Clinical heterogeneity of G6PD deficiency: New variants and correlation between genotype and phenotype, results of a five-year-survey

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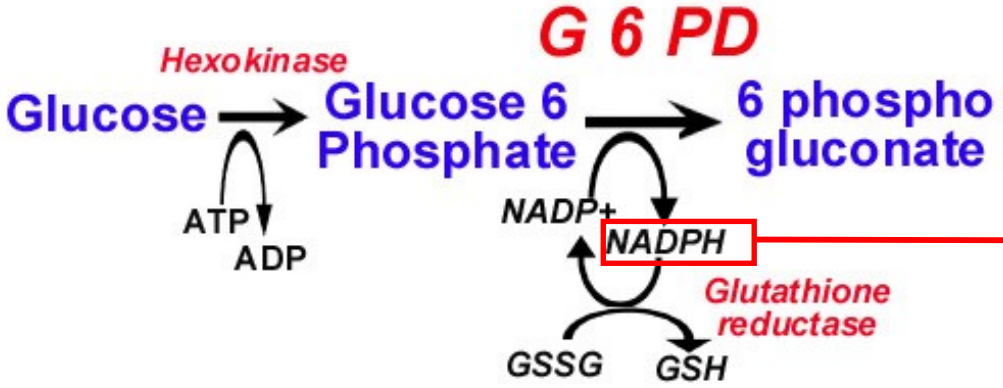
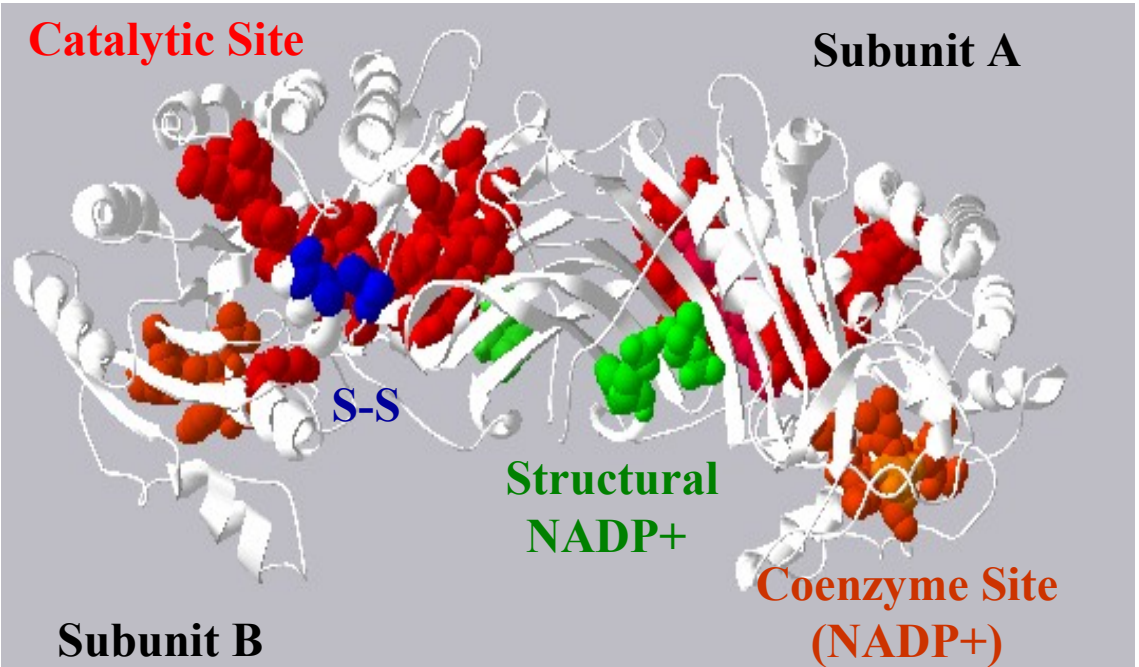
Dorys 2011
Strasbourg

- An enzyme catalyzing the first reaction of the pentose phosphate pathway (PPP).
- Producing reducing agent (NADPH) necesary to all cells to survive
- Absence of mitochondria in RBCs: PPP as their sole source of NADPH



