
Missense Mutation in AR-CGD

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1. Introduction

Chronic granulomatous disease (CGD) is an inherited disorder of the innate immune system characterized by impairment of intracellular microbicidal activity of phagocytes. Mutations in one of four known nicotinamide adenine dinucleotide phosphate (NADPH) -oxidase components preclude generation of superoxide and related antimicrobial oxidants, leading to the phenotype of CGD. Defects in gp91-phox, encoded by *CYBB* gene, lead to X-linked CGD and have been reported to be responsible for approximately 65% of all CGD cases. The autosomal gene in CGD are *CYBA*, encoding p22-phox, *NCF2*, encoding p67-phox, *NCF1*, encoding p47-phox, and *NCF4*, encoding p40-phox (figure 1) (1,2). The mutation in these genes, respectively, abolishes the activity of the oxidase and leads to autosomal recessive chronic granulomatous disease (AR-CGD) which is approximately 35% of all CGD cases (table 1).

2. Phenotype- genotype correlation in CGD

Identification of specific mutations in CGD patients may help to clarify some of the variability in clinical severity seen in this disorder and shows genotype-phenotype correlation. In general, X-CGD patients follow a more severe clinical course than patients with an AR-CGD and exhibit in the first years of life. AR-CGD patients follow a milder clinical course, especially p47-phox defect, and mostly seen in first and second decade of life. AR-CGD patients with missense mutations usually exhibit a mild clinical course, associated with a residual activity of p47-phox and also p22 and p67-phoxs. However, the level of superoxide generation does not always correlate with the clinical course. Some patients suffer from severe and recurrent infections despite having neutrophils with 10–20% of normal oxidase activity (1). Within our study with 40 AR-CGD families, we could not define a direct correlation between the molecular defect and the clinical course of the disease. Either truncations (nonsense and frameshift mutations) or missense mutations could have resulted in severe influence on phenotype.

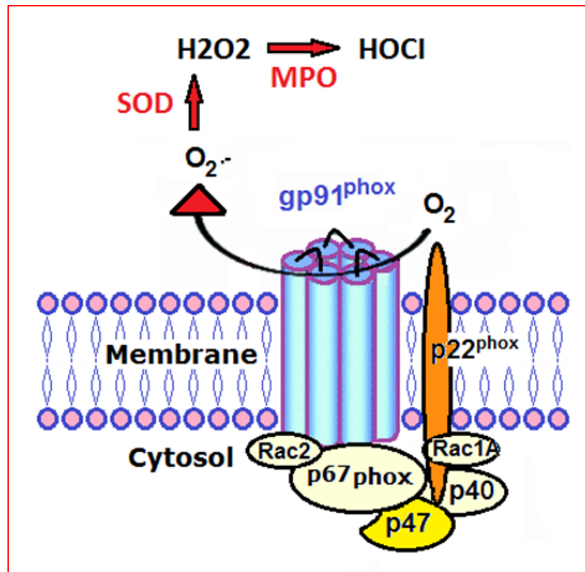


Figure 1. NADPH oxidase enzyme subunits and complex in activation phase.

Components	gp91 phox	p22 phox	p47 phox	p67 phox
Gene	CYBB	CYBA	NCF1	NCF2
Chromosome	Xp21.1	16q24	7q11.23	1q25
Number of Exon	13	6	11	16
The length of bp	30kb	8.5kb	15.3kb	40kb
Genotype	X91, X-linked R	A22, OR	A47, OR	A67, OR
Incidence %	%60	%5	%30	%5
The length of aa. chain	570	195	390	526
Localization	membrane	membrane	cytosol	cytosol

Table 1. The molecular characteristic of NADPH Oxidase components.

3. CYBA (cytochrome *b* alfa chain) gene

Cytochrome *b* is comprised of a light *a*-chain and a heavy *b*-chain. This gene encodes the light, alpha subunit which has been proposed as a primary component of the microbicidal oxidase system of phagocytes. Mutations in this gene are associated with AR-CGD that is characterized by the failure of activated phagocytes to generate superoxide, which is important for the microbicidal activity of these cells. <http://www.genecards.org/cgi-bin/carddisp.pl?gene=CYBA>.

