

## **Male X-chromosome Mosaicism leading to Carrier Phenotype and inheritance of Chronic Granulomatous Disease**

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**Funding:** Nil

**Word count:** 946 words

**Clinical implications:** We present the first description of a male with X-chromosome mosaicism who has clinical features of the X-linked CGD carrier state and passed a *CYBB* mutation to a daughter and subsequently affected grandson: clinicians should be aware of this possibility.

**Key-words:** Chronic Granulomatous Disease; CGD; mosaicism

**Conflicts of Interest:** The authors declare no conflicts of interest.

1 To the Editor,

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3 Chronic Granulomatous Disease (CGD) is a genetic primary immunodeficiency caused by  
4 mutations in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme  
5 complex. This leads to impaired production of reactive oxygen species and impaired killing  
6 of ingested bacteria and fungi. About two thirds of cases are in males with X-linked CGD  
7 caused by mutations in the *CYBB* gene, coding for gp91<sup>phox</sup>, found at Xp21.1-p11.4 [1]; in  
8 approximately 10-12% of families these *CYBB* mutations are believed to arise *de novo*.

9 Female carriers of X-linked CGD exhibit a range of phenotypes, although in one series 37%  
10 were completely asymptomatic and 52% had no features of CGD disease [2]. Common  
11 manifestations in those who are symptomatic include recurrent oral ulceration, photosensitive  
12 rashes and discoid lupus erythematosus (DLE) [2-4], the latter seen in between 9% [2] and  
13 40% [3] depending on population. They can also be affected by gastrointestinal conditions  
14 similar to classic inflammatory bowel disease (approximately 4% [2, 3]) and have an  
15 increased predisposition to other lupus-like autoimmune manifestations. Female carriers can  
16 also present with recurrent or suppurative infections (15% had severe infections in a recent  
17 report [2]) and indeed with extremely skewed lyonisation they can essentially develop the full  
18 CGD syndrome [1]. Thus the carriage state can also confer a significant burden of disease.

19 We present a patient who was identified after his grandson was diagnosed with X-linked  
20 CGD. The grandson, who is the index case, was noted to have abnormal neutrophil functional  
21 tests and a c.1253C>T (p.Ser418Phe) *CYBB* mutation, and has subsequently undergone  
22 haematopoietic stem cell transplantation. During family screening his mother was found to be  
23 a carrier of this variant, but not his maternal grandmother. It was noted that instead his  
24 maternal grandfather had some features consistent with a carrier state. This person (the

25 maternal grandfather of the index case) is 51 years old with a history of asthma, epilepsy,  
26 hypertension and a significant infection history with two previous episodes of pneumonia or  
27 “pleurisy” and two episodes of cellulitis. He reported sun sensitivity, arthralgia and frequent  
28 ‘blistering’ of the lips. He had previously been referred to dermatology with a rash on his  
29 upper chest that was diagnosed on biopsy as Jessner’s lymphocytic infiltrate of the skin  
30 (JLIS) (Figure 1A & 1B) treated successfully with hydroxychloroquine. JLIS demonstrates  
31 very similar characteristics clinically and on histological examination to discoid lupus  
32 erythematosus [5]. He reported recurrent lower respiratory tract infection requiring antibiotics  
33 as a child and a requirement for antibiotics twice a year even in adulthood. A CT chest  
34 showed mild left lower lobe bronchiectasis (Figure 1C).

35 This maternal grandfather of the index case was found to have an abnormal Nitro-blue  
36 tetrazolium (NBT) test and dihydrorhodamine (DHR) oxidation, both of which demonstrated  
37 two populations of neutrophils upon stimulation, one capable of respiratory burst and one  
38 with an impaired burst (Figure 2A & 2B). This was confirmed on repeated testing. Gp91<sup>phox</sup>  
39 expression in these cells showed one positive and one negative peak (Figure 2C). Genetic  
40 analysis from blood and a salivary sample via Ion Torrent™ next generation sequencing  
41 confirmed that he in fact has three X-chromosome variants, two of which carry a novel *CYBB*  
42 mutation at position c.1253 (c.1253C>T [p.Ser418Phe] in 44% of reads, c.1253C>G  
43 [p.Ser418Cys] in 7-8% of reads and the remainder wild-type; Supplementary Figure 1). He  
44 had undergone vasectomy and thus could not provide a semen sample for further analysis.  
45 The brother and the three other daughters of the maternal grandfather of the index case  
46 underwent genetic screening but all were negative for these mutations (Figure 2D).