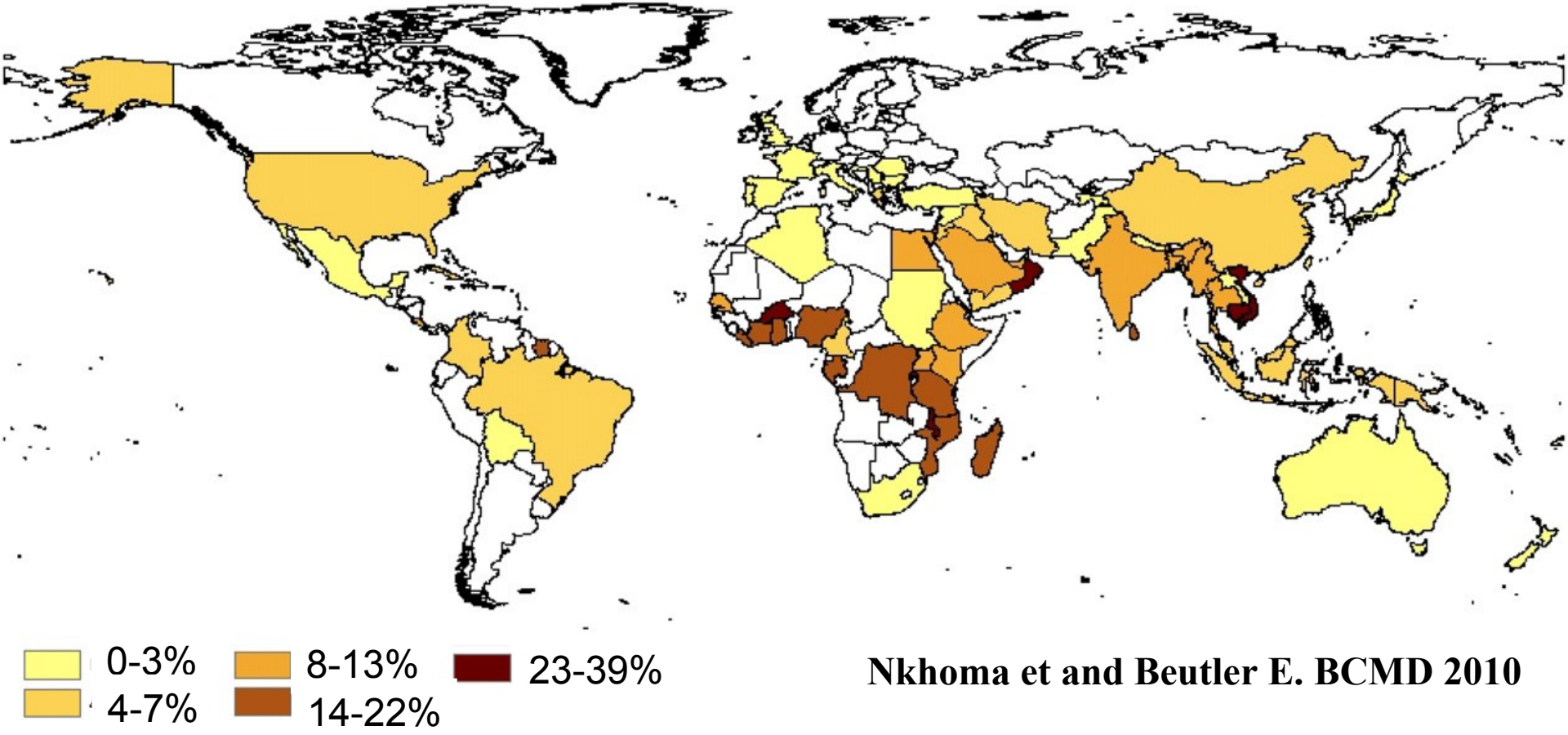
Crucial to protection against oxidative stress



AU SW. et al. 1996

Conserved sites and 3D structure
Protein: 515 AA , 59 KDa
Active enzyme: dimer or tetramer

Coenzyme binding site:
Residus: 34-53
Catalytic site
Residus: 193-218



Nkhoma et and Beutler E. BCMD 2010

Average G6PD prevalence as a percentage across countries

- A superimposition of geographical distribution of G6PD deficiency with that of Plasmodium falciparum malaria
- Confers anti-malarial protection with an unknown mechanism
- Highly prevalent (>23%) in Burkina Faso, Somalia, Vietnam, Cambodia

G6PD deficiency in the world

Global prevalence of G6PD deficiency: 4.9%
about 330 millions affected people worldwide

Africa (1.2-30.7%)	Ivory Coast 30%, Ghana 26%, Burkina Faso 25%, Gabon 22%,
America (1.3-9.7%)	Brazil, Colombie, Jamaica +++, USA (3.2%) Mexico 1.3%
Asia (3.1-28.5%)	Cambodia, Myanmar, Malaysia,
Europe (0.2-11.9%)	Italy, Greece
Middle East (2-17%)	Iran, Saudi Arabia+++
Pacific	really rare

G6PD deficiency variants

Sporadic variants: really rare, prevalence 1/1000 000

Polymorphic variants: frequent

G6PD A-	(frequency of 11% in Afro-Americans) Ivory Coast 30% of population
G6PD Med	(Frequency ranges beween 2 and 20% in different populations). exceptionally high in Kurdish Jews 70%
G6PD Viangchan	Frequent in the Indochina peninsula. 54% in Thailand

Neonatal screening for G6PD deficiency

In USA 25% of kernicterus cases were G6PD deficient

12% of population is Africo-American

-- Watchko JF. 2010 Semin Fetal Neonatal Med.