In about 5% of the CGD patients, the disease is caused by mutations in the cytochrome *b* alfa chain (*CYBA*) gene. The *CYBA* gene encoding p22-phox which contains 195 amino acid, is localized on chromosome 16q24, has a size of about 8,5 and contains six exons and trans-

membrane and proline rich domains [3, 4]. *CYBA* gene encoding p22-phox has 19 different missense mutations in 65 mutated alleles and has more missense mutation than other NADPH oxidase subunit genes (table 2a) (figure 2). Mutations in the *CYBA* gene have been updated by [Roos et al., (2)] and are reviewed in Human Gene Mutation Database, (HGMD; <http://www.hgmd.cf.ac.uk/ac/all.php>).

P22-phox has a key role in the interaction of NADPH-oxidase subunits and any difference in amino acid pattern of this phox protein may change the globular conformation of this protein due to the difference in the electrophoretic characteristic of new amino acid, which prevents the complex formation with other subunits.

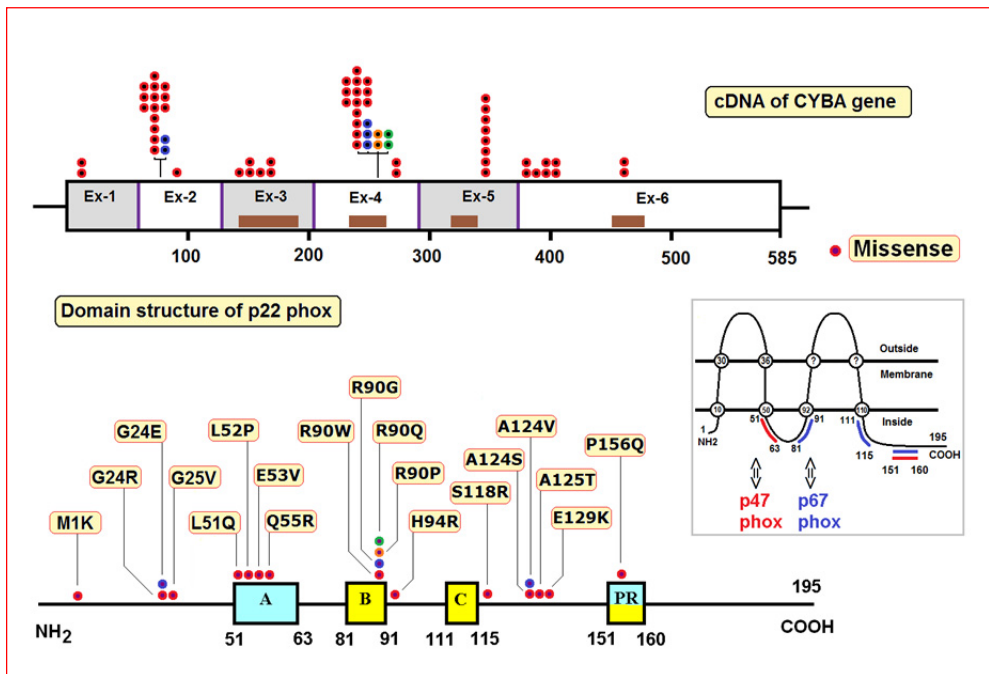


Figure 2. Missense mutations in *CYBA* gene, encoding p22-phox; *CYBA* gene contains 6 exons. P22-phox contains trans-membrane and proline rich domains. Missense mutation points in *CYBA* gene and change in encoded 195 aa. of p22-phox represented in the figure (2).

Missense mutation points may have an important role in the interaction with other subunits, so the amino acid change in that regions may change the property of interactions and prevents or decreases the complex formation, so the activity of NADPH oxidase was abolished.

Total numbers of alleles which have missense mutations are 65 of 173 mutated alleles in *CYBA* gene and the percentage of missense mutations in that mutated alleles are %37,5 (table 2a) (2). Percentage of missense and all mutations of *CYBA* gene in the overall

mutations of AR-CGD is %6.3 and %16.8, respectively (table 3 and 4). Most prevalent missense mutations points in *CYBA* gene are c.70G>A, c.268C>T and c.354C>A which cause p.Gly24Arg, p.Arg90Trp and p.Ser118Arg in p22-phox, respectively (table 5).

4. *NCF1* (Neutrophil Cytosolic Factor 1) gene

In about 25% of the CGD patients, the disease is caused by mutations in the neutrophil cytosolic factor 1 (*NCF1*) gene on chromosome 7q11.23, which encodes p47phox, one of the structural components of the NADPH oxidase and has a size of about 40 kb and contains 11 exons (5, 6). The protein encoded by this gene is a 47 kDa cytosolic subunit of neutrophil NADPH oxidase and is required for activation of the latent NADPH oxidase and contains 390 amino acids and PX, SH3a, SH3b and polybasic domains (figure 3).

