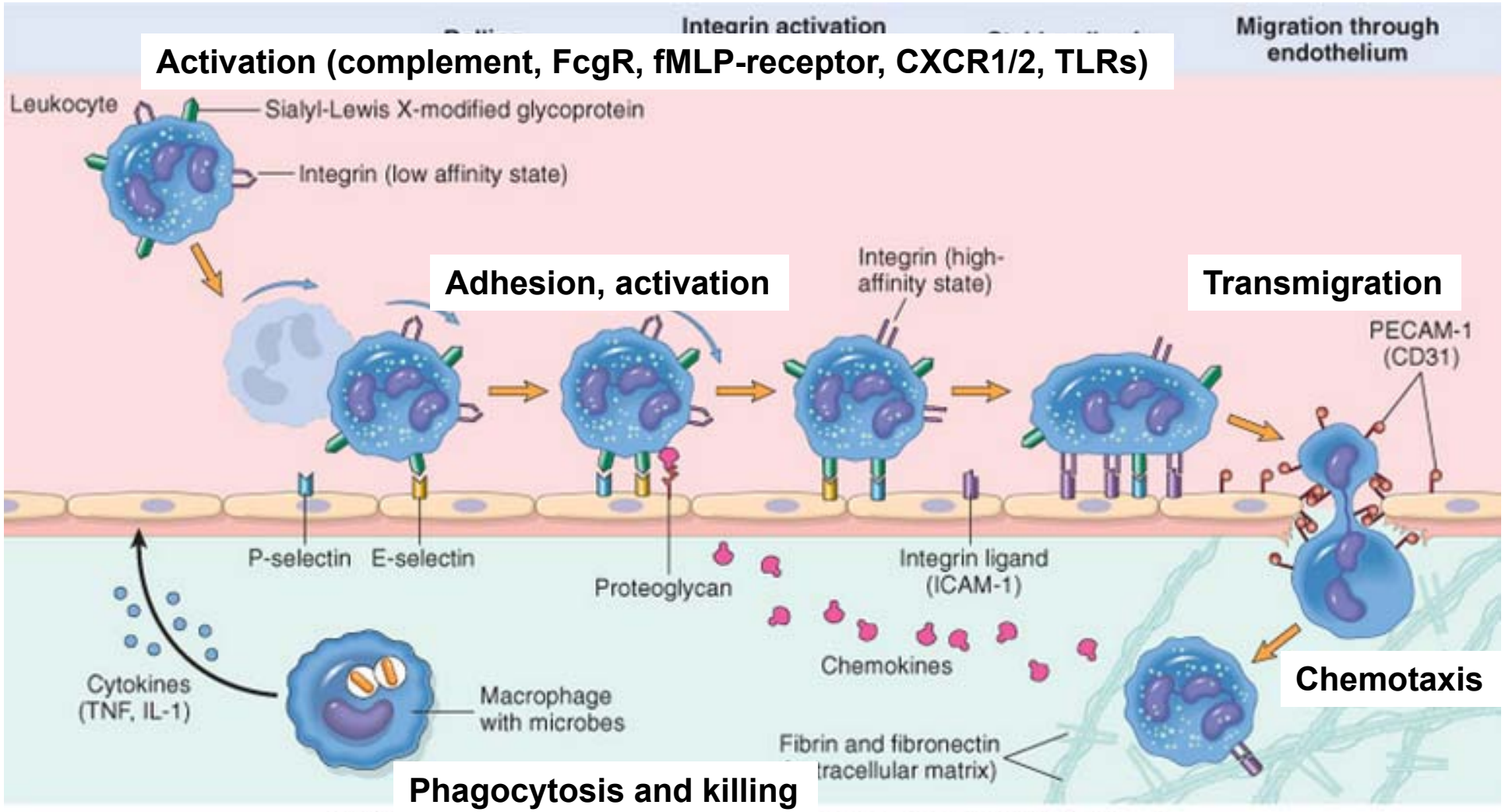
p40^{phox} deficiency

Causing CGD with autoimmunity and skin infections

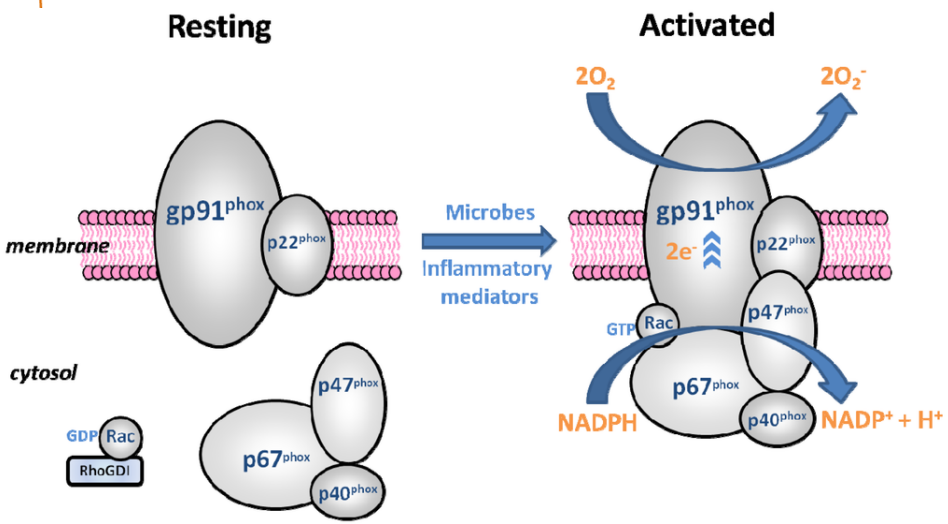
Annemarie van de Geer
MD, PhD student

Dept. of Blood Cell Research,
Sanquin Research
Amsterdam, The Netherlands

Bloed is leven



The NADPH oxidase system: ROS production



Heterodimer (cytochrome b_{558})

Heterotrimer translocating to cytochrome b_{558} upon cell activation

↓

Conformational change in gp91-phox, binding of NADPH

↓

Electron transport within gp91-phox
O_2^- production in phagosome

The NADPH oxidase system: ROS production

Reaction:

$$O_2 + NADPH \xrightarrow{\text{oxidase}} NADP^+ + H^+ + O_2^- \rightarrow H_2O_2 \rightarrow HOCl$$

Chronic Granulomatous Disease

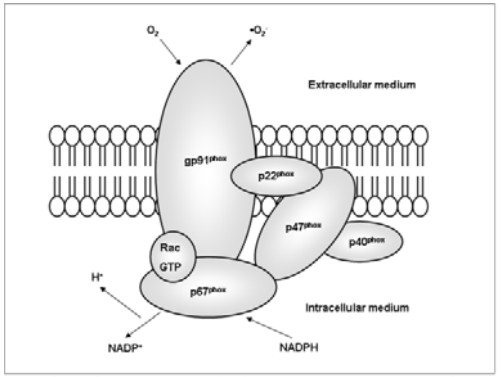


