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Lionization Phenomenon: Random or Predictable Event?

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Lionization Phenomenon: Random or Predictable Event?

Prof.Dr.Hüseyin Onay M.D.,Ph.D.

MULTİGEN **Genetic Diseases Diagnosis Center, Turkey**

GENE2INFO **Health Informatics, Turkey**

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FRASE DE SALVAMENTO

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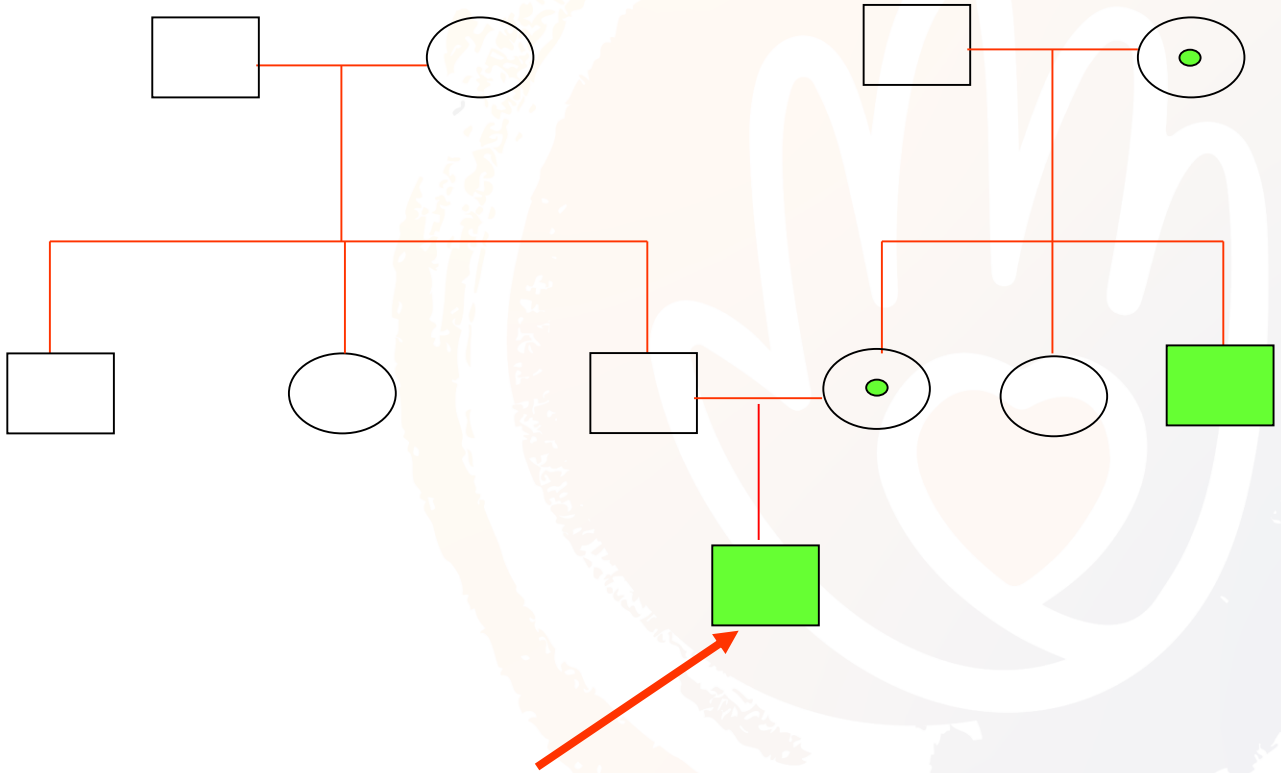
X-Linked Recessive Inheritance



Hemizygous

Thompson&Thompson, Genetics in Medicine, 2016

Female Patients with Heterozygous Mutations

article

January 2007 · Vol. 9 · No. 1

Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life

Raymond Y. Wang, MD[1,2], Alicia Lelis, MS[1], James Mirocha, PhD[3], and William R. Wilcox, MD, PhD[1,2,4]

Purpose: To determine if there is significant symptomatology in women with heterozygous α-galactosidase mutations. **Methods:** Data from medical records of the 44 heterozygous females followed at Cedars-Sinai Medical Center were compiled and analyzed for symptoms of Fabry disease. Quality of life data were also analyzed. **Results:** Seventy-six percent were referred due to an affected male relative; 76% reported acroparesthesias as their first symptom. A mean of 15.7 years elapsed from onset of first symptoms to the diagnosis. Quality of life, measured by the SF-36 survey, was globally reduced. Pain affected mood and enjoyment of life. Central/peripheral nervous, cardiopulmonary, and renal system manifestations of Fabry disease were present far above that predicted for random X-inactivation of the normal allele. Fatigue, present in 59%, was associated with reduced maximum oxygen consumption ($P = 0.049$); exercise intolerance, present in 83%, was associated with reduced maximal heart rate during exercise testing ($P = 0.0089$). Women diagnosed via family history experienced more angina ($P = 0.035$), decreased vibration sense ($P = 0.026$), and had a worse percentage predicted FEF_{25-75} ($P = 0.037$) compared to women diagnosed because of symptoms. **Conclusions:** This study indicates that the asymptomatic female carrier of Fabry disease is the exception, not the rule: heterozygotes suffer from significant multisystemic disease and reduced quality of life and must be monitored and treated accordingly. ***Genet Med* 2007:9(1):34–45.**

Key Words: *Fabry disease, female, heterozygote, natural history, outcome, SF-36, symptom, quality of life*

Neurologic and psychiatric data for the CSMC Fabry heterozygote cohort

	No. positive	N	Prevalence
TIA	9	37	24%
Stroke	8	36	22%
Brain MRI c/w stroke/white matter Δs	8	25	32%
Tinnitus	22	38	58%
Hearing loss	14	38	37%
Hypohidrosis	25	42	60%
Temperature intolerance	19	39	49%
Decreased vibration sense	33	42	79%
Acroparesthesia	26	40	65%
Depression/antidepressant use	21	34	62%
Anxiety/anxiolytic use	13	33	39%

Renal data for the CSMC Fabry heterozygote cohort

	No. positive	N	Prevalence	
CrCl <90 mL/min/1.73 m^2	21	36	58%	
CrCl <60 mL/min/1.73 m^2	7	36	19%	
End-stage renal disease	5	40	13%	
24-h urine microalbumin >30 mg	8	10	80%	
24-h urine protein >150 mg	10	18	56%	
	Mean	SD	Median	N
Blood urea nitrogen (mg/dL)	18.8	15.3	14	39
Serum creatinine (mg/dL)	1.3	1.8	1.8	39
Creatinine clearance (mL/min/1.73 m^2)	80	33.3	83.3	35
24-h urine microalbumin (mg) (normal <30 mg)	621	1540	88	10
24-h urine protein (mg) (normal <150 mg)	1343	2625	191	18

Cardiac data for the CSMC Fabry heterozygote cohort

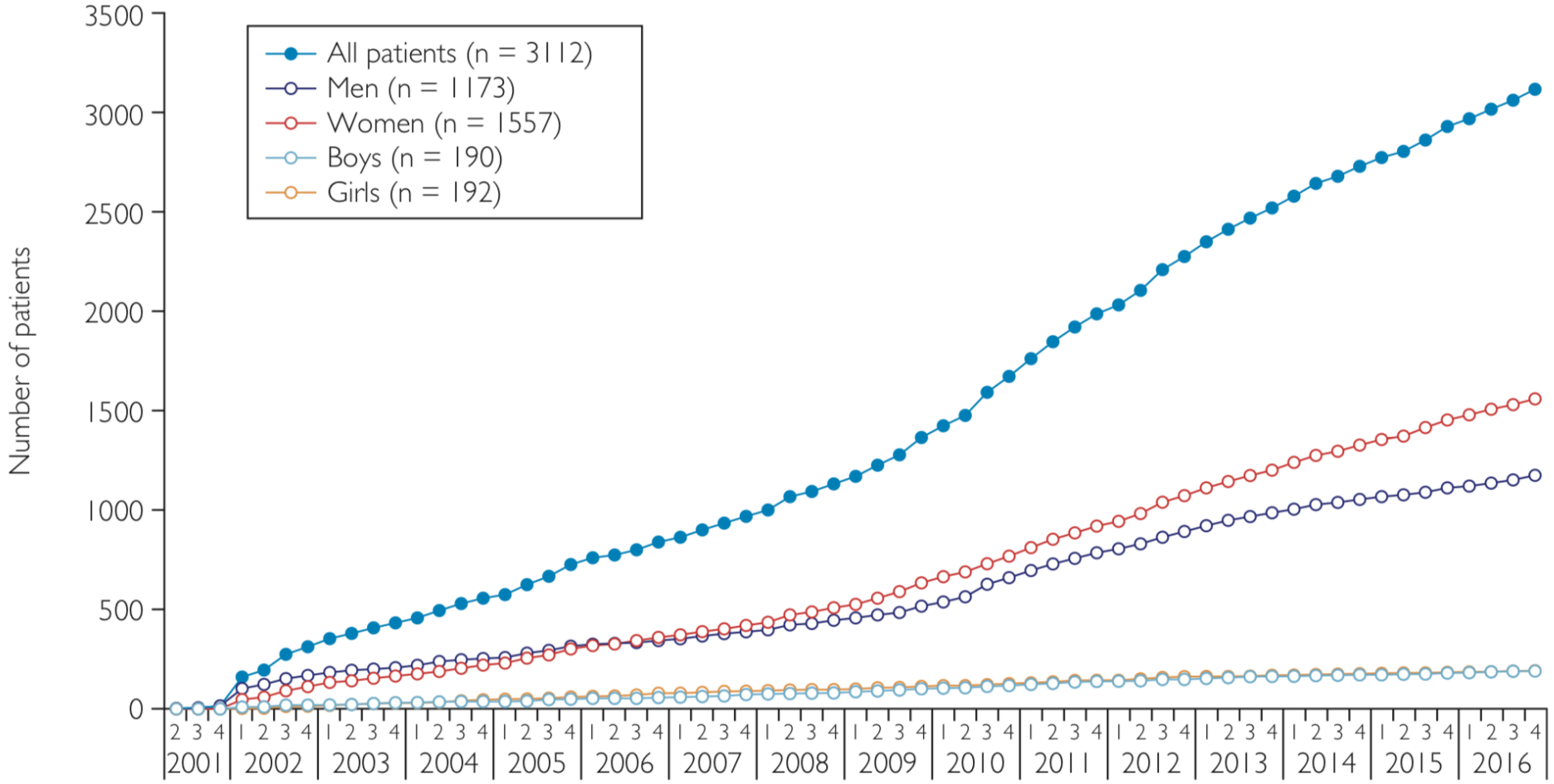
	No. positive	N	Prevalence	
Angina	10	36	28%	
Hypertension	16	37	43%	
Palpitations	8	38	21%	
EKG abnormalities	25	33	76%	
Mitral/aortic insufficiency	18	31	58%	
LVH (by EKG and/or Echocardiogram)	8	34	24%	
	Mean	SD	Median	N
Heart rate (BPM)	67	12	66	39
PR interval (ms)	155	23	152	32
IVS_d (mm)	9.9	1.4	10	28
$LVPW_d$ (mm)	10.1	1.7	11	28
EF%	58	7.6	58	27

