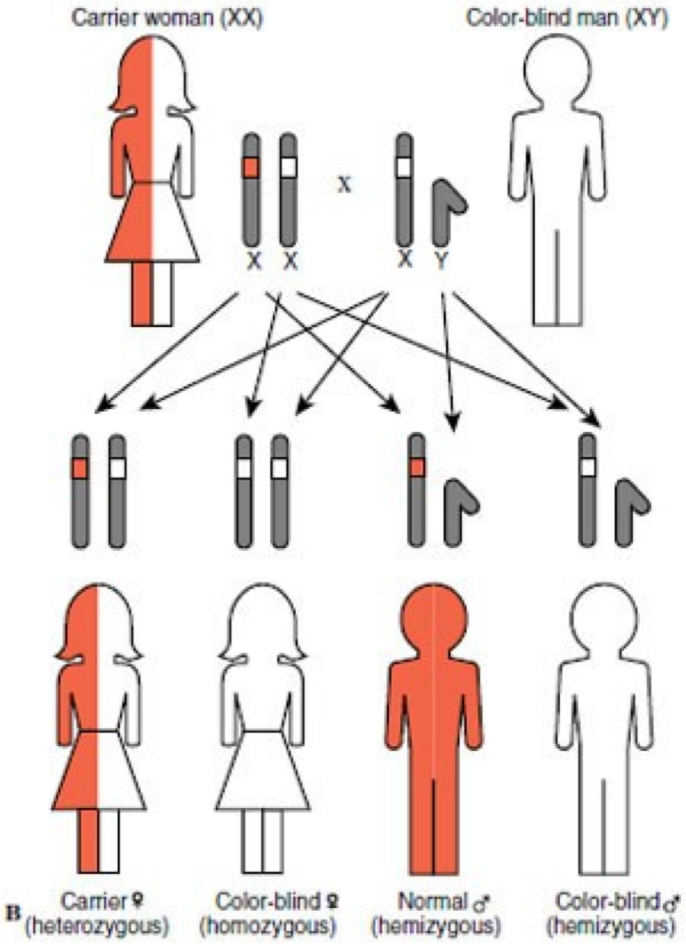
?/100 8/100

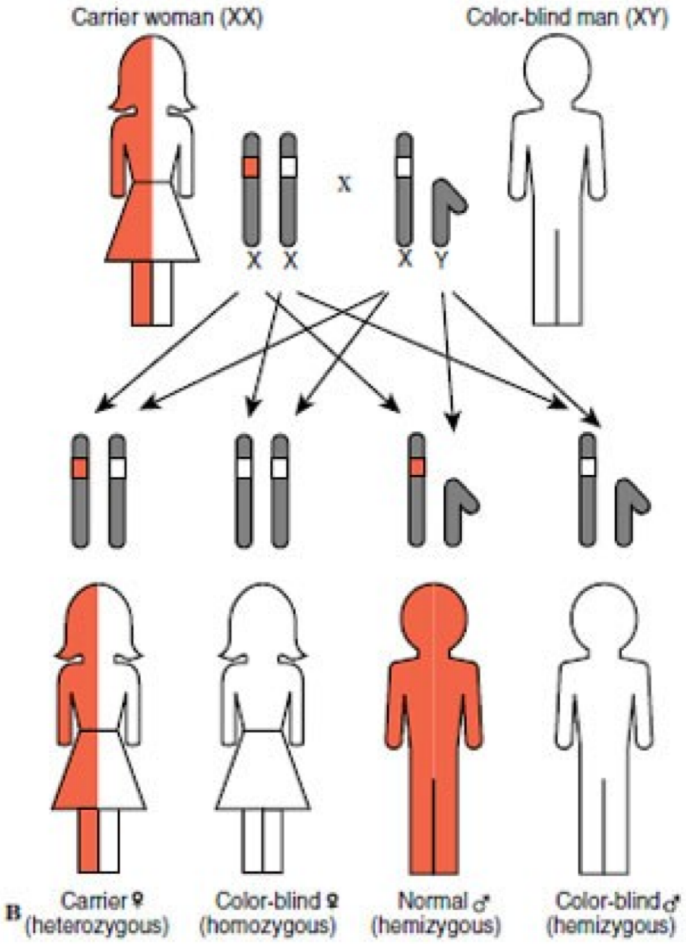
Red/green color blindness



16/100 8/100

Red/green color blindness

128/10 000



64/10 000 (calculated)

50/10 000 (observed)