47

48

49 **Discussion**

50 It has previously been reported that male patients with X-linked CGD can have small  
51 populations of normal cells [6], suggesting mutation in early embryogenesis, or early  
52 reversion in some cell lines after the mutation arose in the oocytes. There has also been a case  
53 report of an X-linked CGD patient's brother with Klinefelter's syndrome having carrier status  
54 [7], due to inheritance of both the mutated allele of *CYBB* and the normal allele. Case reports  
55 of triple mosaic female carriers who had populations of neutrophils with two different  
56 deletion mutations in *CYBB* and the normal variant have been described [8], as has a late-  
57 onset somatic mutation in *CYBB* with subsequent mosaicism in haematopoietic cells in a  
58 female [9]. However, this is the first report to our knowledge of a fertile male acting as a true  
59 'carrier' for X-linked CGD with a compatible clinical syndrome (the 'JLIS' is likely to  
60 represent discoid lupus as seen in the CGD carrier state) and inheritance of the disease-  
61 causing variant in offspring. Both wild-type and mutant forms were present in blood and  
62 saliva: this raises the possibility of either early reversion of a germline mutation during  
63 embryogenesis or a mutation arising during an early multicellular stage with both populations  
64 of cells increasing proportionally. Given that there is no evidence that his brother has the  
65 mutation or clinically that there are other carriers in the family before him we conclude it is  
66 likely that this is a *de novo* mutation.

67 Male carriage of X-linked conditions in this manner would not be picked up via routine  
68 genetic screening of affected families and can lead to unintentional inheritance of the  
69 mutation without appropriate genetic counselling. Indeed in this case the maternal  
70 grandfather passed the gene on to one of his four daughters who subsequently had an affected  
71 child. There is a potential for grandfathers who are mosaics for X-linked conditions to pass  
72 on the genetic mutation to future children and, in diseases such as CGD, carriers themselves  
73 can develop symptoms and significant morbidity. We therefore recommend that clinicians

- 74 are aware of this possibility and consider at least functional testing of maternal grandfathers
- 75 in the presence of compatible symptoms.

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## Figure Legends

**Figure 1.** A. Photograph of the rash diagnosed as Jessner's lymphocytic infiltrate (diameter approximately 2cm). B. Histology from a skin lesion demonstrating superficial and deep dermal perivascular inflammation, normal epidermis on low power (left) and detail of the predominantly lymphocytic infiltrate on higher power (right). C. Axial section from CT thorax showing dilated bronchi larger than accompanying blood vessels, consistent with mild bronchiectasis (arrows).

**Figure 2.** A. NBT test (see Supplementary methods). The maternal grandfather of the index case ('patient', right) demonstrates two populations of cells, one able and one unable to reduce NBT and form blue formazan. B. Dihydrorhodamine test (see Supplementary methods). Rhodamine-1,2,3-FITC results are shown for a control (left) and the maternal grandfather of the index case ('patient', right) without stimulation (red histogram) and after stimulation with PMA (blue histogram), again indicating two cell populations in the maternal grandfather. C. gp91<sup>phox</sup> expression (see Supplementary methods) in neutrophils; isotype control (red histogram) and gp91<sup>phox</sup> stained (blue histogram) results are shown for a control (left) and the maternal grandfather of the index case ('patient', right) who has a population of gp91<sup>phox</sup>-negative cells. D. Family pedigree based on Ion Torrent™ next generation sequencing, indicating transmission of presumed deleterious *CYBB* mutation from the maternal grandfather to one daughter and then to a grand-son (index case) who presented with X-linked CGD.