In Oman 71% of kernicterus cases were G6PD deficient

--Nair PA. 2003 J Trop Pediatr. Review.

Two possibilities

- The neonatal screening in the regions with high frequency of G6PD deficiency to prevent this encephalopathy.

Greek screening program (1977-1989)

detection of 100% of hemizygote and homozygote

but 50% of the heterozygote can not be detected

- Screening for G6PD deficiency for all of the pregnant women and their hausbands in a high risk population

(about11.7% of the heterozygote adults could not be detected by G6PD assay).

Dominique Jolly and Emile Levy Journal d'Economie Médicale 2010

G6PD Deficit: epidemiological and socio-economic arguments in favour of the need for targeted, systematic screening

Screening the G6PD deficiency in blood donors

About 19% of blood donors in Nigeria and 14% in Iran, are G6PD deficient

Isr J Med Sci. 1986 Feb;22(2):120-2.
G6PD-deficient donor blood as a cause of hemolysis in two preterm infants.

Huang CS et al. Am J Hematol. 1998 Mar;57(3):187-92.
Comparison of glutathione content between 97 G6PD-deficient donors and 124 normal donor revealed 33% reduction.
But no delayed hemolytic transfusion reaction was observed.

The application of the precautionary principle to the blood transfuision system in France.

Prevalence of G6PD deficiency in U.S. Army personnel.

The U.S. Army recently mandated that soldiers undergo G6PD testing before deployment to malarious regions. (study realized between October 1, 2004 through January 17, 2005.
Data available for 63,302 (54,874 males and 8,428 females) subjects

Results: 2.5% of males and 1.6% of females were deficient, moderate enzyme deficiency +++
African American males (12.2%), females (4.1%),
Asian males (4.3%),

These results suggest that universal screening for G6PD deficiency is clinically warranted, and particularly essential for those male service members who self-report ethnicity as African American, Asian, or Hispanic.

- **G6PD deficiency:** discovered by Carson et al. (1956) in individuals developing a hemolytic anemia following primaquine intake

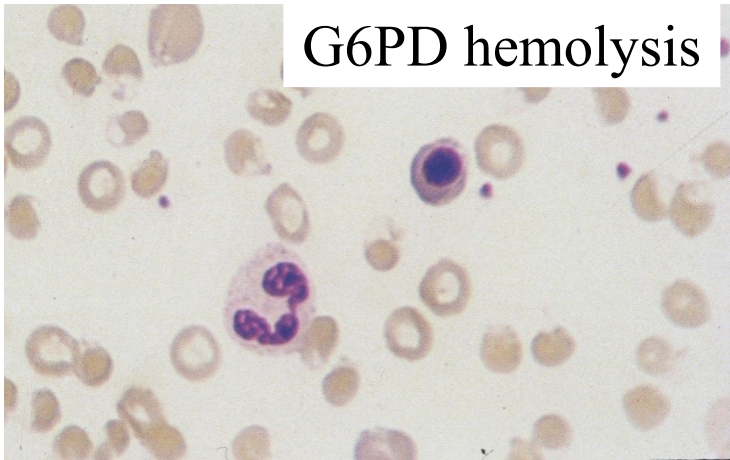
- An X-linked disease (typically affects men)

- The most common enzymatic disorder of RBCs in humans

- Wide range of biochemical and clinical phenotypes: Neonatal jaundice (males++), acute hemolytic anemia (trigerred mainly by exogenous agents; viral or bacterial infections, drugs and fava bean).

Fortunately, most G6PD-deficient individuals are **aysmptomatic** throughout their life.

The illness manifests as acute hemolysis following an oxidative stress (some medications and fava bean intake).



G6PD hemolysis

G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia.

Table 1. Predictive factors for abnormally high velocities (≥ 2m/sec) by univariable models

Variable	Stroke-free SS patients, n = 373	Abnormal TCD n = 62		Normal TCD n = 311		OR (95% CI)	*P*
		Events	n	Events	n		
Male gender	193	32	62	161	311	1.04 (0.61-1.77)	.89
β-globin haplotypes	293		42		251		
Car/Car	118	18		100		0.71 (0.38-1.33)	.28
Ben/Ben	71	10		61		0.60 (0.28-1.30)	.19
Sen/Sen	27	2		25		0.61 (0.20-1.87)	.39
Others	77	12		65			
α-thalassemia	155/325*	12	50	143	275	0.29 (0.15-0.58)	< .001
G6PD deficiency	36/325*	11	55	25	270	2.45 (1.12-5.35)	.024