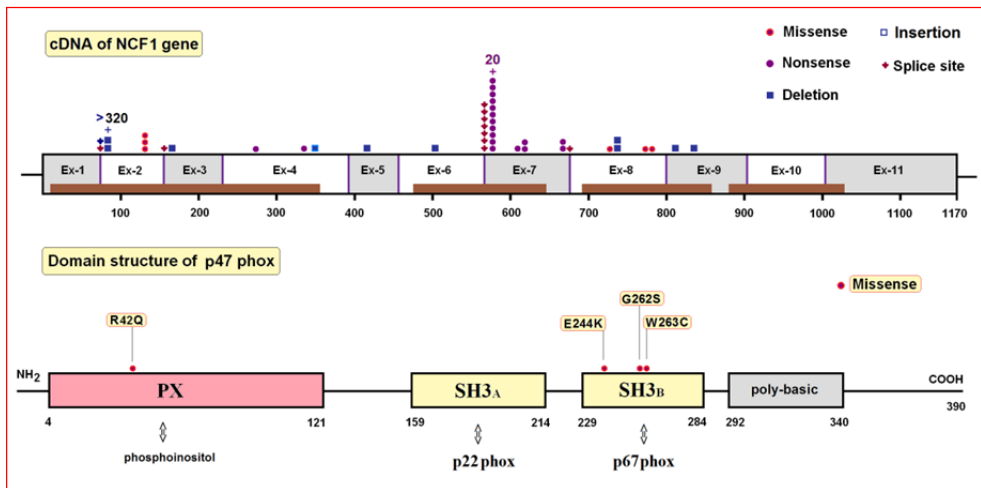


Figure 3. Missense mutations in *NCF1* gene, encoding p47-phox; *NCF1* gene contains 11 exons. p47-phox contains PX, SH3a, SH3b and polybasic domains. Mutation points in *NCF1* gene and change in encoded 390 aa. of p47-phox represented in the figure (2).

A very common mutation found in these patients is a GT deletion in a GTGRepeat sequence at the beginning of exon 2 of *NCF1* (c.75_76delGT) gene (5, 7). *NCF1* gene encoding p47-phox has only 4 different missense mutation in 6 alleles (table 2b) (figure 3) (2). P47-phox has an important role in the interaction of cytoplasmic NADPH-oxidase subunits and any difference in amino acid pattern of this phox protein may abolish the complex formation with other subunits.

Total numbers of alleles which have missense mutations are 6 of 63 (other than delta-GT mutation in exon 2, in more than 620 alleles) mutated alleles in *NCF1* gene and the percentage of missense mutations in that mutated alleles are %9,5. The percentage of

missense and all mutations (including delta-GT mutation) of *NCF1* gene in the overall mutations of AR-CGD is %0.6 and %66.4, respectively (table 3 and 4). So, this high percentage due to the high number of delta-GT mutation in exon 2 of *NCF1* gene and is more than all the mutations in AR-CGD (table 5). This deletion points is hot-spot mutation region for *NCF1* gene.

5. *NCF2* (Neutrophil Cytosolic Factor 2) gene

The neutrophil cytosolic factor 2 (*NCF2*) gene encoding p67-phox is localized on chromosome 1q25, has a size of about 40 kb and contains 16 exons and TRP1-4, AD, SH3a, PB1 and SH3b domains (figure 4) (7, 8). *NCF2* gene, encoding p67-phox, has 41 different missense mutations in 171 mutated alleles (table 2c) (2, 4, 5, 6). Mutations in the *NCF2* gene have been published by [Roos et al., (2)] and are reviewed in Human Gene Mutation Database, (HGMD; <http://www.hgmd.cf.ac.uk/ac/all.php>). P67-phox has a major role in the interaction of NADPH-oxidase subunits in cytoplasm and any difference in amino acid pattern of this phox protein may prevent the complex formation with other subunits (figure 1).