Wang RY et al. Genet Med (2007)

Fabry Registry-2013

	Men	Women
Total Number of Patients Enrolled, N	2144	2338
Regional Enrollment, n (%)		
Europe	843 (39.3)	944 (40.4)
North America	872 (40.7)	1019 (43.6)
Asia Pacific	199 (9.3)	217 (9.3)
Latin America	230 (10.7)	158 (6.8)
Current Age*, All Patients (yrs)		
n	2141	2333
Mean (SD)	40.1 (16.75)	44.4 (17.92)
Median	41.8	45.1
Q1,Q3	27.9, 52.8	30.7, 57.7
Min, Max	1.8, 87.0	1.2, 92.2
Current Age Distribution, n (%)		
Age ≥18 years	1904 (88.8)	2148 (91.9)
Age <18 years	237 (11.1)	185 (7.9)
Age at Fabry Diagnosis (yrs)		
n	2123	2263
Mean (SD)	27.8 (17.20)	33.9 (18.05)
Median	26.4	33.0
Q1,Q3	13.9, 40.2	19.2, 47.4
Min, Max	0.0, 81.1	0.0, 82.4

Fabry outcome survey-2016



Female Fabry Cases

Two different groups of cases

1.Cases who have severe disease like males

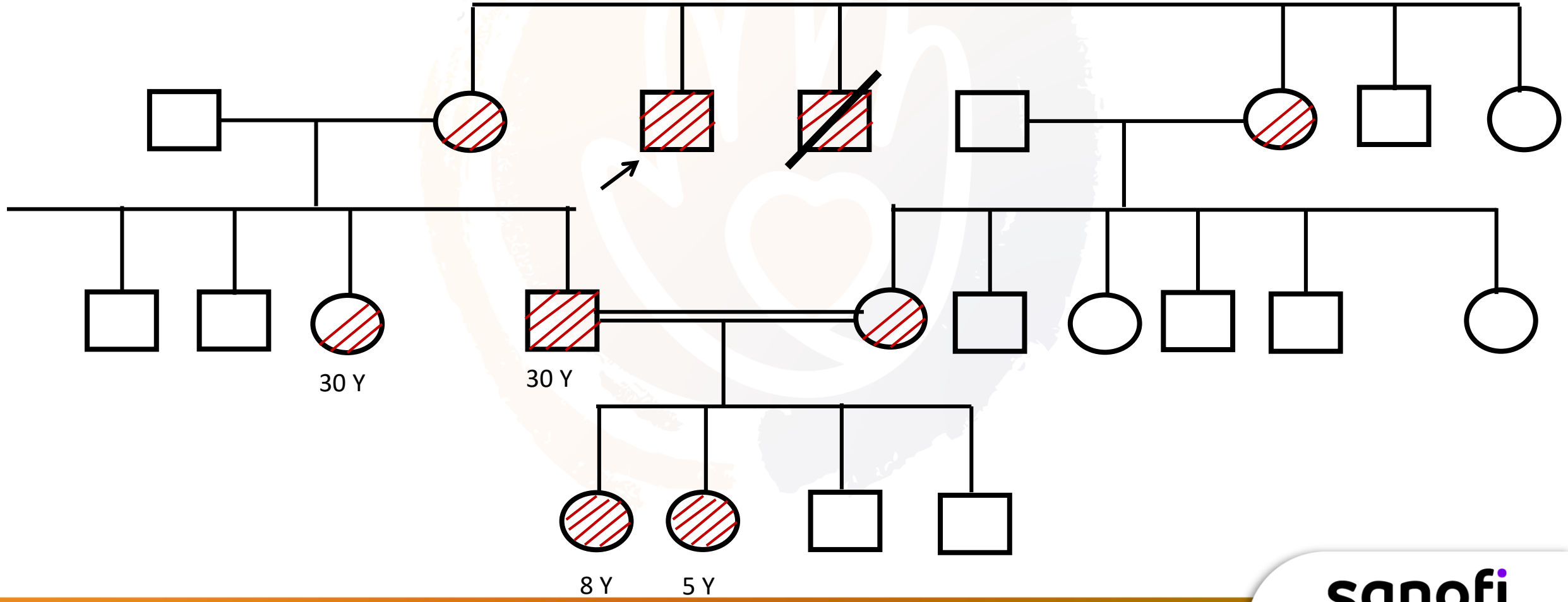
- Affected father, carrier mother
- Turner Syndrome cases (45,X)
- Uniparental isodisomy

2.Heterozygous carriers who present clinical features

- Skewed X inactivation
- Gene specific epialleles

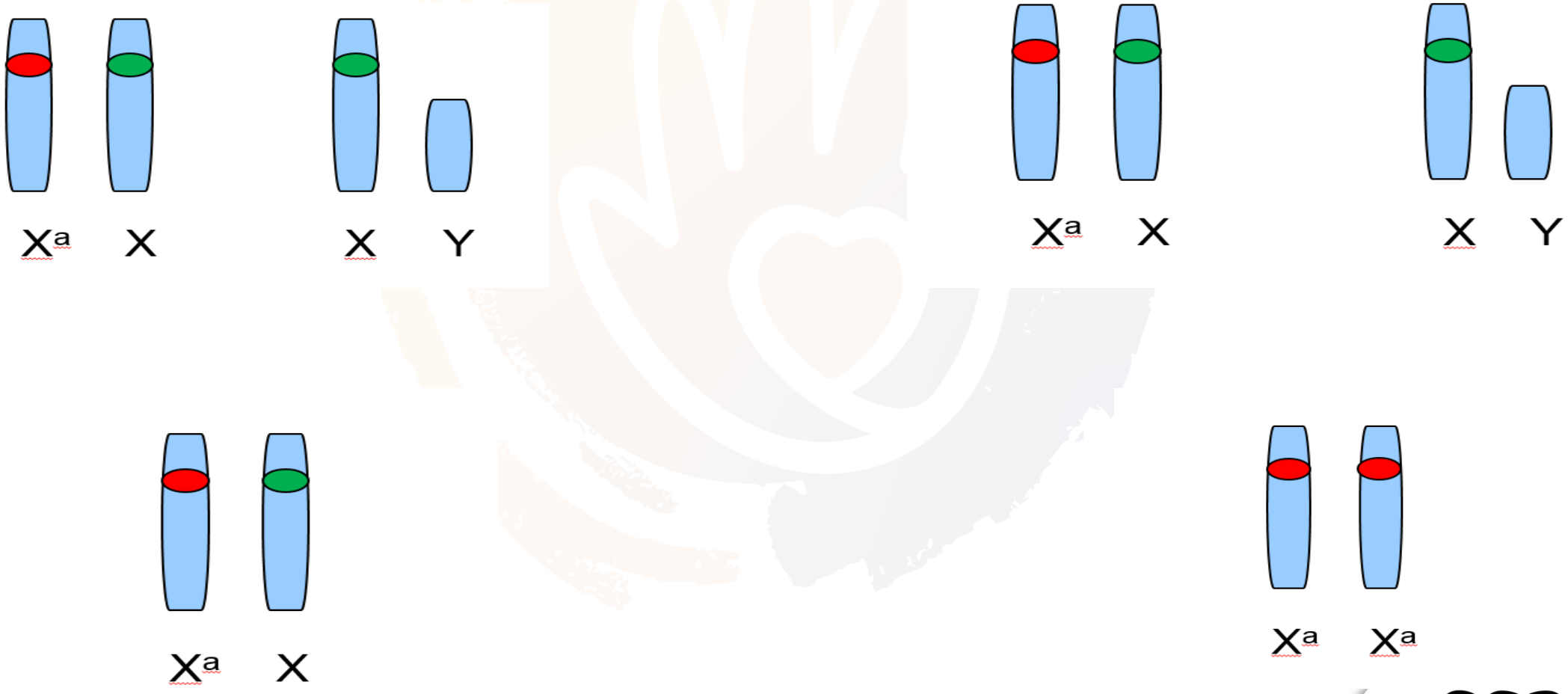
Female Fabry Cases

p.P409L (c.1226 C>T)