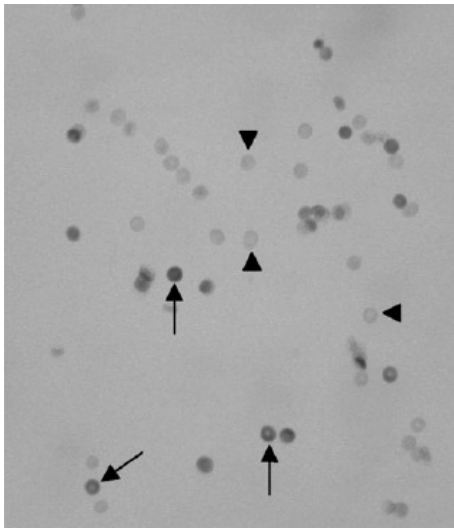
*α gene study and G6PD were available in 325 of 373 patients.

Diagnosis of G6PD deficiency

- Studying the production rate of NADPH
  - Screening test: Fluorescent spot test (semi-quantitative)
  - G6PD assay (quantitative)
    - based on reduction of $NADP^+$ when hemolysate is incubated with G6P (measurement is done at 340 nm)
  - **False negative results:**

    reticulocytosis, state of high regeneration, Iron deficiency and recent blood transfusion
- Review of blood smear stained by tetrazolium salt
  - Useful in heterozygote females

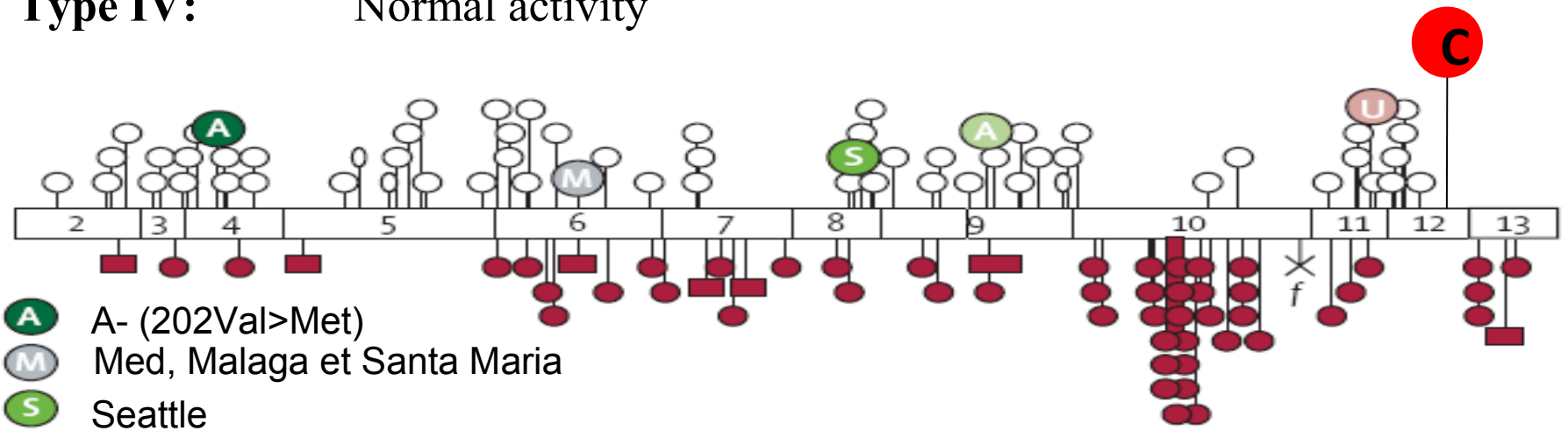
    with normal enzyme acticity assay



Cytochemical detection of heterozygous G6PD deficiency in women. Mixed population of G6PD-containing erythrocytes (arrows) and G6PD-deficient erythrocytes (arrowheads)

- 1986: cloning and sequencing of G6PD gene (a housekeeping gene)
  - 13 exons spanning 20 kb on Xq28
  - molecular characterization of 183 different variants since 1986

Type I: Chronic nonspherocytic hemolytic anemia (CNSHA)
Type II: <10% residual enzymatic activity
Type III: Between 10 and 60% residual enzymatic activity
Type IV: Normal activity



A A- (202Val>Met)
M Med, Malaga et Santa Maria
S Seattle
A A- (323Leu>Pro)
U Union
C Canton

Distribution of G6PD gene mutations:

○ **Type II et III mutants** ● **Sporadic mutants (type I)** ○ **Type IV mutants**