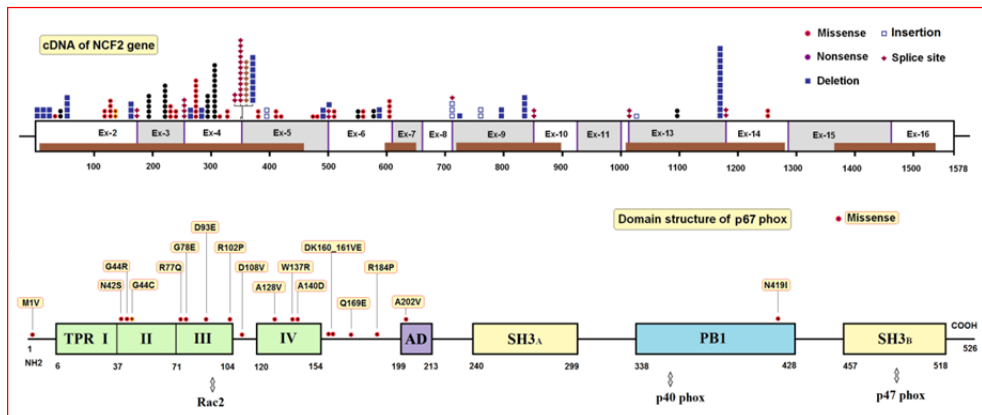


Figure 4. Missense mutations in *NCF2* gene, encoding p67-phox; *NCF2* gene contains 16 exons. P67-phox contains TRP1-4, AD, SH3a, PB1 and SH3b domains. Mutation points in *NCF2* gene and change in encoded 526 aa. of p67-phox represented in the figure (2).

Total numbers of mutated alleles leading AR-CGD in *NCF2* gene are 171 and 41 of them are missense and percentage of missense mutations in that mutated alleles are %24 (table 2c) (2). Percentage of missense and all mutations of *NCF2* gene in the overall mutations of AR-CGD is %4 and %16.6, respectively (table 3 and 4). Most prevalent missense mutations points in *NCF2* gene is c.279C>G which causes p.Asp93Glu in p67-phox (table 5).

6 Mutations in Human Genetic Disease

Nucleotide change	Amino acid change	Amino acid	# of families (alleles)
c.2T>A	p.Met1Lys	M1K	1(2)
c.70G>A	p.Gly24Arg	G24R	9(14)
c.71G>A	p.Gly24Glu	G24E	1(2)
c.74G>T	p.Gly25Val	G25V	1(1)
c.152T>A	p.Leu51Gln	L51Q	1(1)
c.155T>C	p.Leu52Pro	L52P	1(2)
c.158A>T	p.Glu53Val	E53V	1(1)
c.164C>G	p.Pro55Arg	Q55R	1(2)
c.268C>T	p.Arg90Trp	R90W	8(14)
c.268C>G	p.Arg90Gly	R90G	1(2)
c.269G>A	p.Arg90Gln	R90Q	2(3)
c.269G>C	p.Arg90Pro	R90P	1(2)
c.281A>G	p.His94Arg	H94R	1(2)
c.354C>A	p.Ser118Arg	S118R	4(8)
c.370G>T	p.Ala124Ser	A124S	1(2)
c.371C>T	p.Ala124Val	A124V	1(1)
c.373G>A	p.Ala125Thr	A125T	1(2)
c.385G>A	p.Glu129Lys	E129K	1(2)
c.467C>A	p.Pro156Gln	P156Q	1(2)
19 different alleles			65 alleles

(a)

Nucleotide change	Amino acid change	Amino acid	# of families (alleles)
c.125G>A	p.Arg42Gln	R42Q	3(3)
c.730G>A	p.Glu244Lys	E244K	1(1)
c.784G>A	p.Gly262Ser	G262S	1(1)
c.789G>C	p.Trp263Cys	W263C	1(1)
4 different alleles			6 alleles

(b)

Nucleotide change	Amino acid change	Amino acid	# of families (alleles)
c.1A>G	p.Met1Val	M1V	1(1)
c.125A>G	p.Asn42Ser	N42S	1(2)
c.130G>C	p.Gly44Arg	G44R	2(4)
c.130G>T	p.Gly44Cys	G44C	1(2)
c.230G>A	p.Arg77Gln	R77Q	3(3)
c.233G>A	p.Gly78Glu	G78E	1(2)
c.279C>G	p.Asp93Glu	D93E	4(8)

c.305G>C	p.Arg102Pro	R102P	1(1)
c.323A>T	p.Asp108Val	D108V	1(2)
c.383C>T	p.Ala128Val	A128V	1(2)
c.409T>A	p.Trp137Arg	W137R	1(2)
c.419C>G	p.Ala140Asp	A140D	1(1)
c.[479A>T; 481A>G]	p.AspLys160_161ValGlu	DK160_161VE	1(1)
c.505C>G	p.Gln169Glu	Q169E	1(2)
c.551G>C	p.Arg184Pro	R184P	1(2)
c.605C>T	p.Ala202Val	A202V	2(4)
c.1256A>T	p.Asn419Ile	N419I	1(2)
17 different alleles			41 alleles

(c)

Table 2. (a) Missense Mutation in *CYBA* gene. (b) Missense Mutation in *NCF1* gene. (c) Missense Mutation in *NCF2* gene.