Figure 2 - Structure of phagocytic NADPH oxidase. The gp91phox is the NADPH binder with electron transport function in active NADPH oxidase. The extracellular production of $^{\bullet}O_2$ by reduction of an electron of O_2 by gp91phox, using β- reduced nicotinamide adenine dinucleotide phosphate (NADPH). Adapted from Dusting et al, 2005.

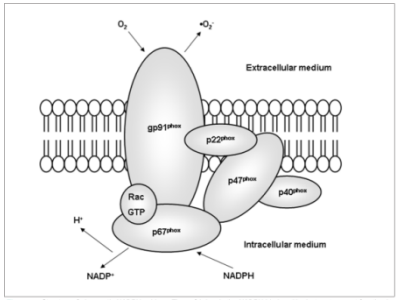
- **<u>Absent/reduced ROS production by NADPH oxidase system</u>**
  → Recurrent bacterial and fungal infections

- Mutation in genes encoding NADPH oxidase subunits

Gene	Subunit
CYBB (X-linked, 70%)	gp91-phox
CYBA (AR, 5%)	p22-phox
NCF1 (AR, 25%)	p47-phox
NCF2 (AR, 5%)	p67-phox
NCF4 (AR, ?)	p40-phox

- 1:250,000, male>female.

A novel mutation



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A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40*phox* and selective defects in neutrophil NADPH oxidase activity

