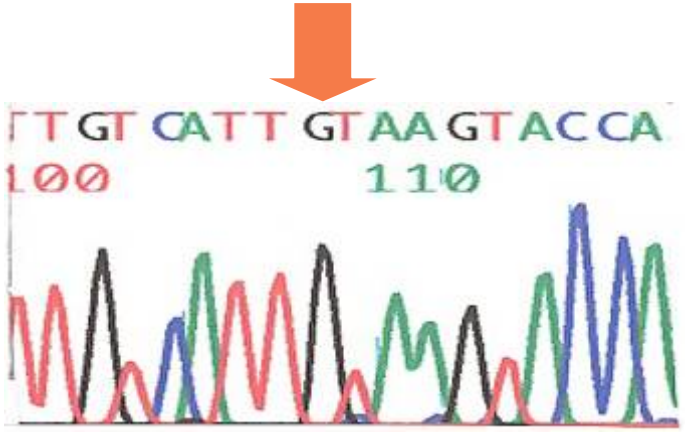
Mutations detected in the CYBB gene

Patient	Age (yrs)	Exon	Nucleotide change	Mutation type	Protein change
1	13.5	1	ds+1 G→A	Splice site	-
2	4.9	3	197 T→C	Missense	Leu66Pro
3	27	3	217 C→T	Nonsense	Arg73Stop
4*	23.4	8	882 T→C	Nonsense	Arg290Stop
5*	20.2	8	882 T→C	Nonsense	Arg290Stop
6	18.9 †	9	1038 del T	Deletion	346 fs 384Stop
7	23.1	13	1598 del GAG	Deletion	533 del Gly