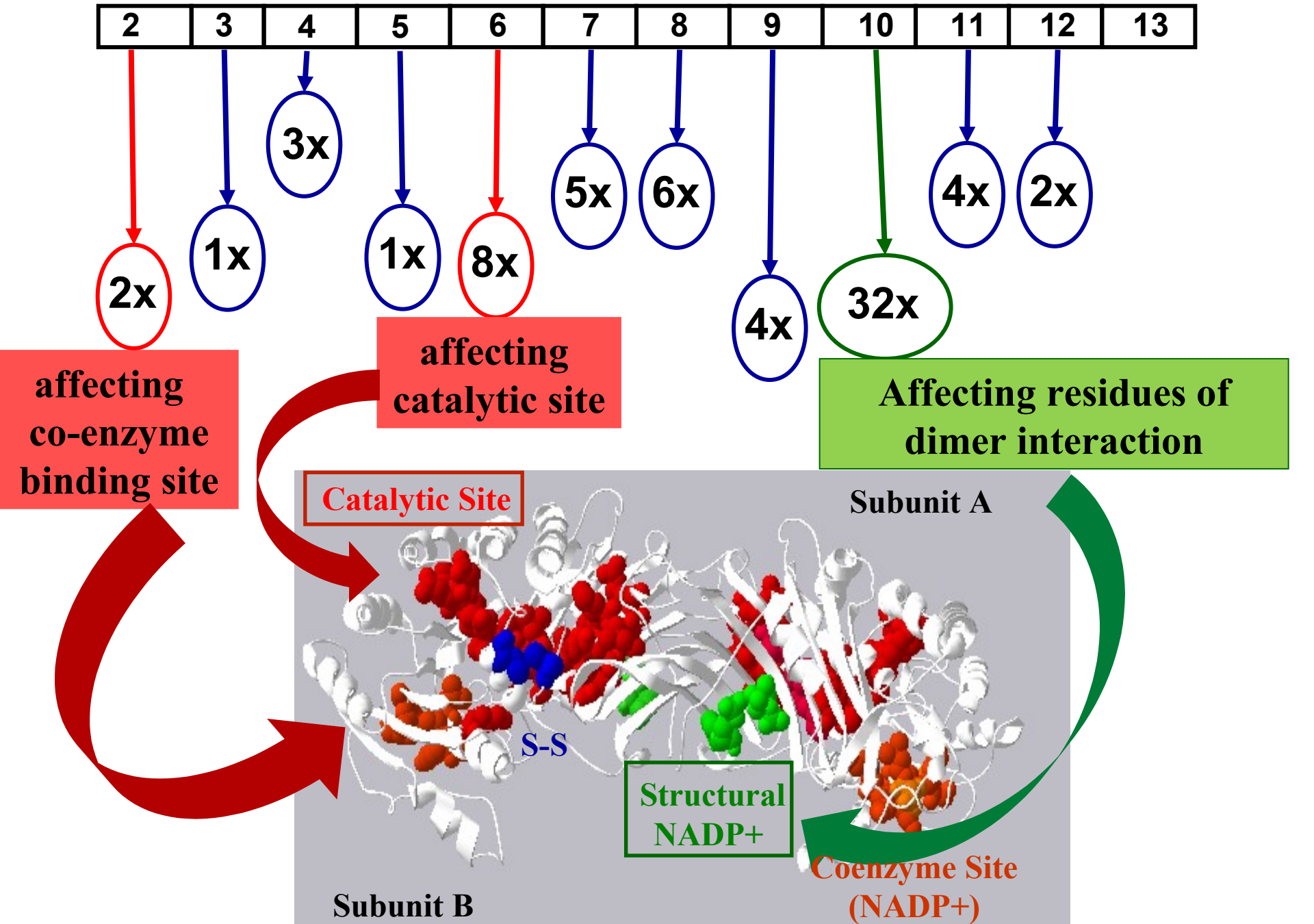
▬ **small deletions** **x** **nonsense mutation** ***f*** **splicing mutation**

Type of mutation	Nomber of cases
Single missense	164
Double or triple missense	8
In frame deletions	9
Splice Mutation (IVS-X)	1
Nonsense Mutation (G6PD Georgia)	1
Total	**183**

including	70 mutants	type I.
	40 mutants	type II
	36 mutants	type III
	Others	type IV

Note: Maternally transmitted severe G6PD deficiency is embryonic lethal. Longo L *et al*. The EMBO journal 16: 4229 – 4239, 2002

G6PD Exons | **Mapping of G6PD mutants « Type I » leading to a chronic hemolytic anemia**