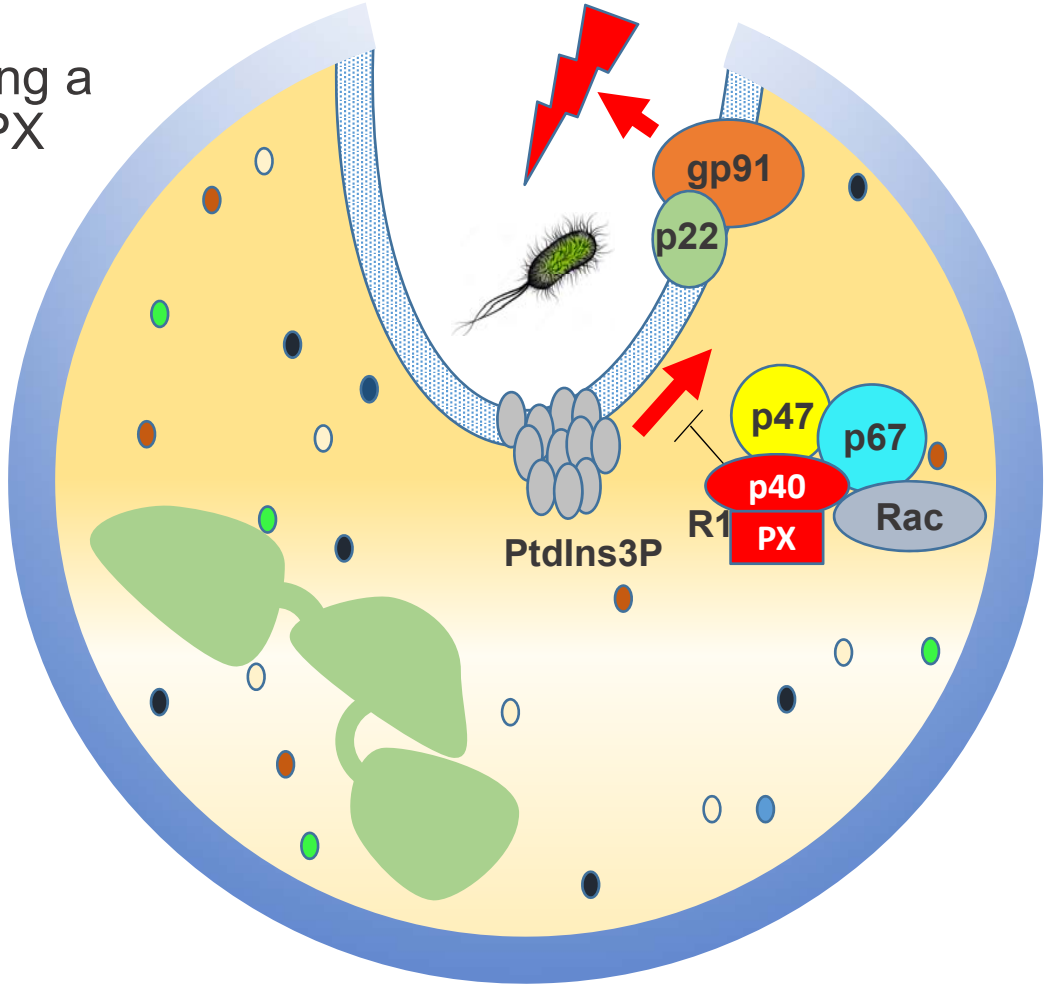
*Juan D. Matute,[1] *Andres A. Arias,[1] Nicola A. M. Wright,[2] Iwona Wrobel,[2] Christopher C. M. Waterhouse,[2] Xing Jun Li,[1] Christophe C. Marchal,[1] Natalie D. Stull,[1] David B. Lewis,[3] MacGregor Steele,[2] James D. Kellner,[2,4] Weiming Yu,[5] Samy O. Meroueh,[6] William M. Nauseef,[7] and Mary C. Dinauer[1,8]

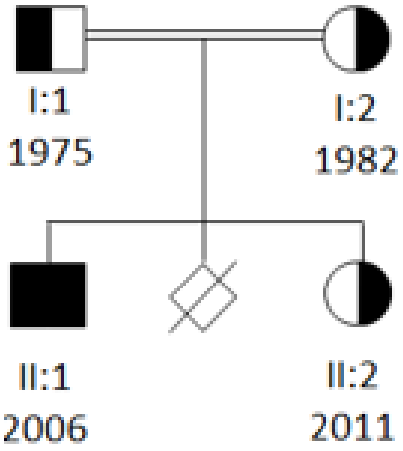
Blood. 2009 Oct 8;114(15):3309-15. doi: 10.1182/blood-2009-07-231498.

New CGD subtype?

Phagocytosis-induced ROS production

- Missense mutation predicting a R105Q substitution in the PX domain
- No binding PX domain p40*phox* to phosphatidylinositol 3-phosphate (PtdIns(3)P) on the inner membrane of the phagosome
- No phagocytosis induced superoxide production

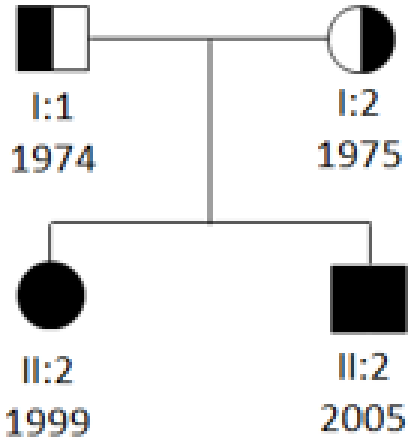




c.118-1G>A

Phenotype:

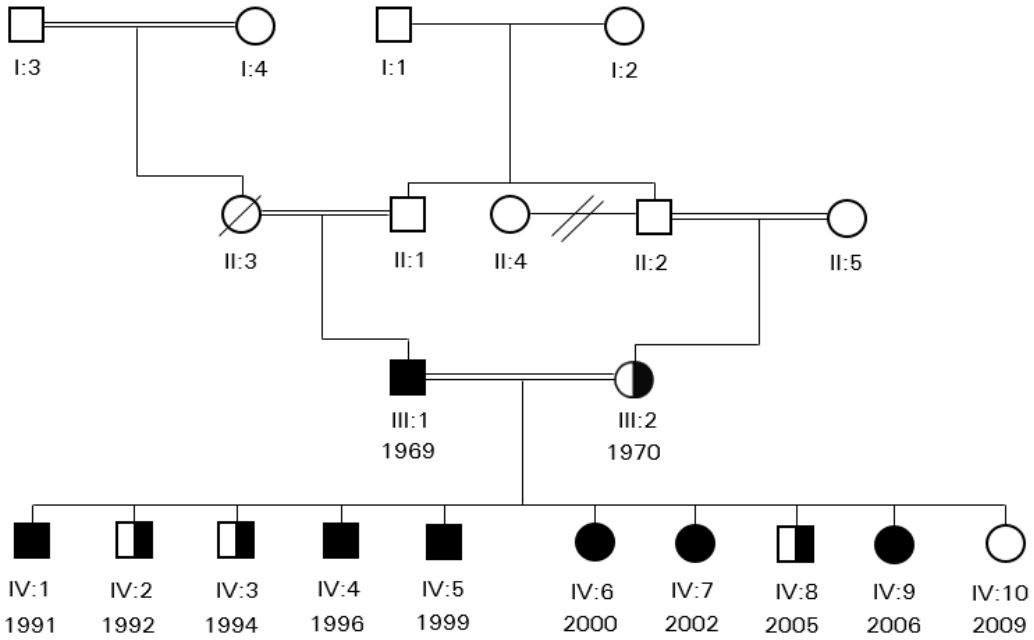
- Mouth ulcers and Crohn-like granulomata in large intestine
- Recurrent purulent lymphadenitis *(S. aureus)*
- Stable on cotrimoxazole/ itraconozal prophylaxis.



15bp deletion

Phenotype:

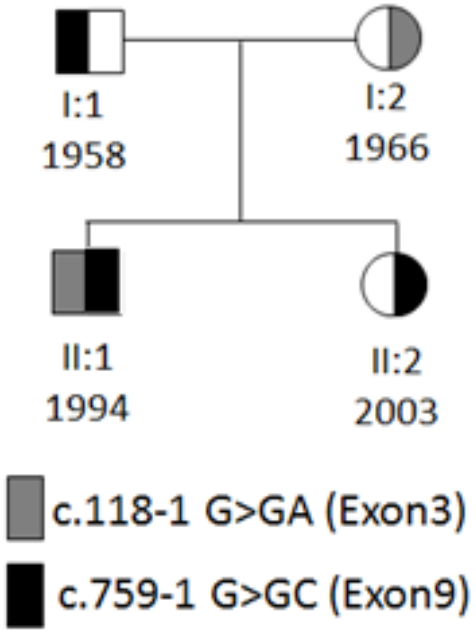
- Folliculitis
- Oral candidiasis
- Oral abscesses
- Skin lupus



c.314G>A

Phenotype:

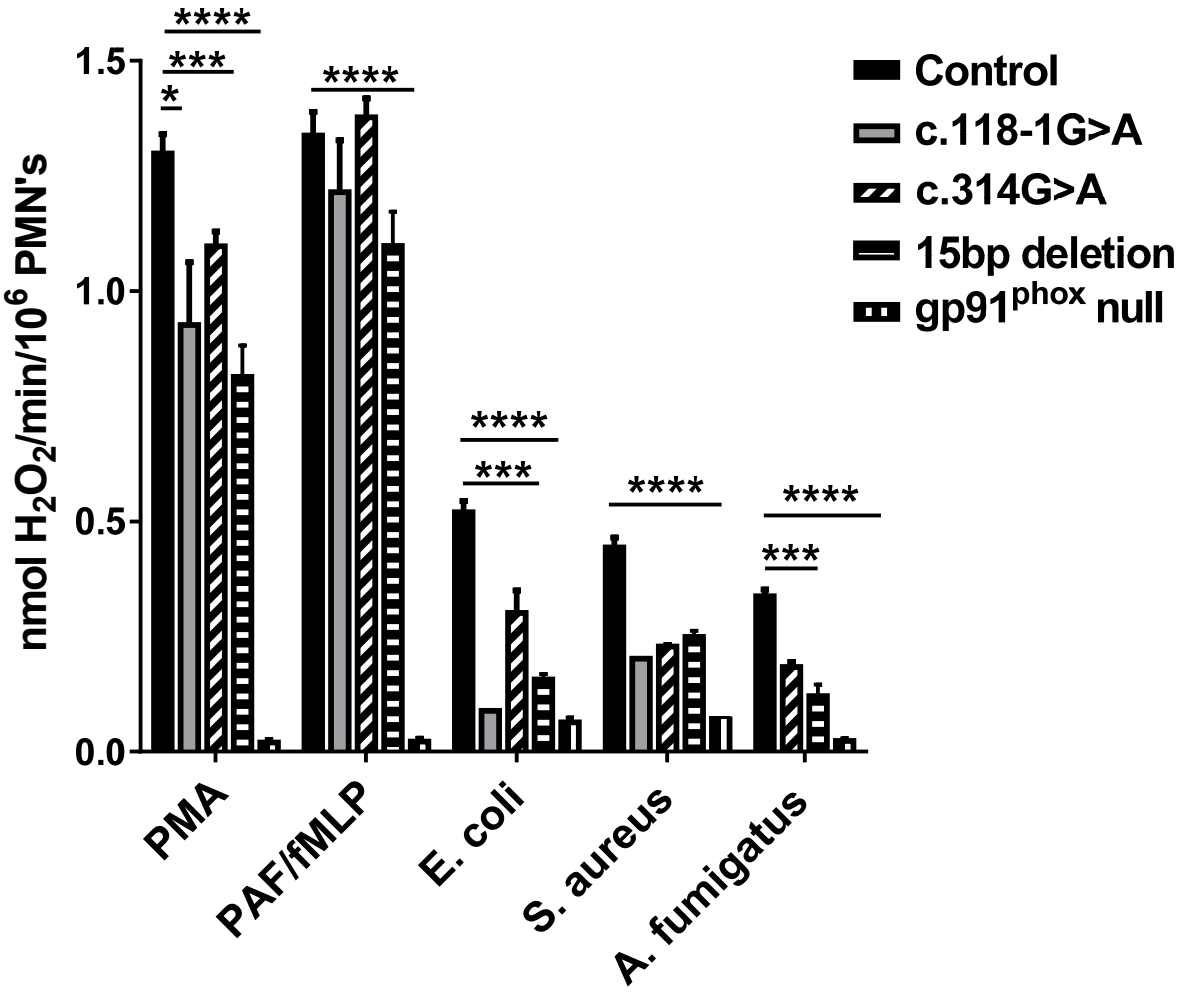
- Skin lupus
- Skin infections
- No IBD
- ANCA negative
- Stable on cotrimoxazole prophylaxis.



Phenotype:

- *S. aureus* skin infections
- Mouth ulcers, abdominal pain, diarrhea, anal fissures.