Autosomal Gene	Alleles with missense mutations		Alleles with mutations ^{&}		In the all mutations of AR-CGD	
	#	%	#	%	Missense %	Total mutations %
<i>CYBA</i>	65	37,5	173	100	6.3	16.8
<i>NCF1</i>	6	9,5*	63 +620*	100	0.6	66.4
<i>NCF2</i>	41	24	171	100	4	16.6
<i>NCF4</i>	1	50	2	100	0.1	0.2
In AR-CGD	113	27.6	409+620*	100	11	100

*: (delta-GT mutations in exon 2, not included)

&: Including nonsense, missense, splice site, deletion and others.

Table 3. Distribution of number and percentage of missense and all mutations in genes (*CYBA*, *NCF1*, *NCF2* and *NCF4*) of AR-CGD.

Autosomal Gene	# of different missense mutations	Total # of different mutations ^{&}	Different missense / different all mutations in that gene
<i>CYBA</i>	19	55	%34.6
<i>NCF1</i>	4	23	%17.4
<i>NCF2</i>	17	54	%31.5
<i>NCF4</i>	1	2	%50
In AR-CGD	41	134	%30

Table 4. Number and percentage of different missense mutations in genes (*CYBA*, *NCF1*, *NCF2* and *NCF4*) of AR-CGD.

Autosomal Gene	Nucleotide change	Aa change	Number of family (alleles)
<i>CYBA</i>	c.70G>A	p.Gly24Arg	9(14)
"	c.268C>T	p.Arg90Trp	8(14)
"	c.354C>A	p.Ser118Arg	4(8)
<i>NCF1</i>	c.125G>A	p.Arg42Gln	3(3)
<i>NCF2</i>	c.279C>G	p.Asp93Glu	4(8)

Table 5. Most prevalent missense mutation in the genes of AR-CGD.

6. *NCF4* (Neutrophil Cytosolic Factor 2) gene

NCF4 gene encoding p40-phox with 339 amino acids is localized on chromosome 22q13.1 has a size of about 4,4 kb and contains 10 exons. P40-phox interacts primarily with p67-phox. Up to know, the first mutation in *NCF4* gene was founded in a family with compound heterozygote mutations and one of the mutations was a missense with c.314G>A in one allele, which causes change in p.Arg105Gln amino acid in the structure of p40-phox (2, 9).

7. Conclusion

19 different alleles in *CYBA* gene, 4 different alleles in *NCF1* gene, 17 different alleles in *NCF2* gene and one allele in *NCF4* gene have missense mutations which cause change in amino acid patterns of NADPH oxidase subunits and results in AR-CGD. The percentage of missense mutations in the overall mutations of AR-CGD is %11 (table 3). One of the most prevalent missense mutations in AR-CGD is in *CYBA* gene with c.70G>A, in 14 alleles of 9 families, which causes p.Gly24Arg in p22-phox (table 5).

In p22-phox the first interaction with p67-phox occur in B part (domain) which is located between 81-91 amino acids in p22-phox. There are 4 different missense mutations (in 21 alleles of *CYBA* gene) change amino acid (arginine) at position 90. So, this position is highly susceptible to any conformational changes which may prevent the interaction with p67-phox. So, the change in the molecular structure of this part may abolish the stability and function of p22-phox and latent NADPH oxidase could not be activated leading to AR-CGD. P22-phox has more different missense mutation than other NADPH oxidase components. The ratio of the number of different missense mutation and the number of amino acid in the chain is approximately 19/195 (%9.74). The different missense mutation to overall amino acid chain length in p67-phox is 17/526 (%3.23). But, the ratio in p47-phox is 4/390 (%1). This result shows that p67-phox has 3 times and p22-phox has approximately 10 times high incidence of different missense mutations than p47-phox in their primary amino acid structure. The underlying reason for this may be the highly specific interaction and function of p22-phox which is vulnerable to any change in the globular structure of protein.

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