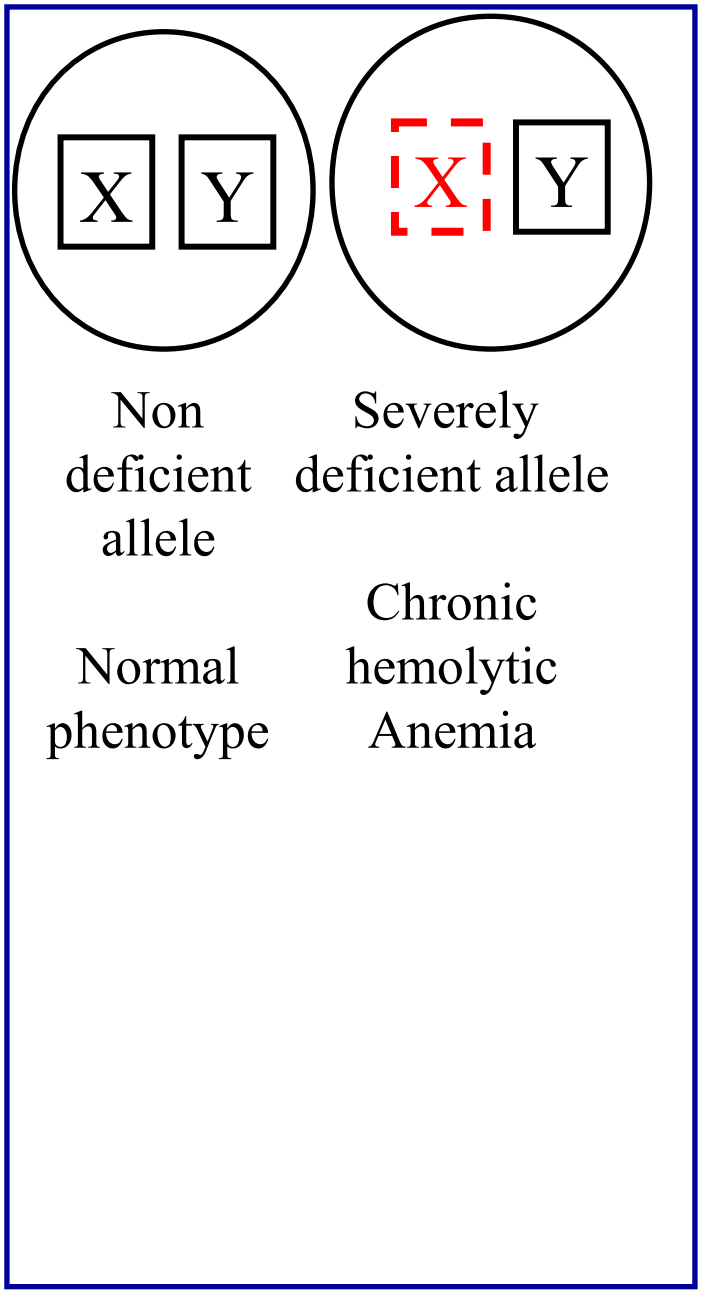
G6PD deficiency in females

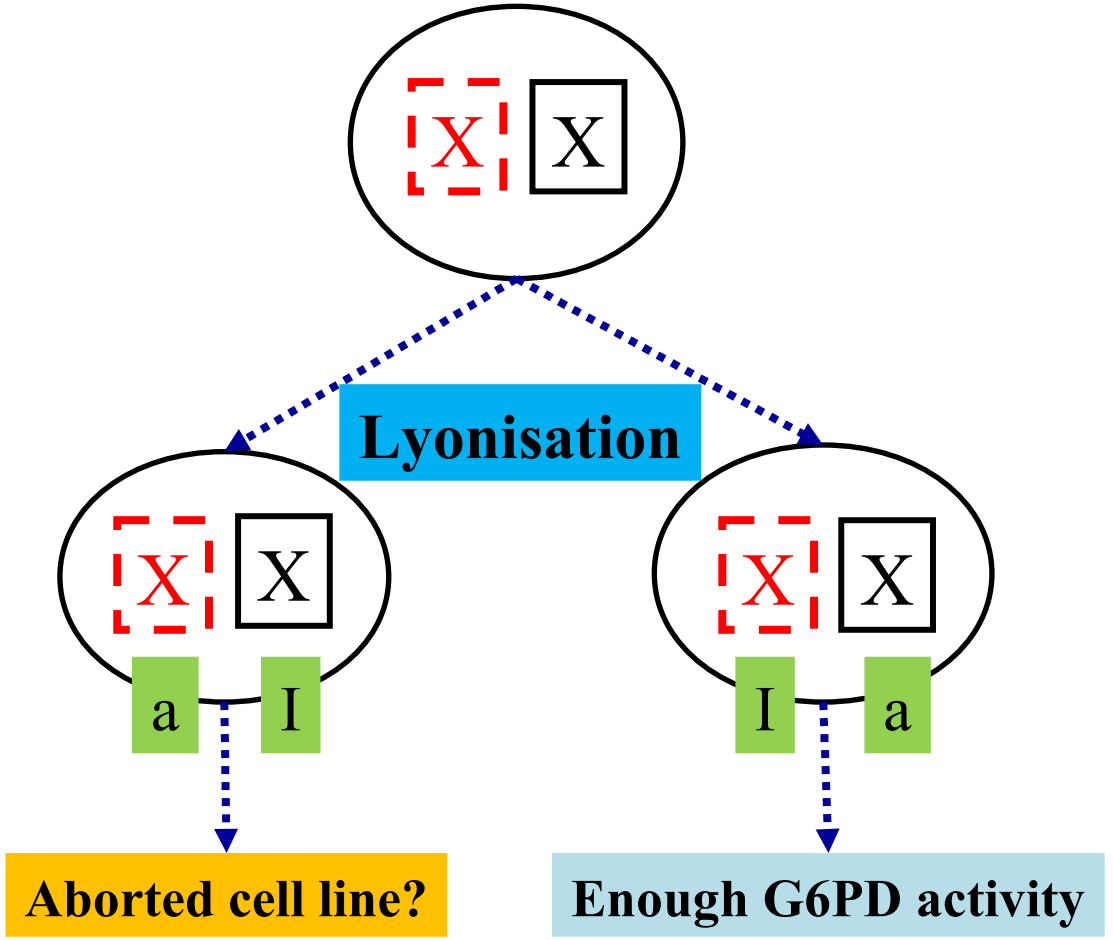
- Compound heterozygous or homozygous is not rare in the regions with highly frequent of deficient G6PD alleles
    - The situation similar to that of the G6PD-deficient males
- Less clinical manifestations in heterozygote status:
- Problem of neonatal screening in heterozygote females
    - **Around 50% of heterozygotes have normal G6PD activity**
- Adult heterozygote females with iron deficiency can have a normal G6PD activity
- Extreme Lyonization (non random X-inactivation)
- after about 55 years of age, the frequency of skewed X inactivation increases.