NADPH oxidase activity: H_2O_2 production



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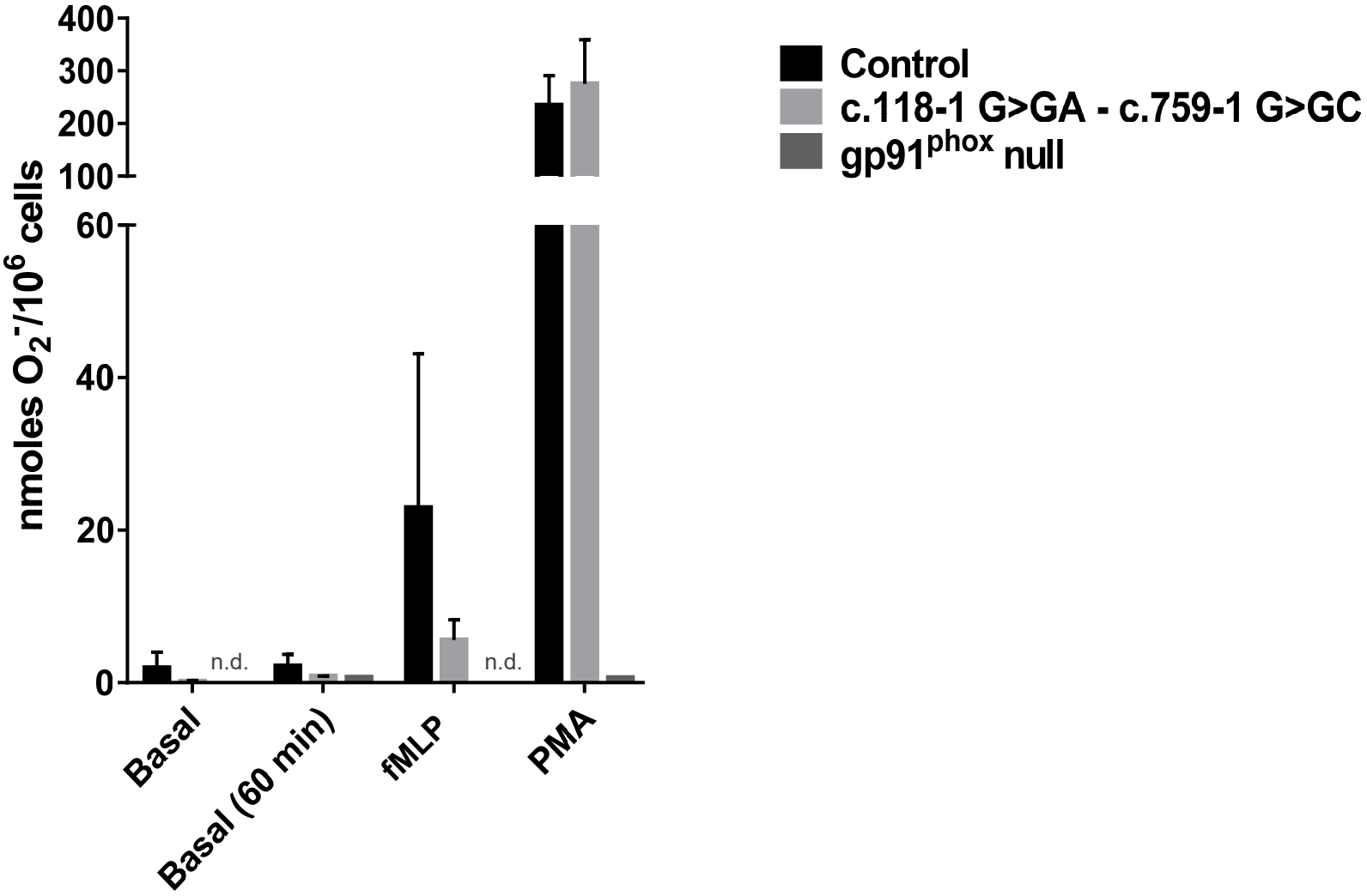