Color blindness prevalence

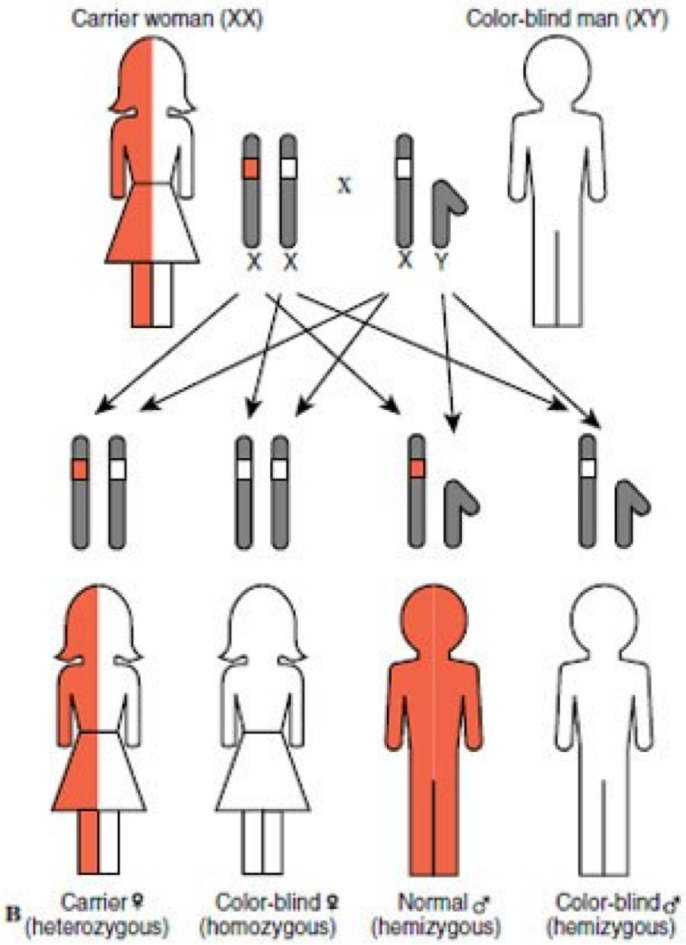
1/12 ~ 8 /100

1/200

2/20 000 1/20 000

A rare disease

Couple : 1/ 200 000 000



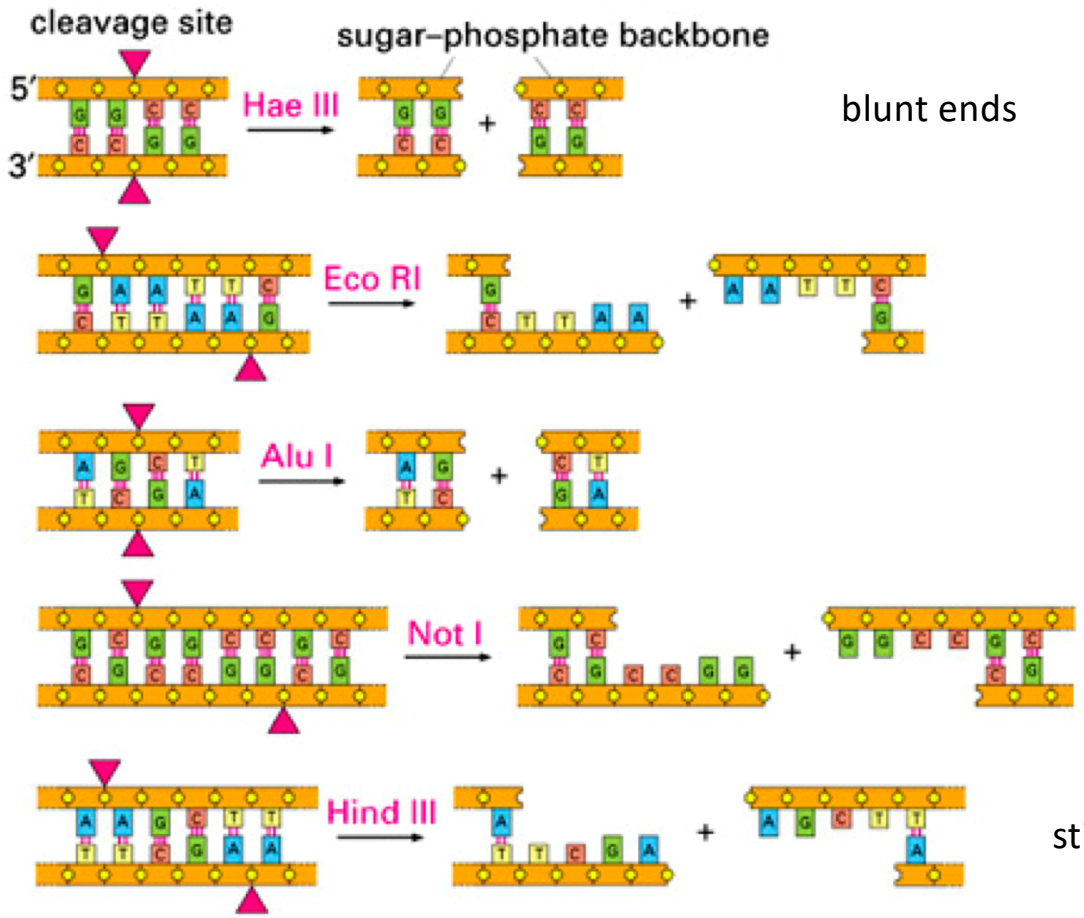
Disease :
usually the father is **not affected**

a ***de novo*** mutation must be envisioned

When a girl presents with a **rare** X-linked recessive disease, what is going on ?