Blood Cells Mol Dis. 2011 Mar

Chronic hemolytic anemia is associated with a new G6PD in-frame deletion in an older woman.

G6PD Tondela: 18-bp in-frame deletion mapping in exon 10 (residues: LNERKA)





Non random X inactivation

Late onset chronic hemolytic anemia

Awareness of G6PD deficiency in elderly females related to acquired skewing of lyonization with age

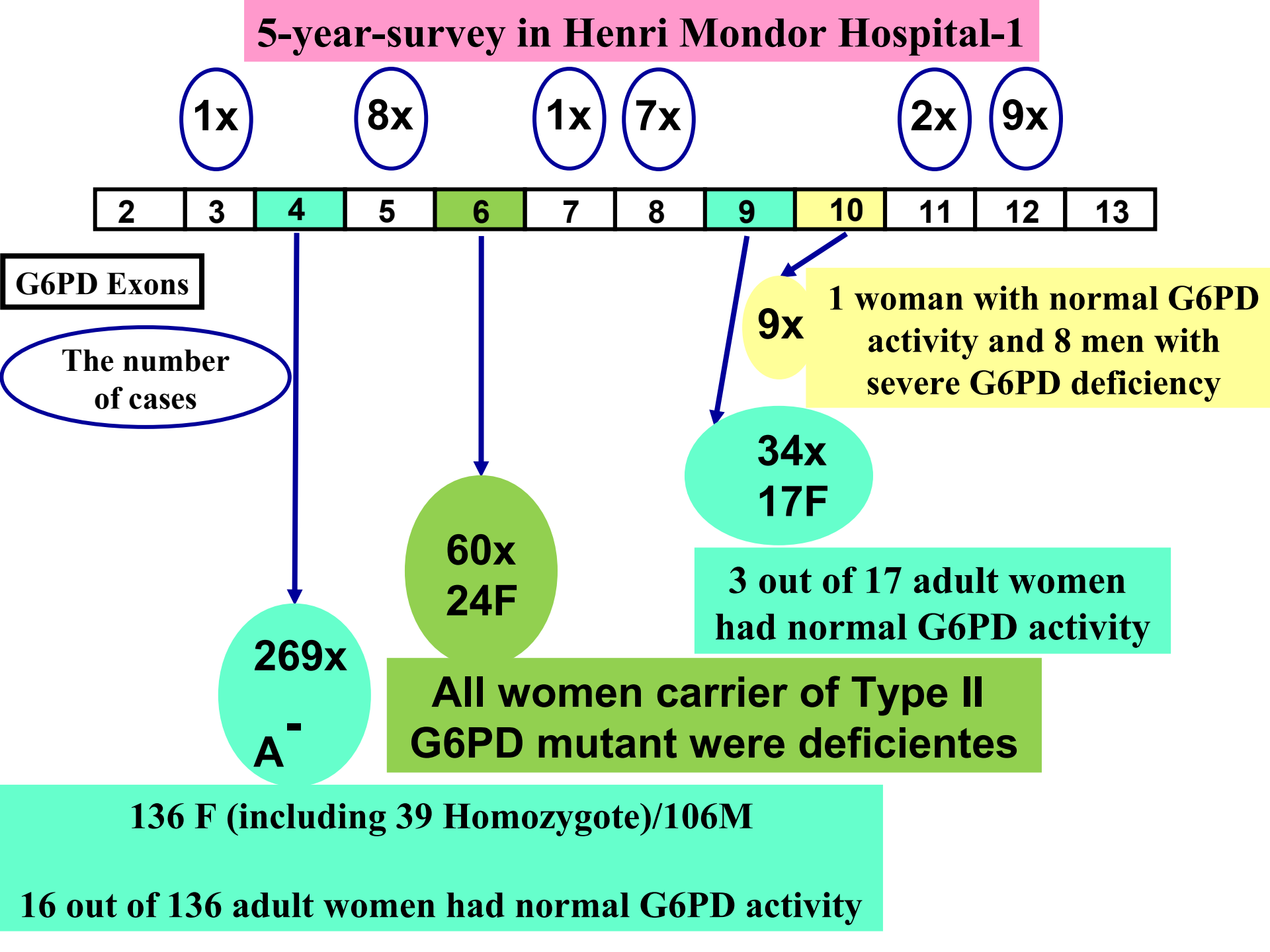
Cell selection: In the most majority of the heterozygote G6PD class I