**Figure 1**

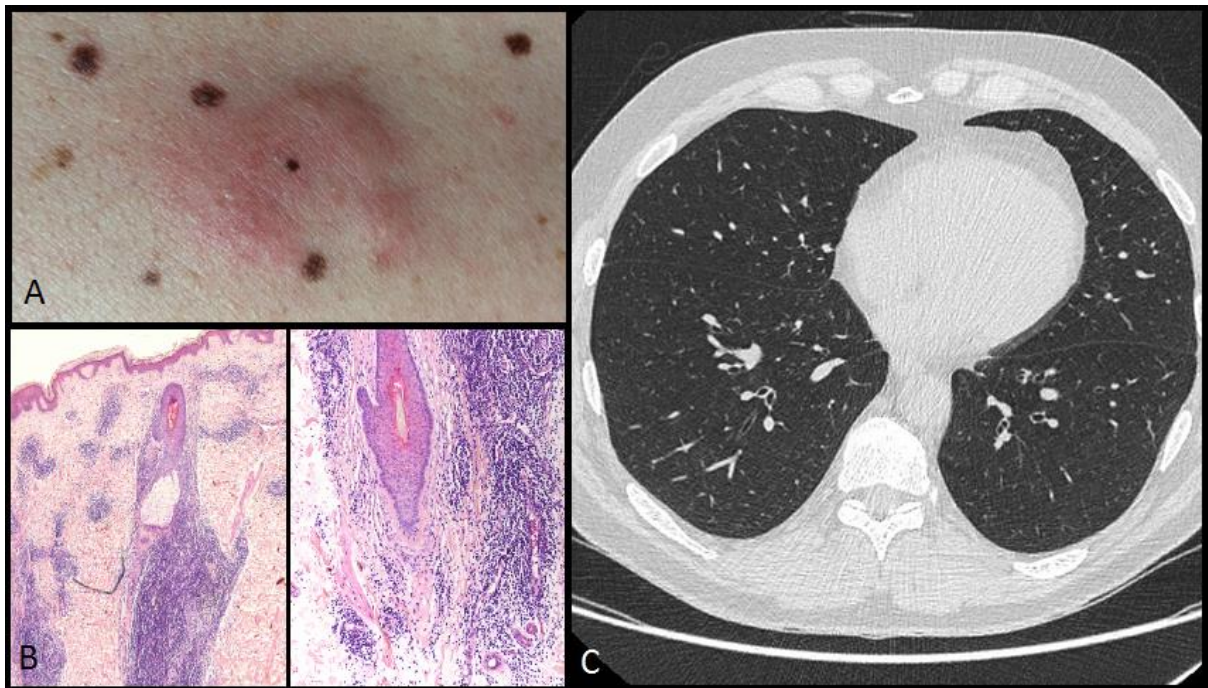
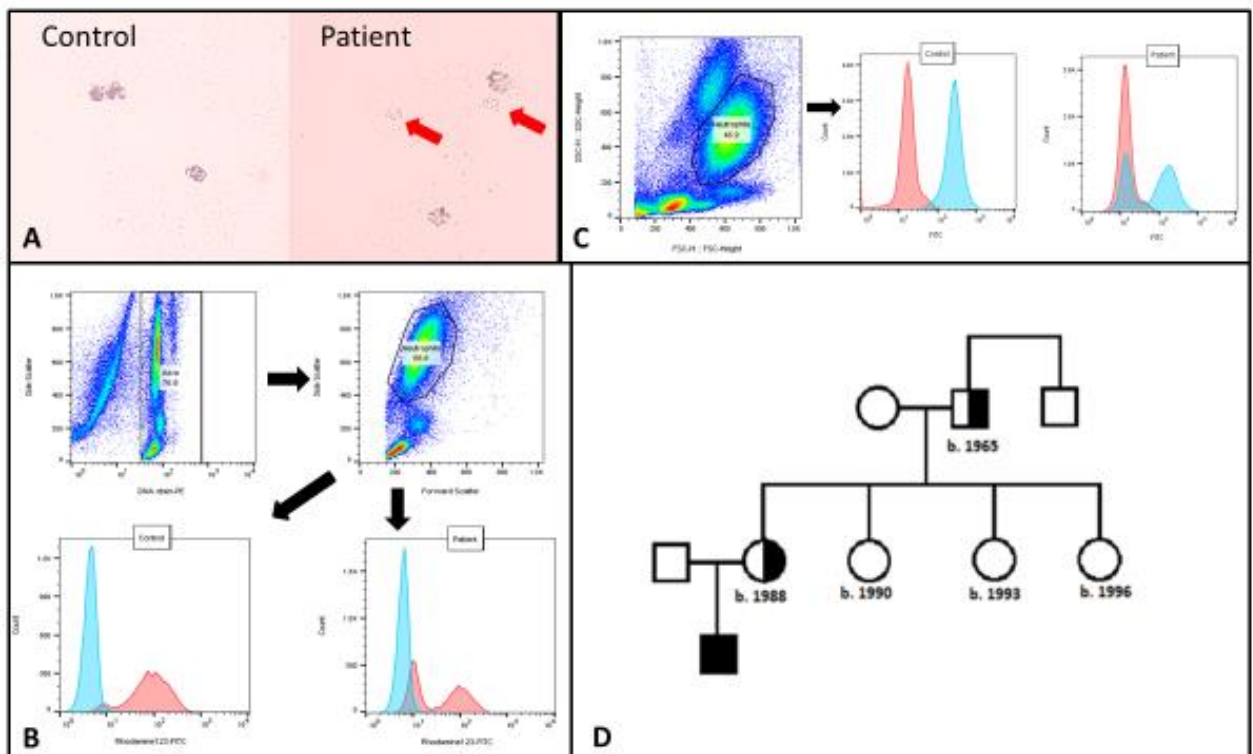