Patient # 1



Patient



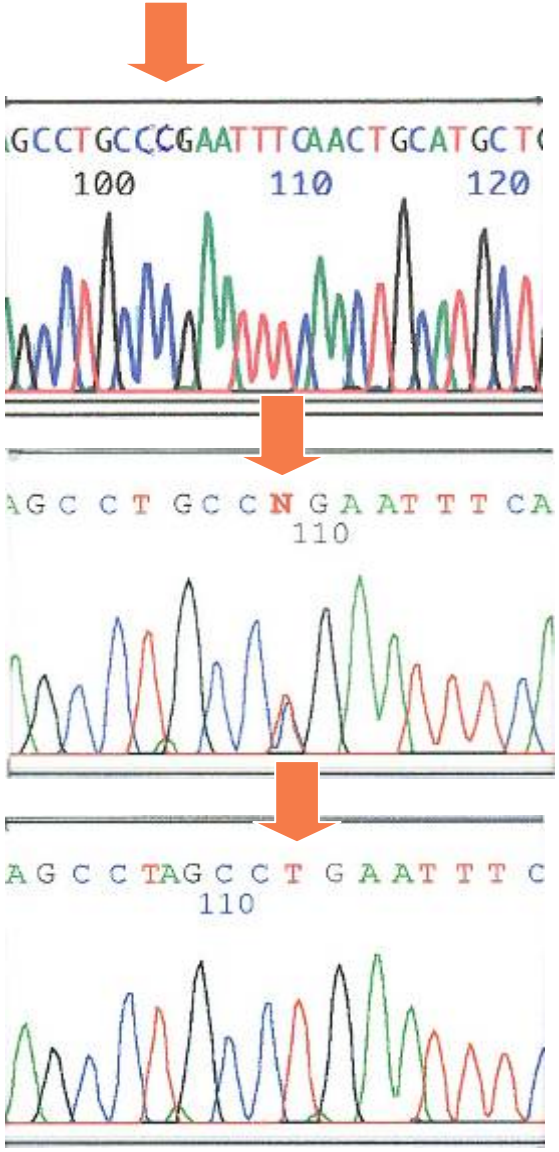
Mother

Mutation: CYBB exon 1

ex1 ds+1 G→A

De novo mutation

Patient # 2



Patient

Mother

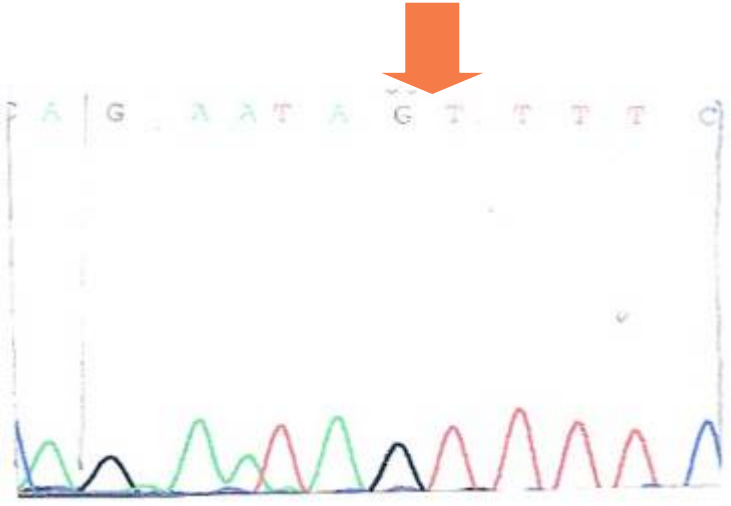
Sister, BM donor

Mutation: CYBB exon 3

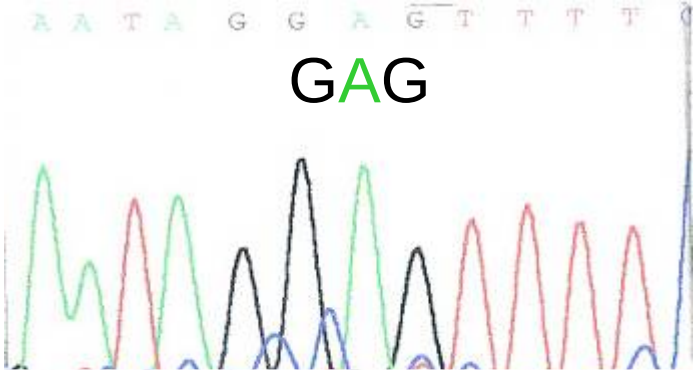
197 T→C

L66P

Patient # 7



Patient



GAG

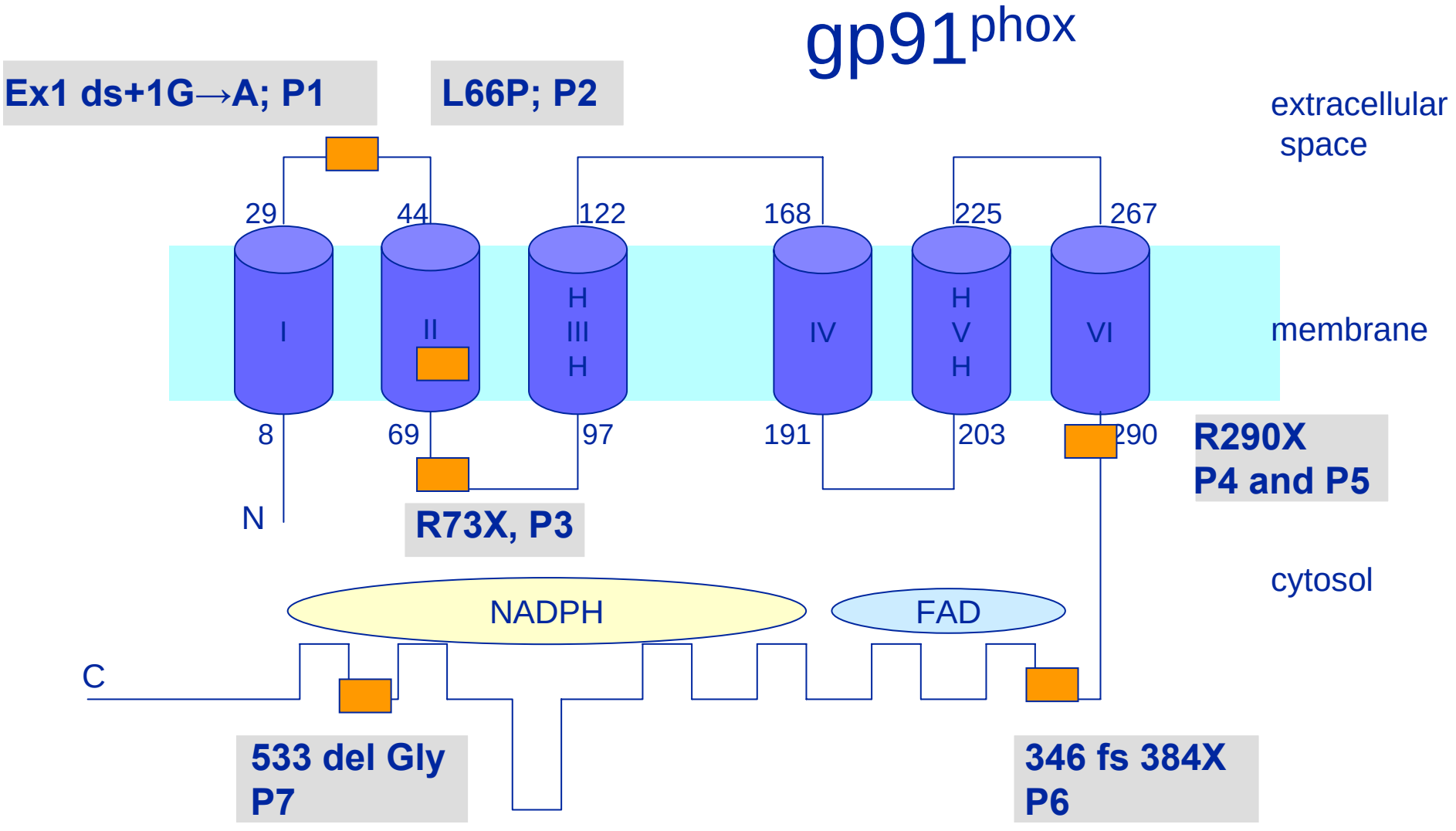
Wt

Mutation: CYBB exon 13

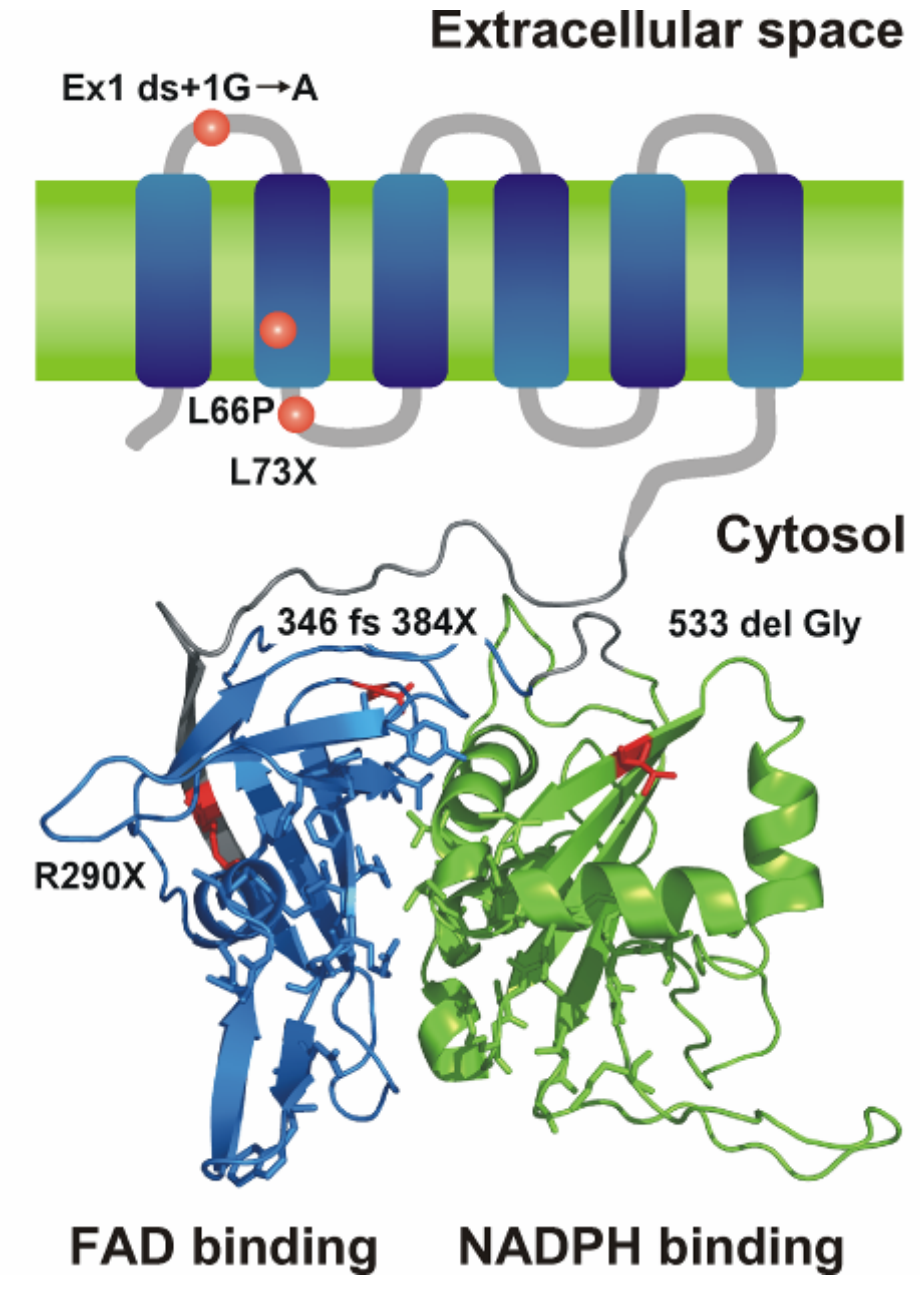