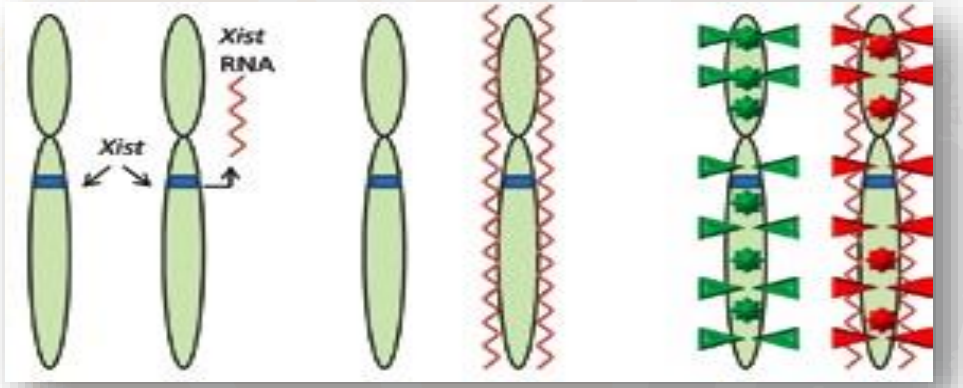
Female Fabry Cases

- Uniparental isodisomy

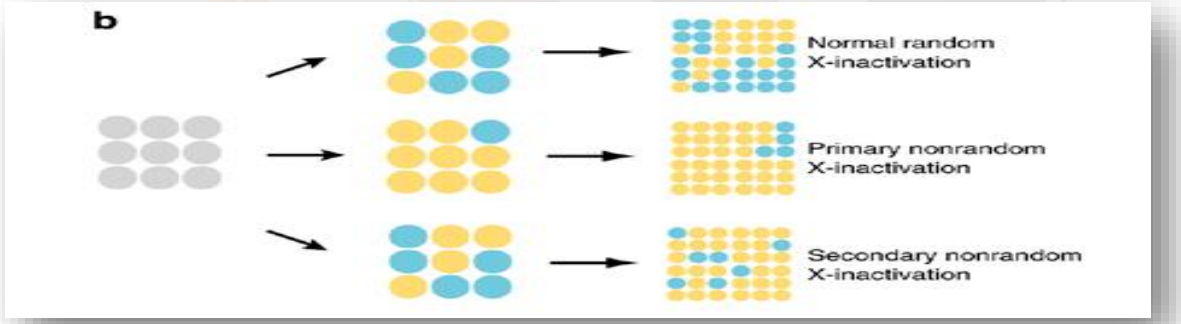


Female Fabry Cases

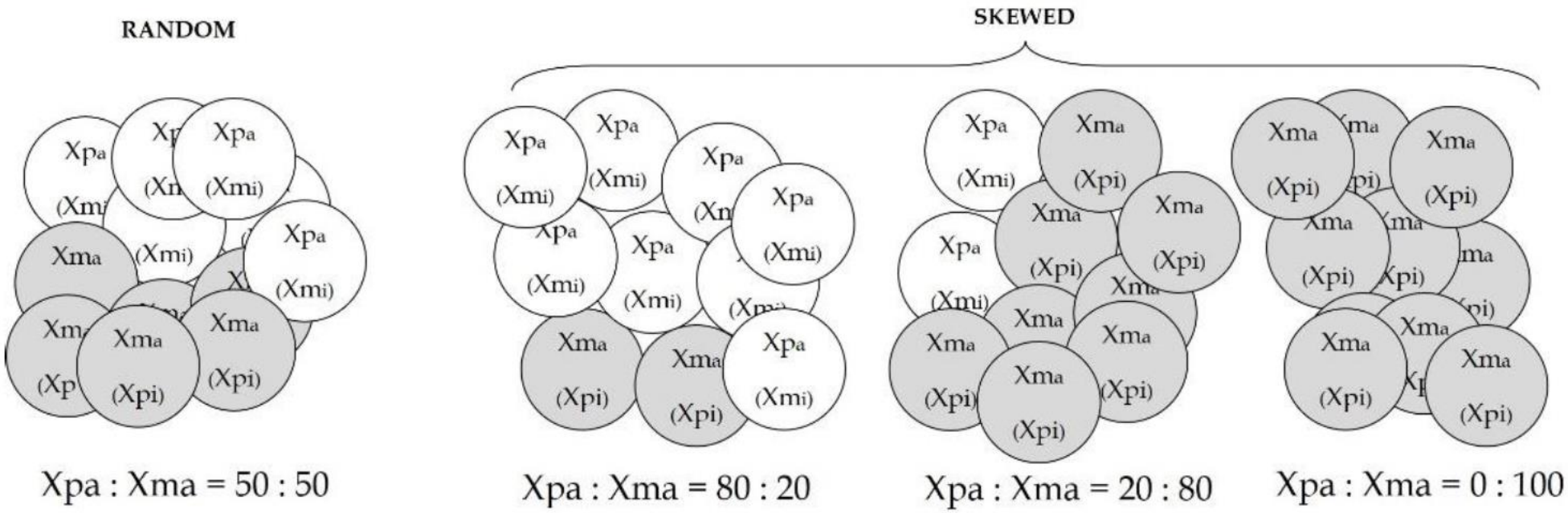
Random X inactivation



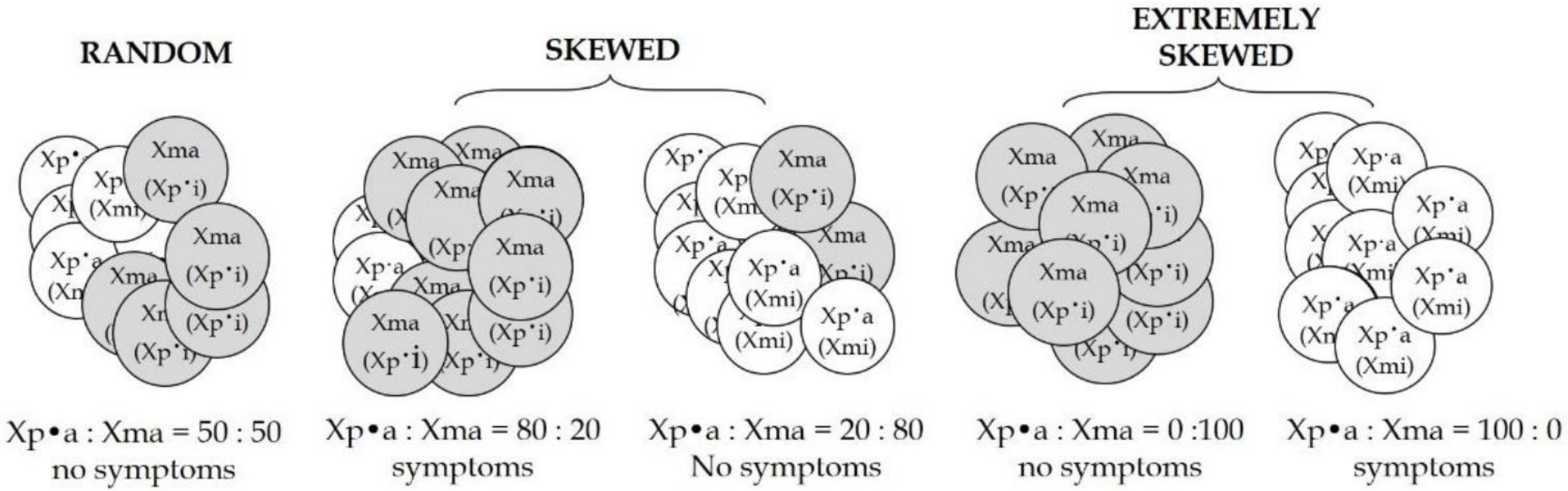
Skewed X inactivation



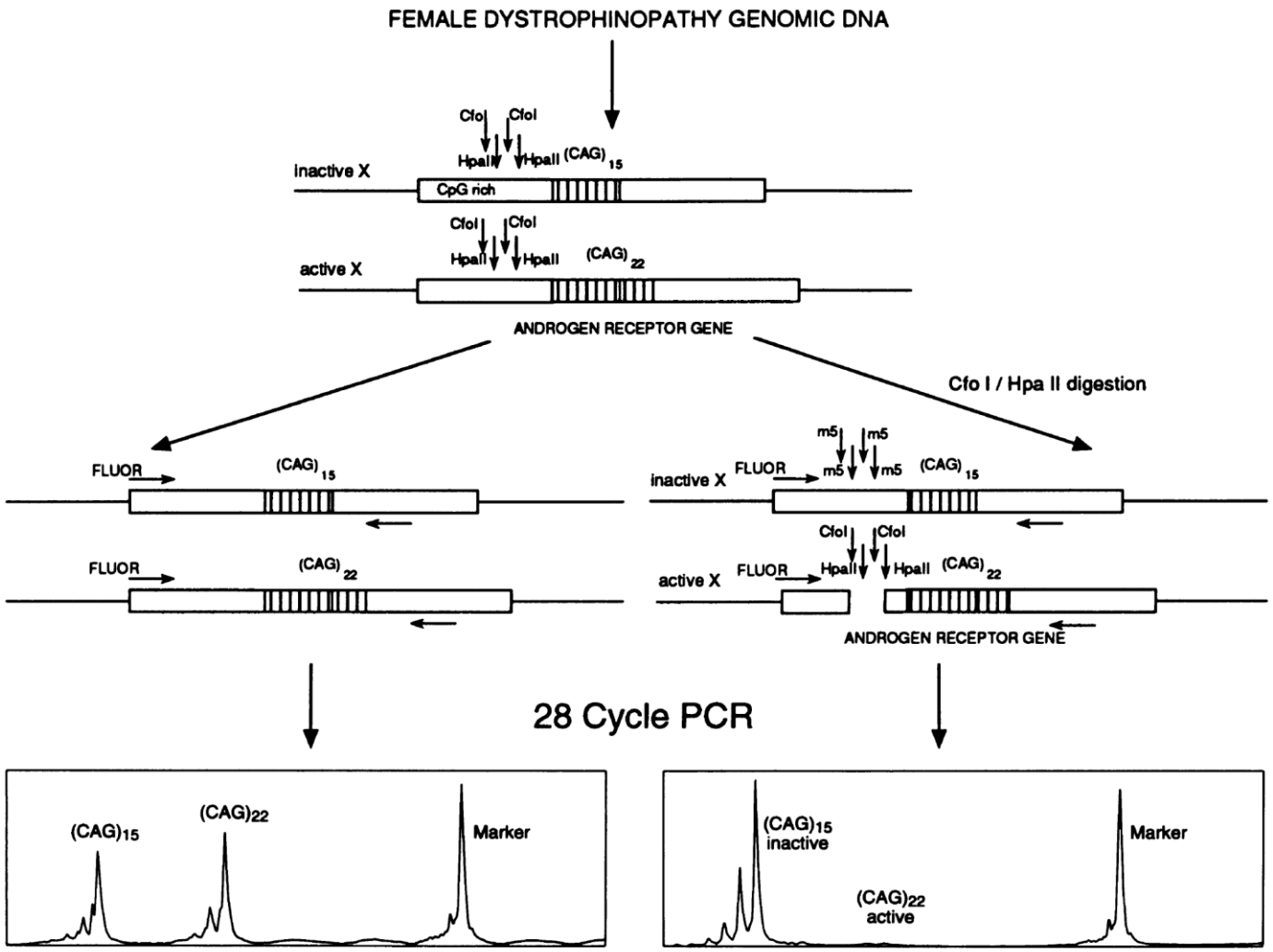
Thompson&Thompson, Genetics in Medicine, 2016