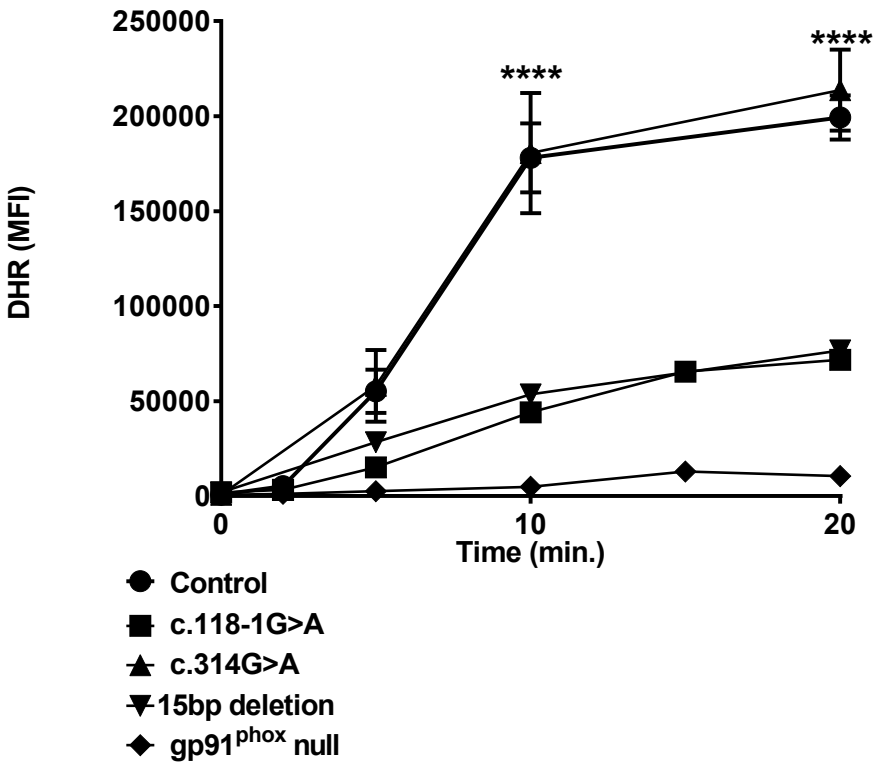
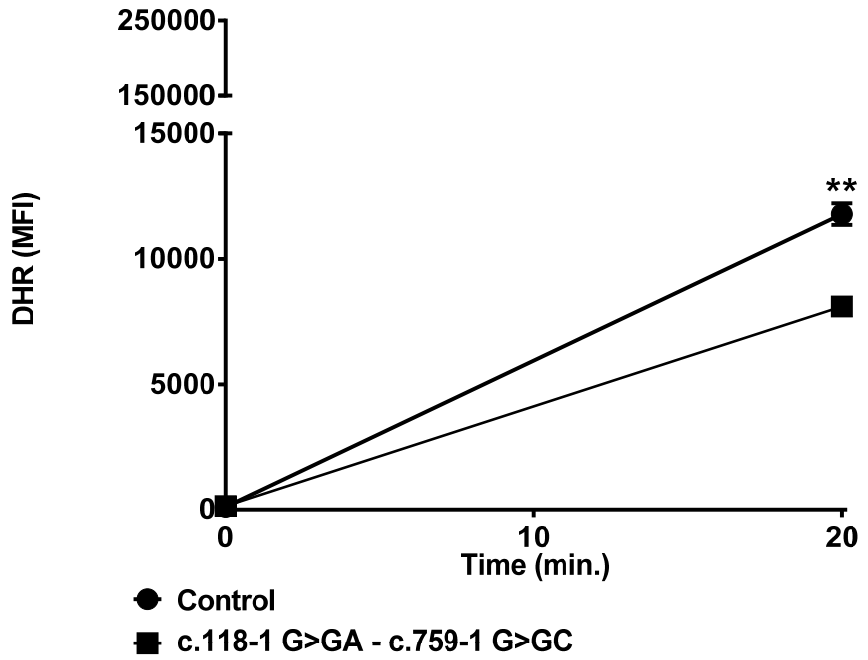
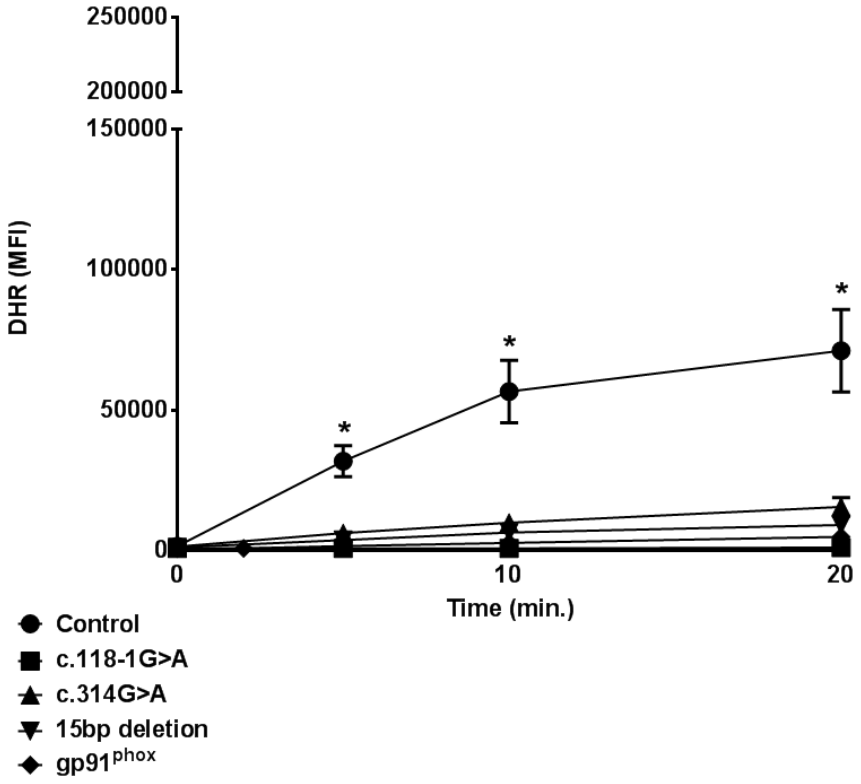