Filosa S et al. Am. J. Hum. Genet. 59:887-895, 1996

Previously Published Data on the Expression of G6PD Class I Variants in Heterozygotes

G6PD Variant[a]	G6PD Activity (% of Normal[b]) Hemizygote(s)	Heterozygote(s)	Reference
Albuquerque	0, 0	90, 94	Beutler et al. (1968)
Barcelona	0	40[c]	Vives Corrons et al. (1982)
Bari	<1	100, 100	Filosa et al. (1994)
Chicago	9	28	Beutler et al. (1968)
Duarte	10	101	Beutler et al. (1968)
Genova	1.3	113[d]	Gaetani et al. (1990)
Harilaou	<1[e]	82.1	Town et al. (1990)
Nagano	1.7	86	Takahashi et al. (1982)
Portici	.86	89, 100	Filosa et al. (1992)
San Francisco	0	70, 63	Mentzer et al. (1980)
Walter Reed	7	87.2	Beutler et al. (1986)
Wayne	6, 10	80	Ravindranath and Beutler (1987)

Genova (Type I) **82%** **Present study**


5-year-survey in Henri Mondor Hospital-1
1x
8x
1x
7x
2x
9x
2
3
4
5
6
7
8
9
10
11
12
13
G6PD Exons
The number of cases
9x
1 woman with normal G6PD activity and 8 men with severe G6PD deficiency
34x
17F
3 out of 17 adult women had normal G6PD activity
60x
24F
All women carrier of Type II G6PD mutant were deficientes
269x
A-
136 F (including 39 Homozygote)/106M
16 out of 136 adult women had normal G6PD activity

5-year-survey in Henri Mondor Hospital-2

Sixteen heterozygote females for G6PD A^- with normal G6PD Activity

Iron deficiency in the majority of cases (aged from 20 to 40 years old)

About 50 cases at homozygotes state and One case of compound heterozygous

(G6PD A^-, Med, Viangchan and Canton)

Familial study of G6PD

Three cases of heterozygote females for G6PD A^- (ages: 74, 63 and 50 year-old) **with very low enzyme activity (similar to that of homozygotes)**

no abnormality in cytogenetic study

X-Inactivation

Two cases of hemizygote G6PD variant type II (G6PD Med) with normal G6PD activity (Post-hemolysis regeneration).

Conclusions

Evaluation of enzymologic tests must be done regarding to age, sex, familial and clinical history (**iron deficiency**, associated regererative anemia, …).

In the case of incomptability between phenotype and genotype, the other causes of hemolytic anemia (ex. compound heterozygous, other RBC enzymopathies, membrane abnormalities, …).

Molecular analysis of G6PD locus in the mothers of the male individus with non spherocytic chronic hemolytic anemia. The result of molecular study is useful for genetic counseling; germinal or somatic mutation.

Acknowledgements

Ms. Catherine Bimet
Ms. Claire Albert
Ms. Nathalie Le Metayer

Pr. Michel Goossens
Dr. Marie-Odette Balleygier
Dr. Claude Préhu
Dr Serge Pissard
Dr. Michel Rahuau
Dr. Henri Wajcman

Pr. Frédéric Galactéros
Dr. Dora Bachir
Dr ..Anoocha Habibi

All of the physiciens for providing blood and DNA samples
Dr. Patricia Martinez, Dr Anne-Marie Soummer, Dr Anne Lambilliote, Pr. Christian Rose, …