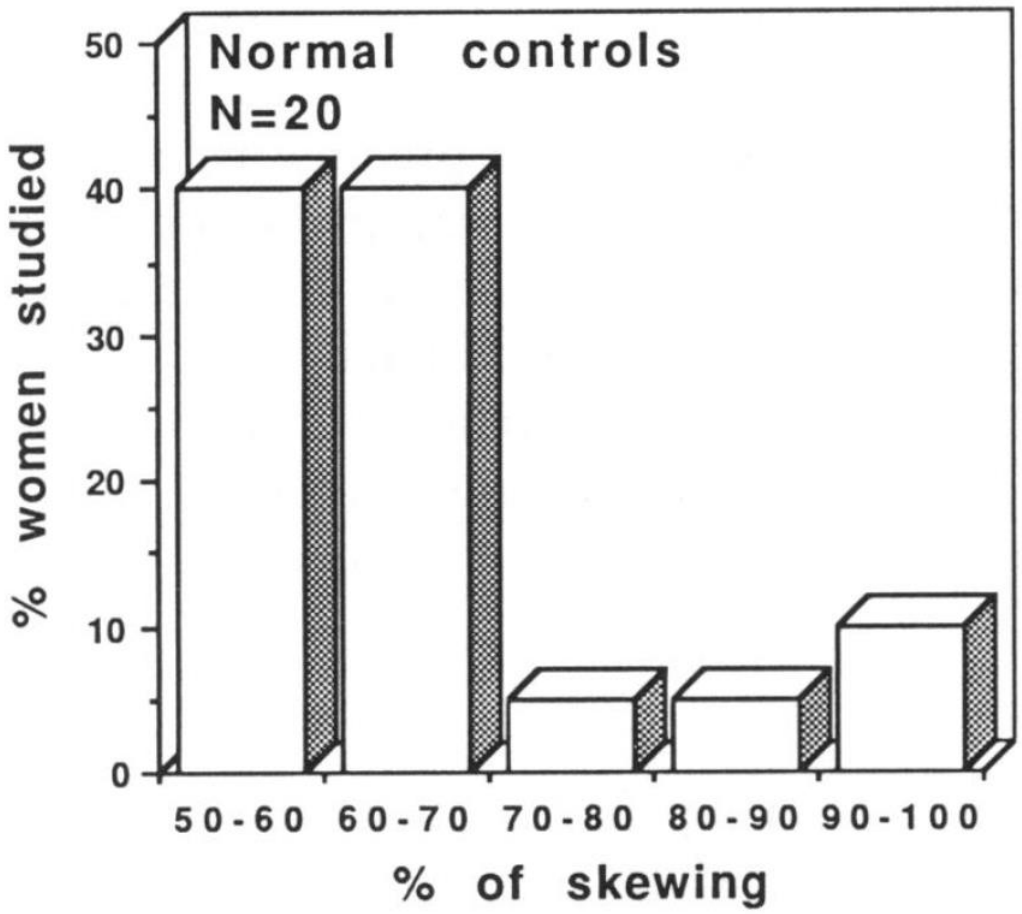
Viggiano E et al., *Int. J. Mol. Sci. 2021*



Viggiano E et al., *Int. J. Mol. Sci. 2021*



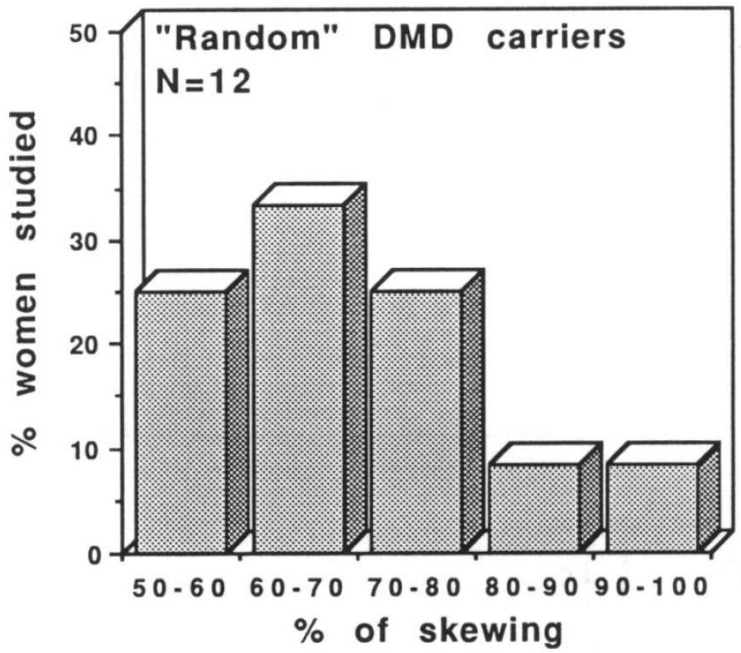
Pegoraro E et al., Am.J. Hum. Genet., 1994

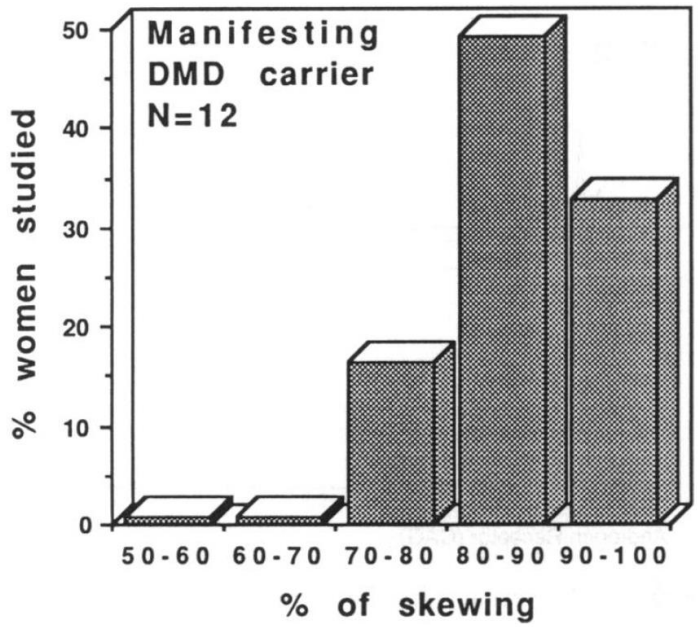


-10-15% of females have nonrandom XCI by chance.

-Increases with age

Pegoraro E et al., Am.J. Hum. Genet., 1994





-Nonrandom XCI ratio: 86:14 to 100:0

- Suggests nonrandom pattern of XCI in tissue type tested

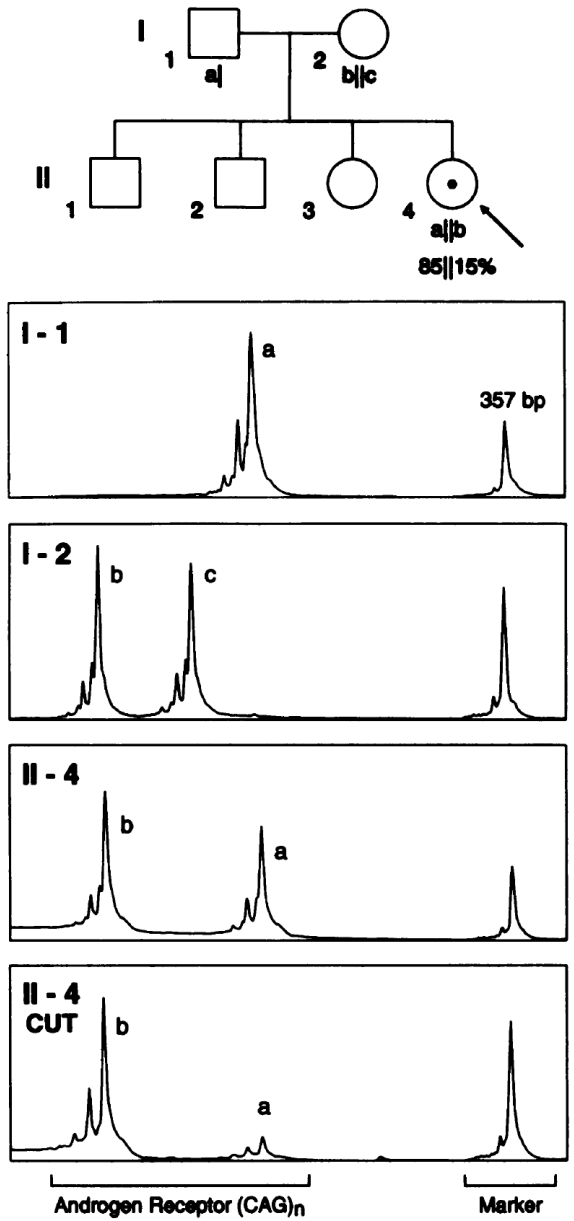
Random XCI ratio: 50:50 to 74:26

- Suggests random pattern of XCI in tissue type tested

-Uninformative result: XCI ratio cannot be determined.

- Maternally and paternally derived X chromosomes could not be distinguished

Pegoraro E et al., Am.J. Hum. Genet., 1994

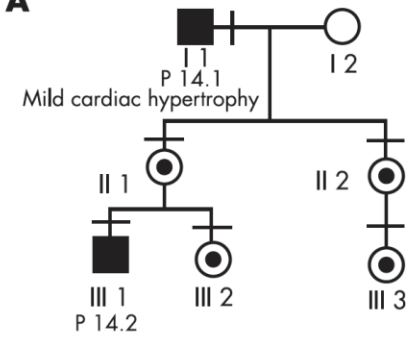


Pegoraro E et al., Am.J. Hum. Genet., 1994

Limitations

- Testing is limited to XX females only
- The assay will be uninformative in up to 20% of females due to homozygosity for the polymorphic *AR* gene locus analyzed
- XCI patterns may differ among tissues
- Test will not determine if the X-inactivation pattern is associated with rearrangements of the X chromosome
- If nonrandom XCI pattern is present, the parent of origin of the active X cannot be determined without testing parental samples
- Test is not recommended for prenatal diagnosis because XCI levels may differ in prenatal specimens and whole blood.