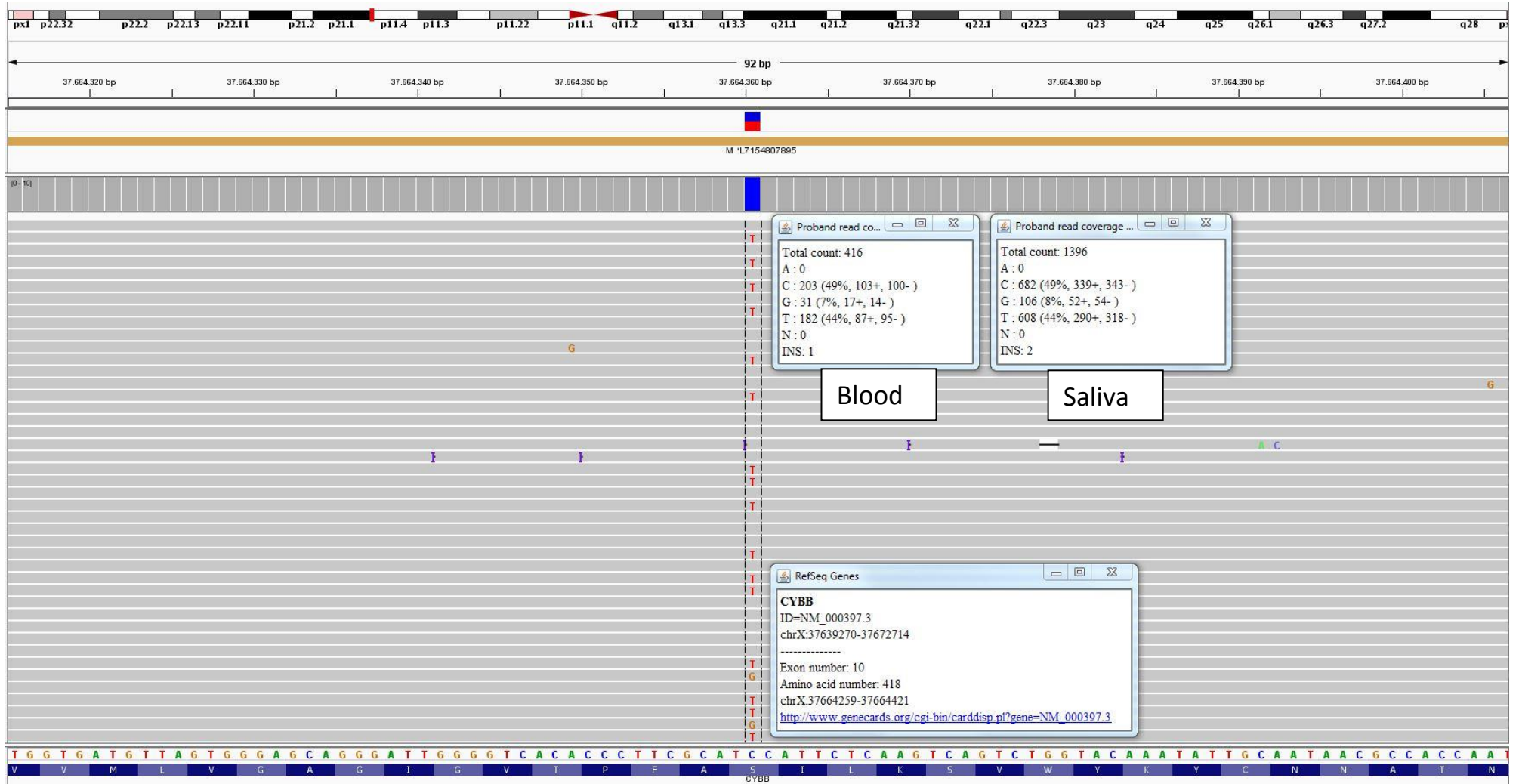


Figure 2



# Supplementary Information

## Supplementary Figure 1



## Supplementary Methods

**NBT test.** This was performed in accordance with published reports [1]. Briefly, 100 µl of EDTA whole blood was incubated with 100 µl of 0.2% NBT (Sigma) and 100 µl of 10 µg/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) for 25 minutes at 37°C, then 25 minutes at room temperature before mixing with 500 µl of 2% acetic acid and trace crystal violet; 10-µl aliquots were added to counting slides and examined at x40 magnification.

**DHR test.** This was performed in accordance with published reports [2]. The PhagoBurst™ kit (Glycotope Biotechnology) was used on heparinised whole blood according to manufacturers' instructions and samples processed on a FACSCalibur flow cytometer. For analysis a DNA stain was used to identify live cells and then neutrophils were gated by forward and side scatter (Figure 2).

**Gp91<sup>phox</sup> expression.** This was determined in neutrophils (gated by forward and side scatter; Figure 2) via flow cytometry using 7D5 mAb against gp91<sup>phox</sup> (IgG1, LifeSpan BioSciences) and a FITC-conjugated rabbit-anti-mouse IgG.

## References

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## Supplementary Figure Legend

**Supplementary Figure 1.** Results of Ion Torrent™ next generation sequencing of the CYBB gene in the patient's blood and saliva.