1598 del GAG

533 del Gly

De novo mutation


gp91phox
Ex1 ds+1G→A; P1
L66P; P2
extracellular space
membrane
cytosol
29
44
122
168
225
267
I
II
H III H
IV
H V H
VI
8
69
97
191
203
290
N
C
R73X, P3
R290X P4 and P5
NADPH
FAD
533 del Gly P7
346 fs 384X P6

Structural model of the mutated protein of the gp91 enzyme system



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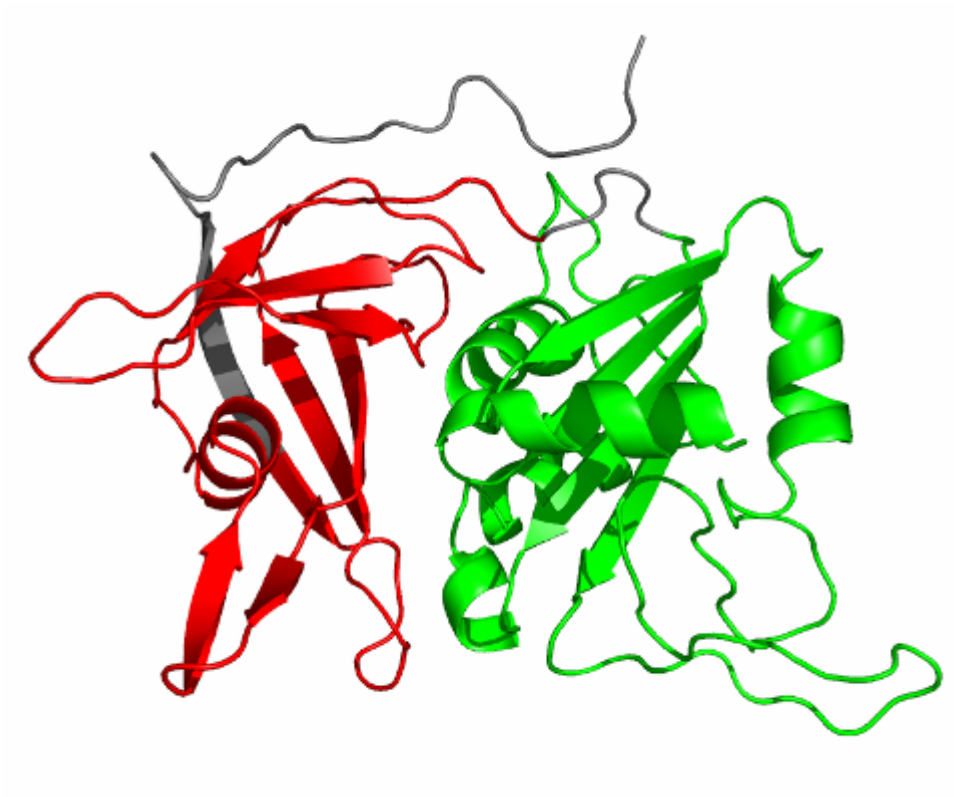

Conclusions

- The type of infections present in CGD patients from Slovenia similar to prior reports
  - in our patients higher frequency of infections with *Aspergillus sp.*
- Seven out of 9 patients (78%) had mutation in the CYBB gene
  - three novel mutations
  - two *de novo* mutations
- No correlation existed between the type of mutation and the clinical phenotype of the disease


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J Project Meeting (16-17 Nov 2007)