A

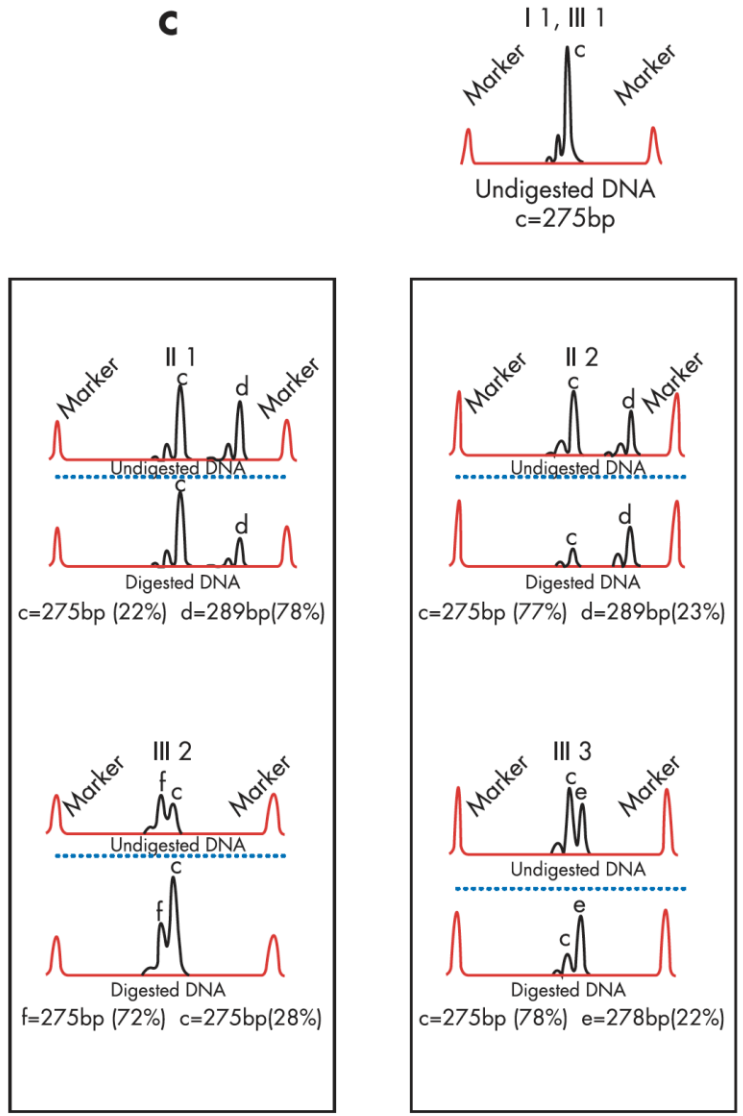


Pedigree of family 14

B

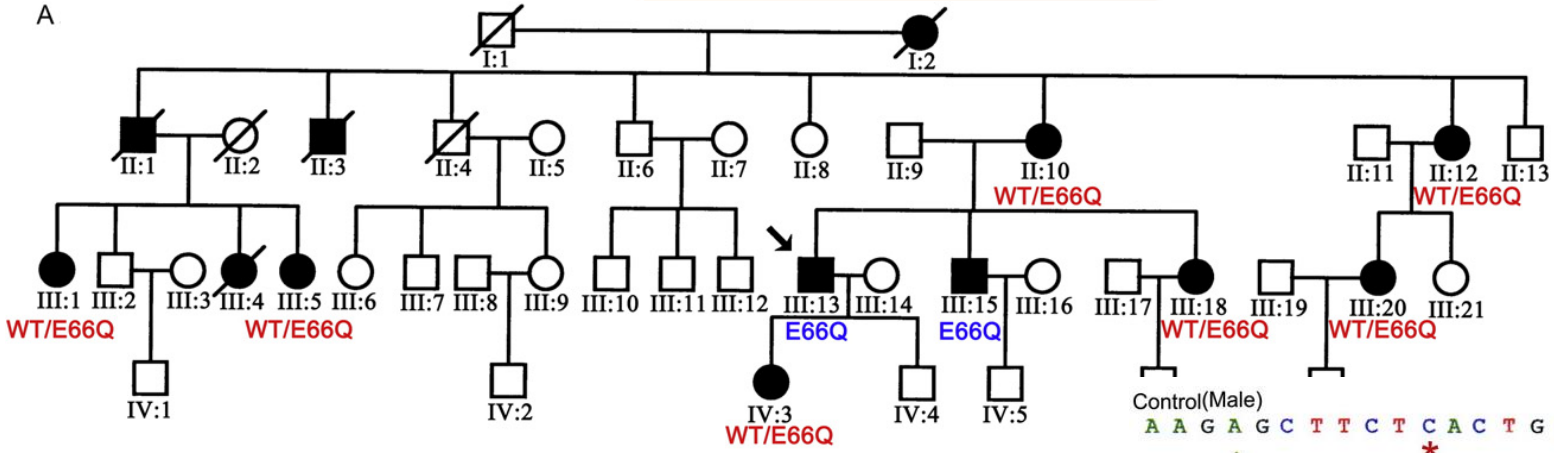
Carrier female	II 1	II 2	III 2	III 3
Age at present	39 y	38 y	8 y	6 y
Age at diagnosis	38 y	37 y	7 y	5 y
Hypohidrosis	No	No	No	No
Pains	No	Yes, since 6 y	No	Yes, since 4 y
Fever crisis	No	No	No	No
Angiokeratoma	No	No	No	No
Cerebrovascular involvement	No	No	No	No
Cornea verticillata	+/-	+++	No	+
Renal insufficiency	No	No	No	No
Cardiac involvement	No	No	No	No
Lymphoedema	No	No	No	No
Enzymatic activity(%)	Normal	45	53	24
Hearing impairement	No	No	No	No

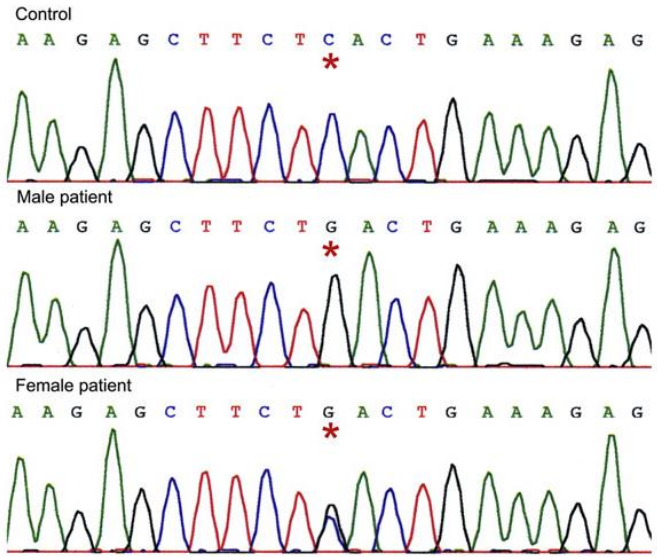
C

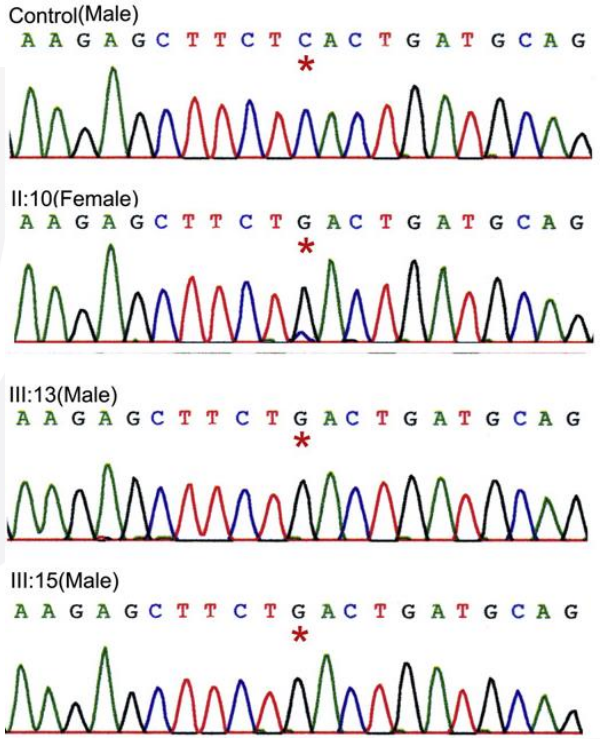


Morrone A et al.,J. Med. Genet., 2003

X Chromosome Inactivation





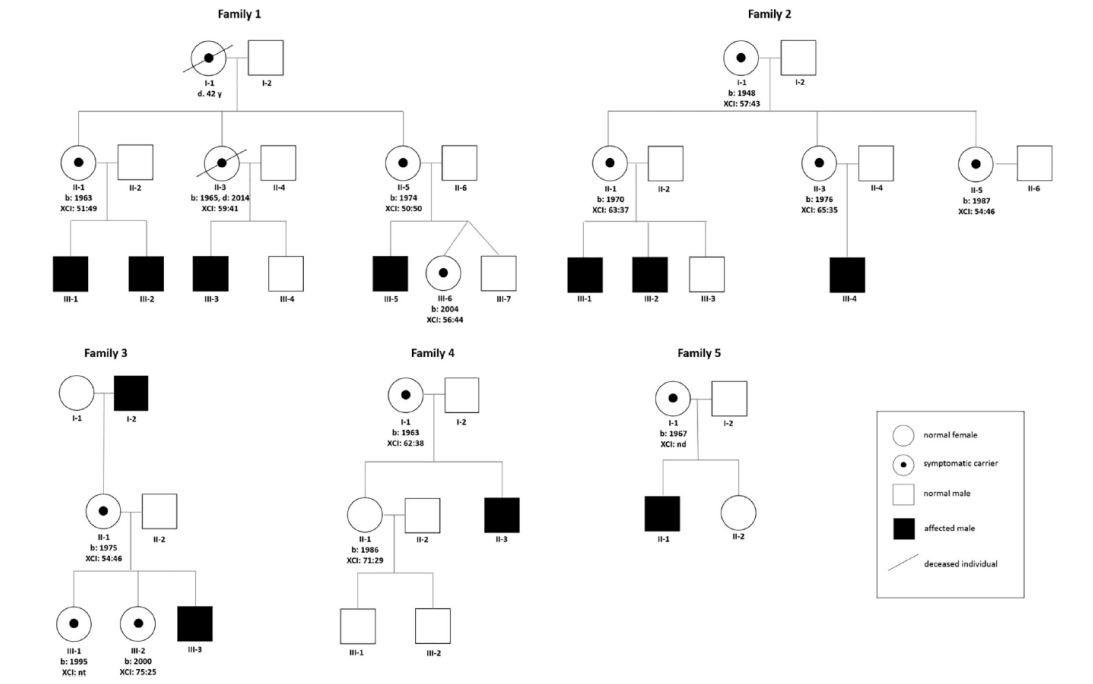


XCI analysis for female patients in the FD pedigree.