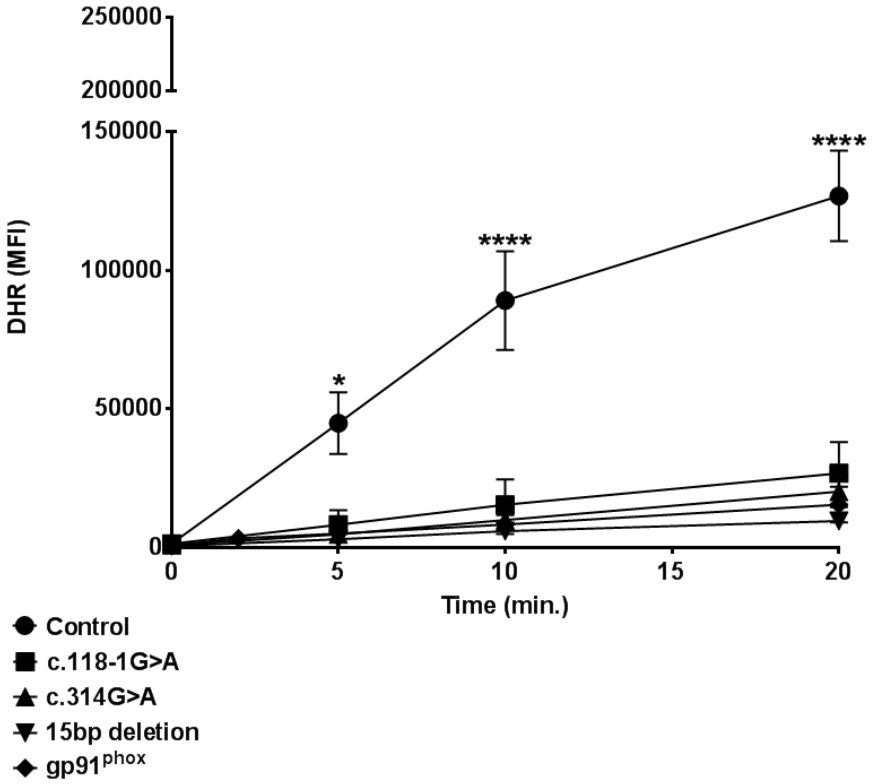
NADPH oxidase activity: PMA DHR





NADPH oxidase activity: *S. aureus, E. coli* DHR





Conclusion I: NADPH oxidase activity

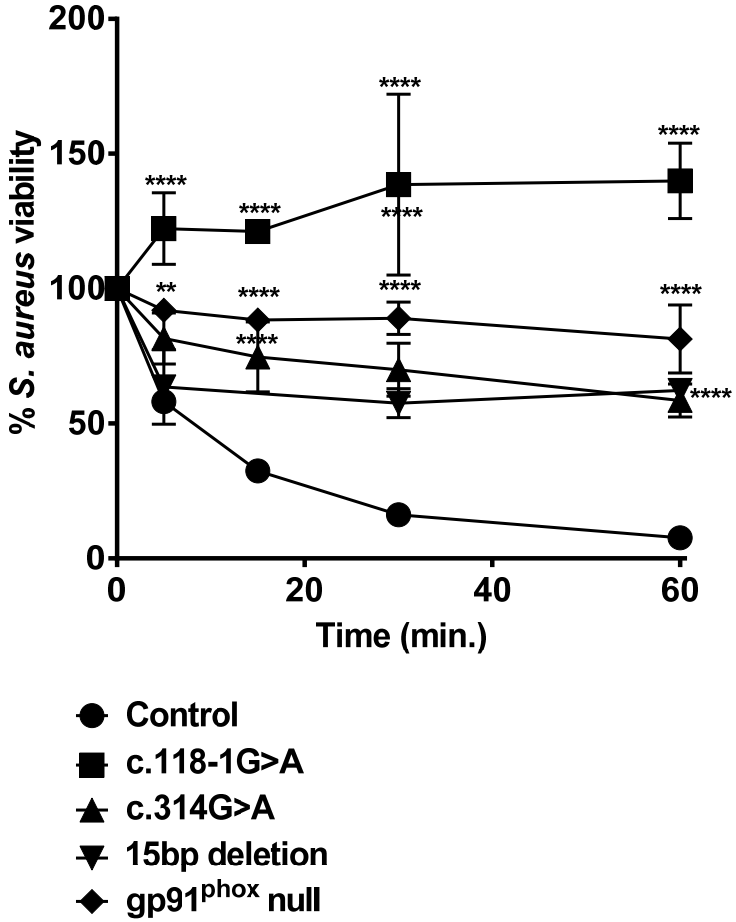
- p40^{phox} deficiency results in:
  - Severely reduced *E. coli* and *S. aureus* induced NADPH oxidase activity and ROS production;
  - Sustained soluble stimulus-induced H_2O_2 release; intracellular production dependent on presence of p40^{phox}

    → Impaired phagocytosis-induced ROS production

    → CGD subtype

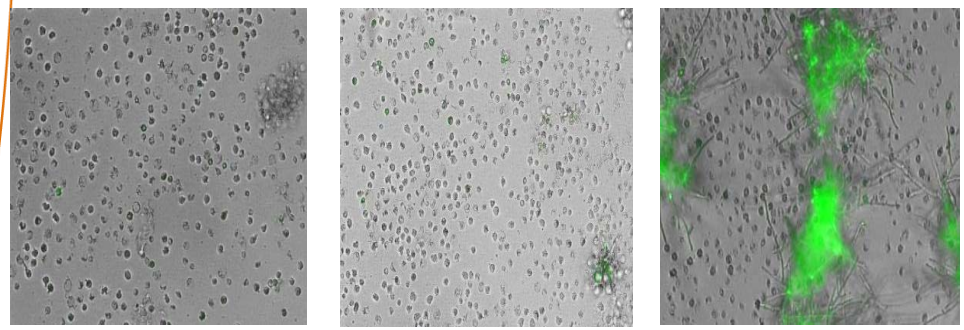
Pathogen killing - bacteria

- *E. coli, S. aureus*
- CFU assay



Pathogen killing - fungi

- *Candida albicans*
- conidia killling, hyphae killing, inhibition of germination



control **p40*phox*** **gp91*phox* *null***

Conclusion II: pathogen killing

- Bacterial killing equally affected compared to classical CGD
- Fungal killing – especially *Aspergillus* - surprisingly less affected compared to classical CGD

Conclusions

- Clinically heterogeneous CGD subtype

- p40^{phox} deficiency results in severely diminished particle-induced ROS production and a normal soluble stimulus-induced ROS production.
  - Severely affected *S. aureus* killing, *E. coli* killing less affected
  - *S aureus* killing = ROS-dependent
  - *E coli* killing = both ROS and granule-dependent

- Partially sustained *Aspergillus*- and *Candida*-induced ROS production
  - Killing partially affected - if at all
  - residual NADPH oxidase activity or other mechanisms?

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