Patients NO.	II:10	II:12	III:1	III:5	III:18	III:20	IV:3
XCI ratios	25/75	28/72	26/74	32/68	38/62	37/63	30/70
Expressed mutant X allele ratios	75%	72%	74%	68%	62%	63%	70%

Peng H et al. Gene (2016)

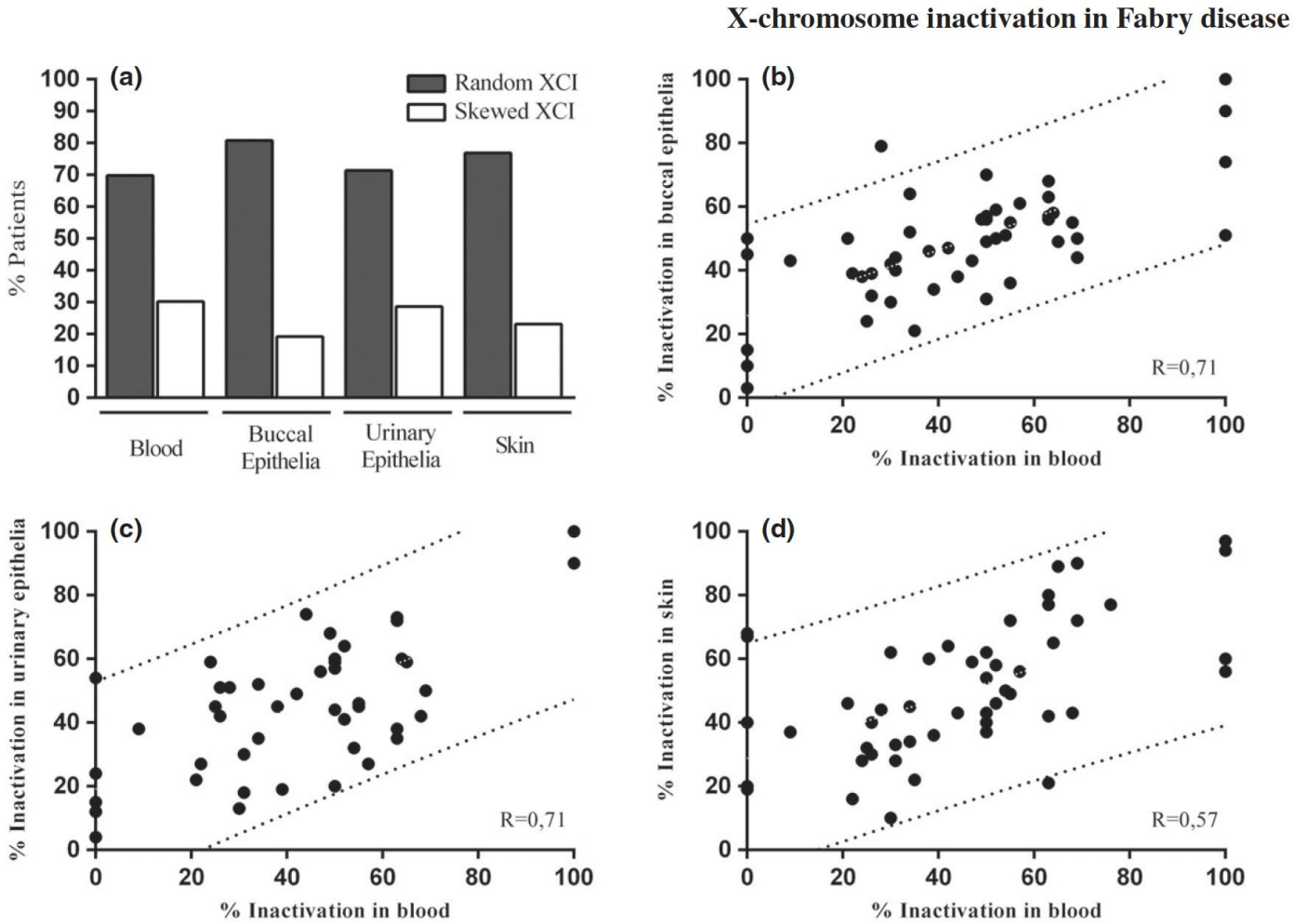
X Chromosome Inactivation



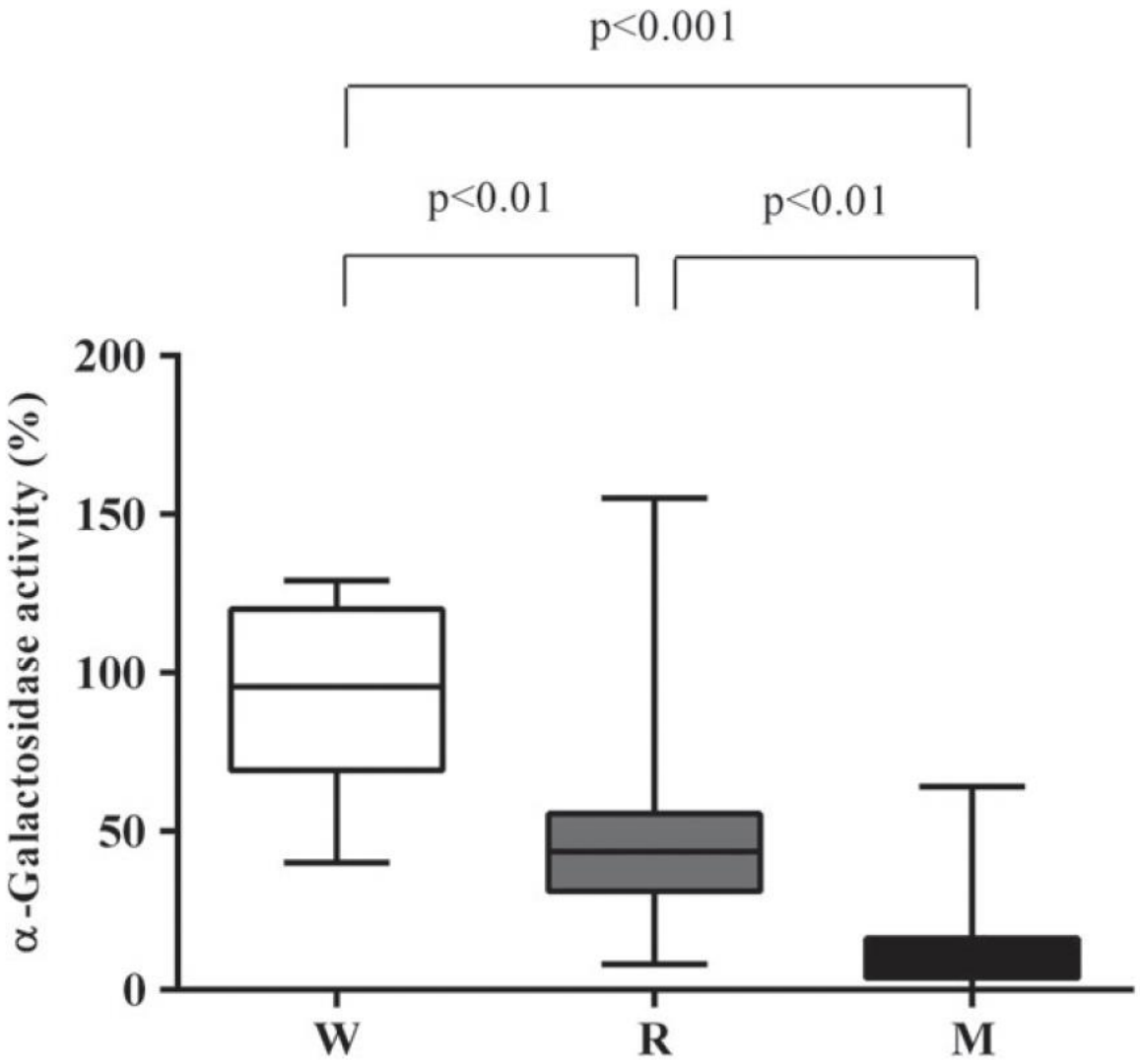
Family	Gene mutation	Location	Protein change
1	c.71G > A	Exon 1	p.Trp24Ter
2	c.803_806delTAGT	Exon 6	p.Leu268Ter
3	c.1055_1056delCT	Exon 7	p.Ala352AspfsTer22
4	c.1057_1058delAT	Exon 7	p.Met353AspfsTer21
5	c.1235_1236delCT	Exon 7	p.Thr412SerfsTer38

Family	Patient symbol	MSSI score[a]						FOS-MSSI score[b]						Age- and sex-adjusted score[c]	DS3[d]	XCI (saliva)
		G	N	C/V	R	Total	Severity	G	N	C/V	R	Total	Severity			
1	II-1	14	16	10	0	40	Moderate	13,5	12	12	0	37.5	Moderate	25.5	15.3	51:49
1	II-3	11	15	9	8	43	Severe	10,5	9	9	8	36.5	Moderate	25.2	14.3	59:41
1	II-5	6	9	8	4	27	Moderate	6	6	8	4	24.0	Moderate	15.5	8.7	50:50
1	III-6	2	1	0	0	3	Mild	2	0	0	0	2.0	Mild	0.0	1.6	56:44
2	I-1	13	10	20	4	47	Severe	11,5	3	18	4	36.5	Moderate	19.6	14.6	57:43
2	II-1	13	10	2	4	29	Moderate	13,5	6	1	4	24.5	Moderate	14.8	8.0	63:37
2	II-3	4	13	0	4	21	Moderate	4,5	8	0	4	16.5	Mild	8.9	4.3	65:35
2	II-5	8	11	0	4	23	Moderate	8	6	0	4	18.0	Mild	13.1	6.7	54:46
3	II-1	4	10	3	4	21	Moderate	4	7	3	4	18.0	Mild	9.2	5.0	54:46
3	III-2	1	5	0	0	6	Mild	1	4	0	0	5.0	Mild	2.2	2.0	75:25
4	I-1	7	10	11	0	28	Moderate	7	9	13	0	29.0	Moderate	16.7	7.3	62:38
5	I-1	11	7	0	0	18	Mild	9	6	0	0	15.0	Mild	4.4	9.6	nd

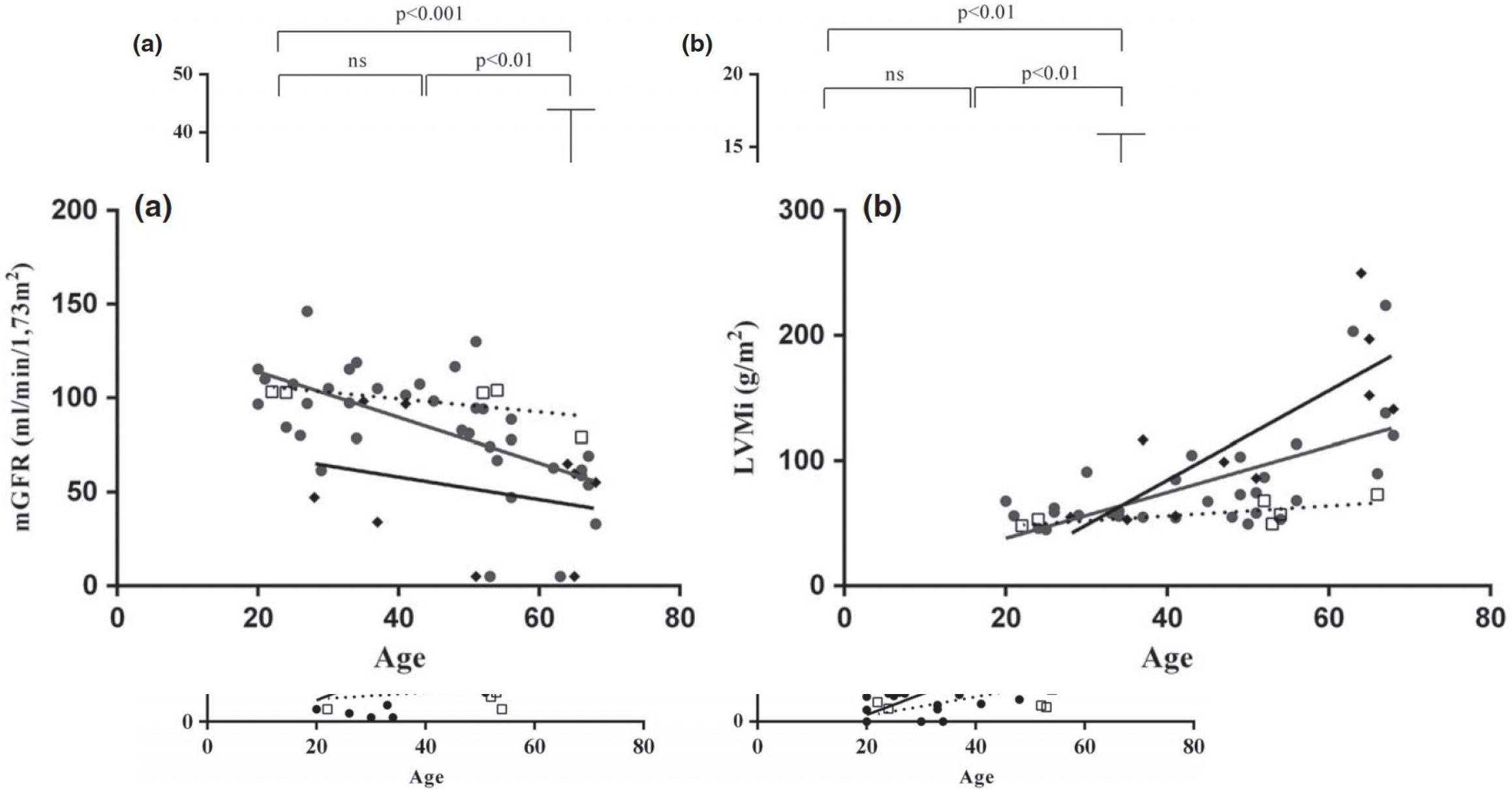
Juchniewicz P et al. Gene, 2018



Echevarria L et al., *Clin Genet 2016*



Echevarria L et al., *Clin Genet 2016*



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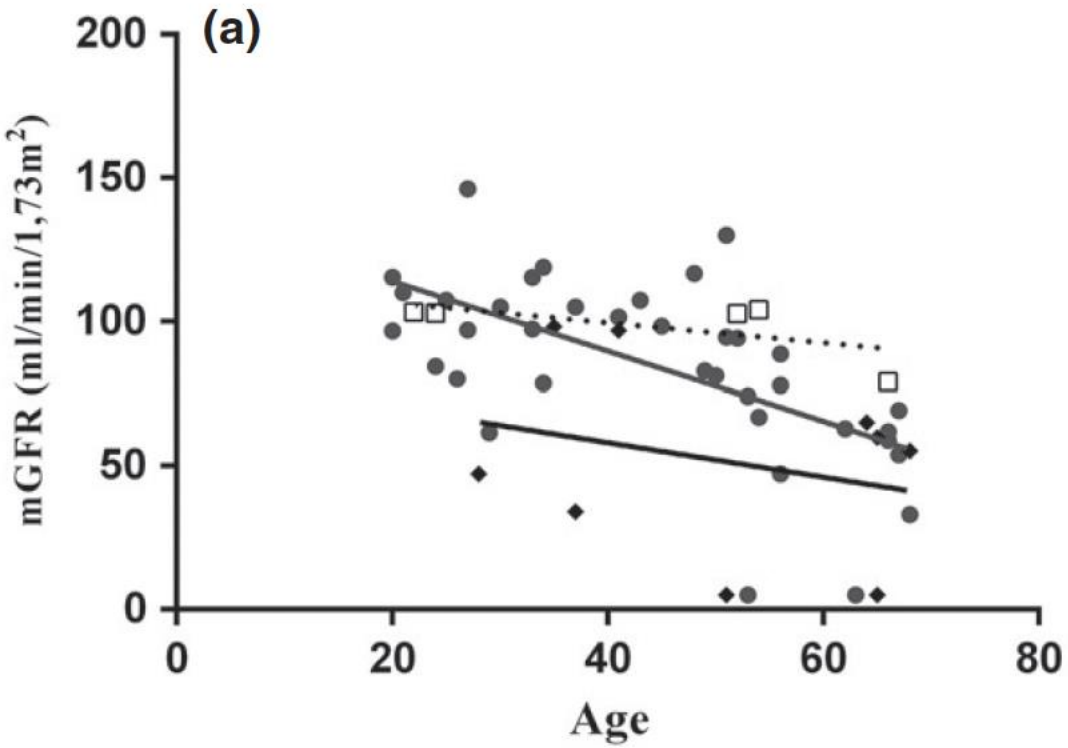
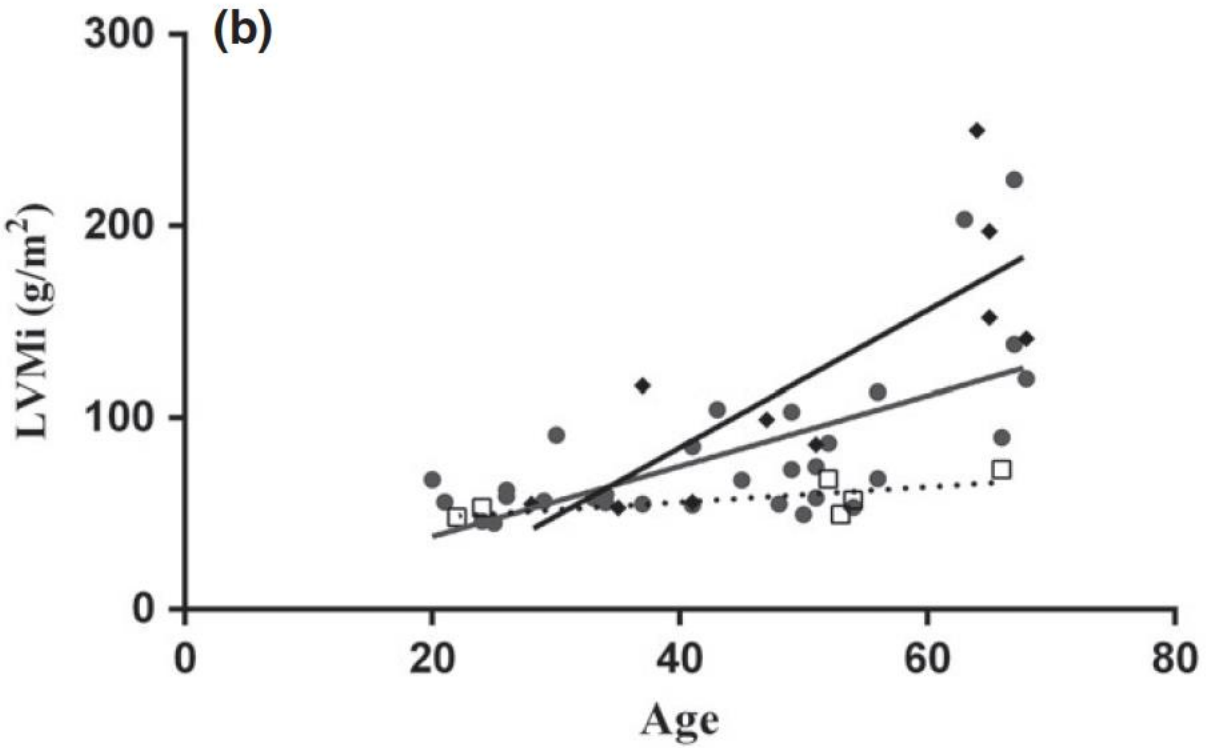




Echevarria L et al., *Clin Genet 2016*

References	Samples	Type of sample	Method	Main findings
Redonnet-Vernhet et al. [15]	2 FD women	Peripheral blood leukocytes; Fibroblasts	HUMARA assay	Unbalanced XCI in monozygotic twins' fibroblasts in opposite direction. This is the first documented case of female twins discordant for FD
Maier et al. [19]	28 FD women	Blood	HUMARA assay	Fabry heterozygous females showed a random X inactivation and no significant correlation was found between X inactivation patterns and clinical phenotype
Elstein et al. [20]	77 FD women	Peripheral blood leukocytes	HUMARA assay	XCI did not correlate with signs and symptoms of classic Fabry disease
Hübner et al. [51]	9 FD patients	Peripheral blood leukocytes	CALCR Methylation-specific PCR- High-resolution melting CALCR sequencing	A specific CpG of autosomal CALCR gene is differentially methylated in ERT treated and non-ERT-treated FD patients indicating that this CpG could be an epigenetic biomarker of FD
Echevarria et al. [18]	56 FD women	Peripheral blood; Mouth epithelial cells; skin biopsy; Urine	HUMARA assay	XCI significantly impacted the phenotype and natural history of FD in females, supporting the correlation between XCI and clinical phenotype
Hossain et al. [44]	4 FD women	Peripheral blood; spinal fluid	GLA Methylation-sensitive restriction enzymes analyses GLA Bisulfite Sanger sequencing	Allele-specific GLA methylation correlated with the severity of FD phenotype
Juchniewicz et al. [21]	12 FD women	Saliva	HUMARA assay	XCI pattern did not correlate with Fabry disease severity scores
Hossain et al. [46]	36 FD women	Peripheral blood; skin fibroblasts	GLA Methylation-sensitive restriction enzymes analyses GLA Bisulfite Sanger sequencing	Methylation of the GLA non-mutated allele was proportionally correlated with the clinical severity score (FASTEX score)
Yanagisawa et al. [45]	4 FD women	Fibroblast from Skin tissue	Allele-specific GLA expression (RT-PCR)	mRNA expression level of the GLA mutant allele correlated with disease severity
De Riso et al. [55]	3 FD women	Peripheral blood	High coverage-amplicon bisulfite sequencing (HC-ABS) versus HUMARA	Substantial concordance in direction and entity of the methylation imbalance between AR and GLA genes. Clearly distinct allele-specific epiallele profiles were obtained by epiallele distribution analysis
Rossanti et al. [56]	9 FD women	Blood leukocytes; Urine sediments	HUMARA assay GLA Ultra-deep targeted RNA Sequencing	Skewed XCI explained the severity of FD in only limited number of female cases

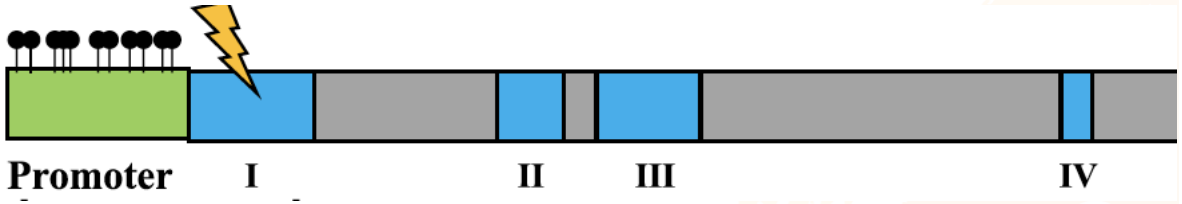
Di Risi et al. Clin Epigenet (2021)

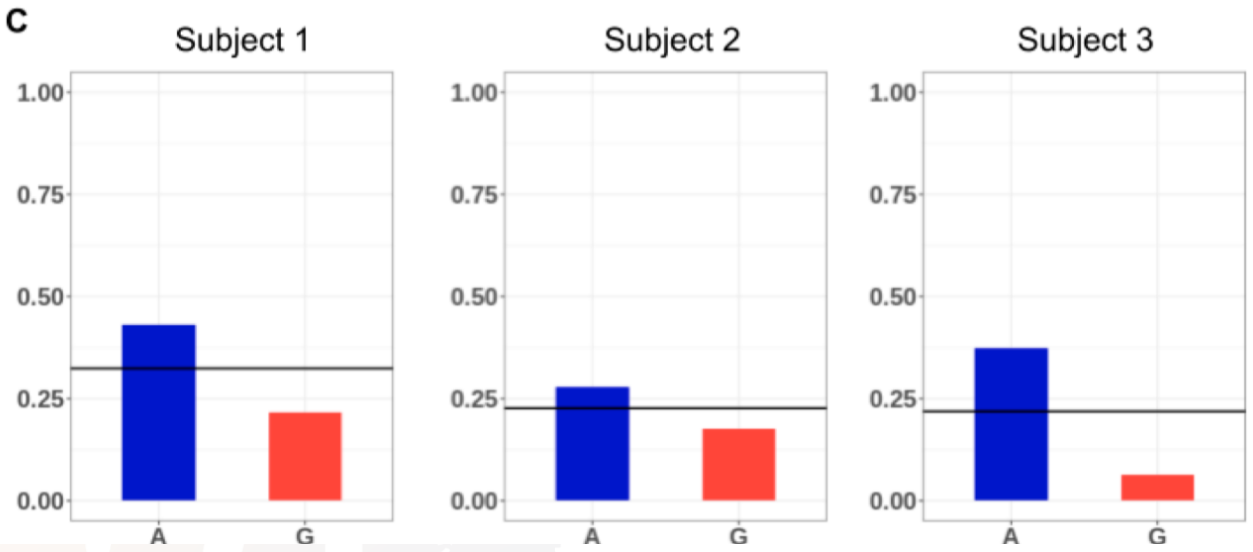
Table 1. Results of studies on the XCI in Fabry carriers.

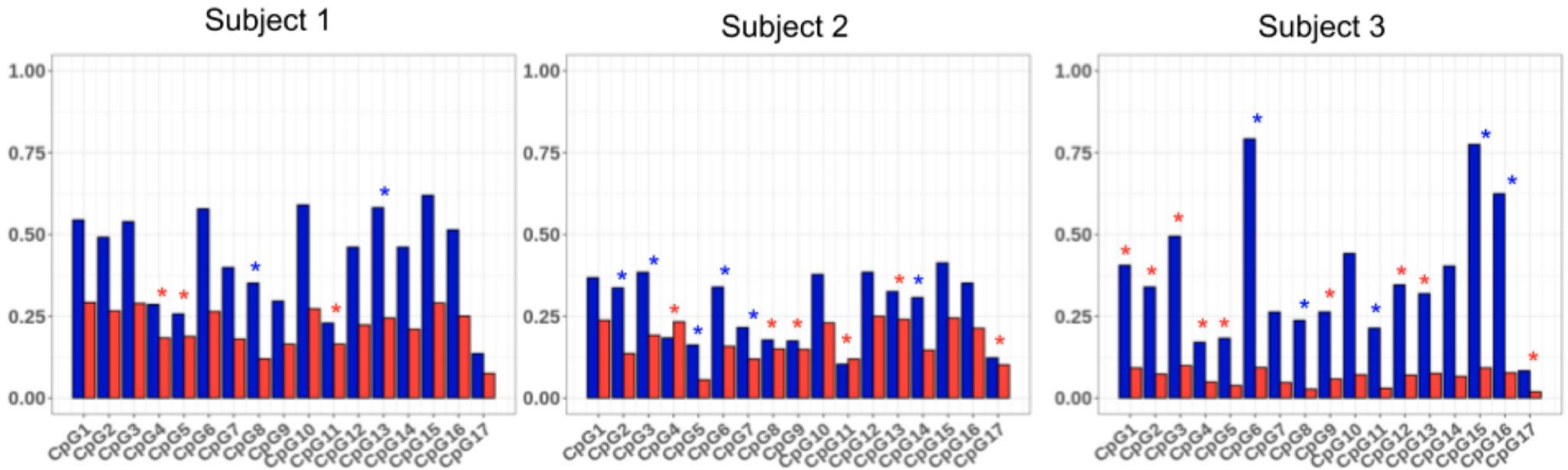
Articles	Age	Skewed XCI				
		Tissue Analyzed	Mild MSSI Score (Total Subjects)	Moderate-Severe MSSI Score (Total Subjects)	Cardiac Involvement (Total Subjects)	No Cardiac Involvement (Total Subjects)
Dobrovolny et al., 2005	Young/Adult	L, U, SE	7 (24)	4 (14)	n.d.	n.d.
Maier et al., 2006	Young/Adult	L	5 (10)	5 (18)	6 (16)	4 (12)
Echeivarra et al., 2015	Young/Adult	L, U, SE, skin	3 (35)	7 (21)	7 (41)	3 (8)
Morrone et. al., 2003	Young/Adult	L	n.d.	n.d.	2 (0)	2 (4)
Rossanti et al., 2021	Adult	L,	n.d.	n.d.	0 (5)	1 (2)

n.d. = not determined; L = peripheral blood leukocytes; SE = salivary epithelia; U = urinary sediment cells; skewed XCI is referred to preferential inactivation of wild X chromosome with a ratio of 75:25.

Viggiano E et al. Int. J. Mol. Sci. 2021





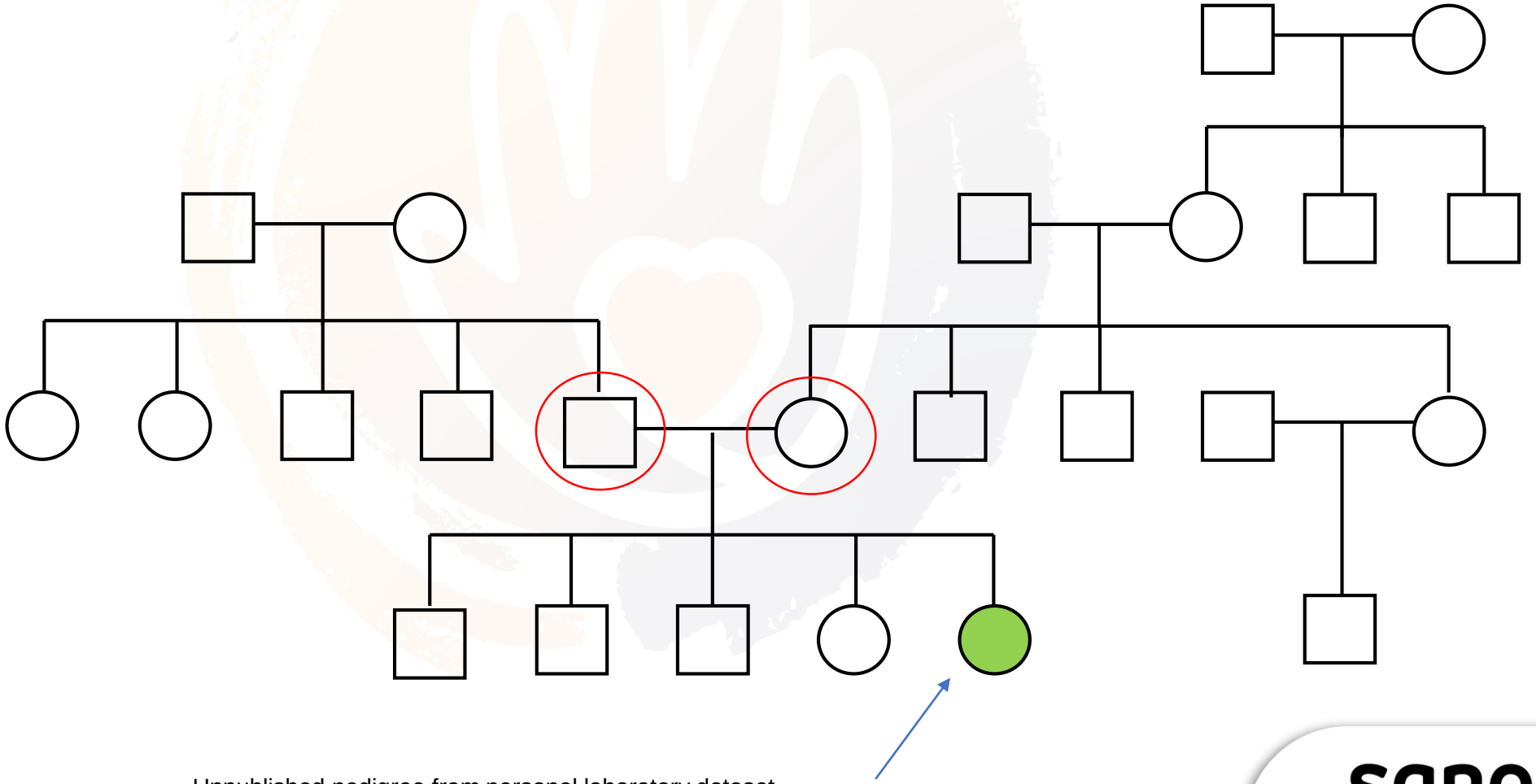


Di Risi et al. Clin Epigenet (2021)

De Riso G et al. Genes (2020)

Family Screening Strategy in Fabry

Female Proband



Unpublished pedigree from personel laboratory dataset

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¡GRACIAS!

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