Affected woman : 1/ 400 000 000

DNA can be cut by restriction nucleases



- cut double-stranded DNA
- at particular sites: recognition sites
- highly specific sequences of
  4 nucleotides: frequent cutters
  6 nucleotides: medium cutters
  8 nucleotides: rare cutters
- from bacteria; defense mechanism against phages

Figure 10-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Isoschizomeres

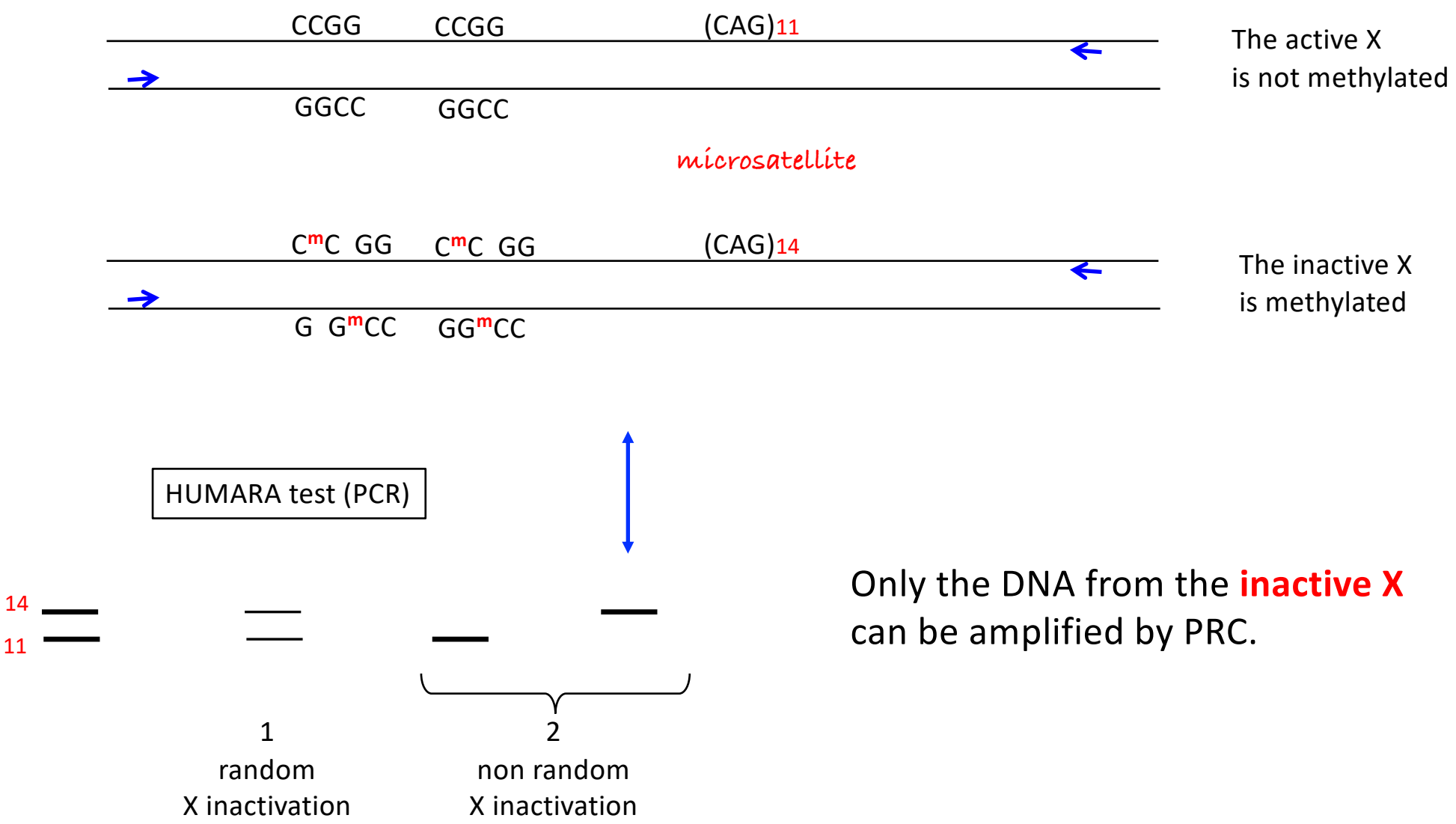
	cut	no cut
Hpa II recognizes	5’- C C G G – 3’ 3’- G G C C – 5’	5’- C ^{m}C G G – 3’ 3’- G G ^{m}C C – 5’
Msp I recognizes	5’- C C G G – 3’ 3’- G G C C – 5’	5’- C ^{m}C G G – 3’ 3’- G G ^{m}C C – 5’
	cut	cut

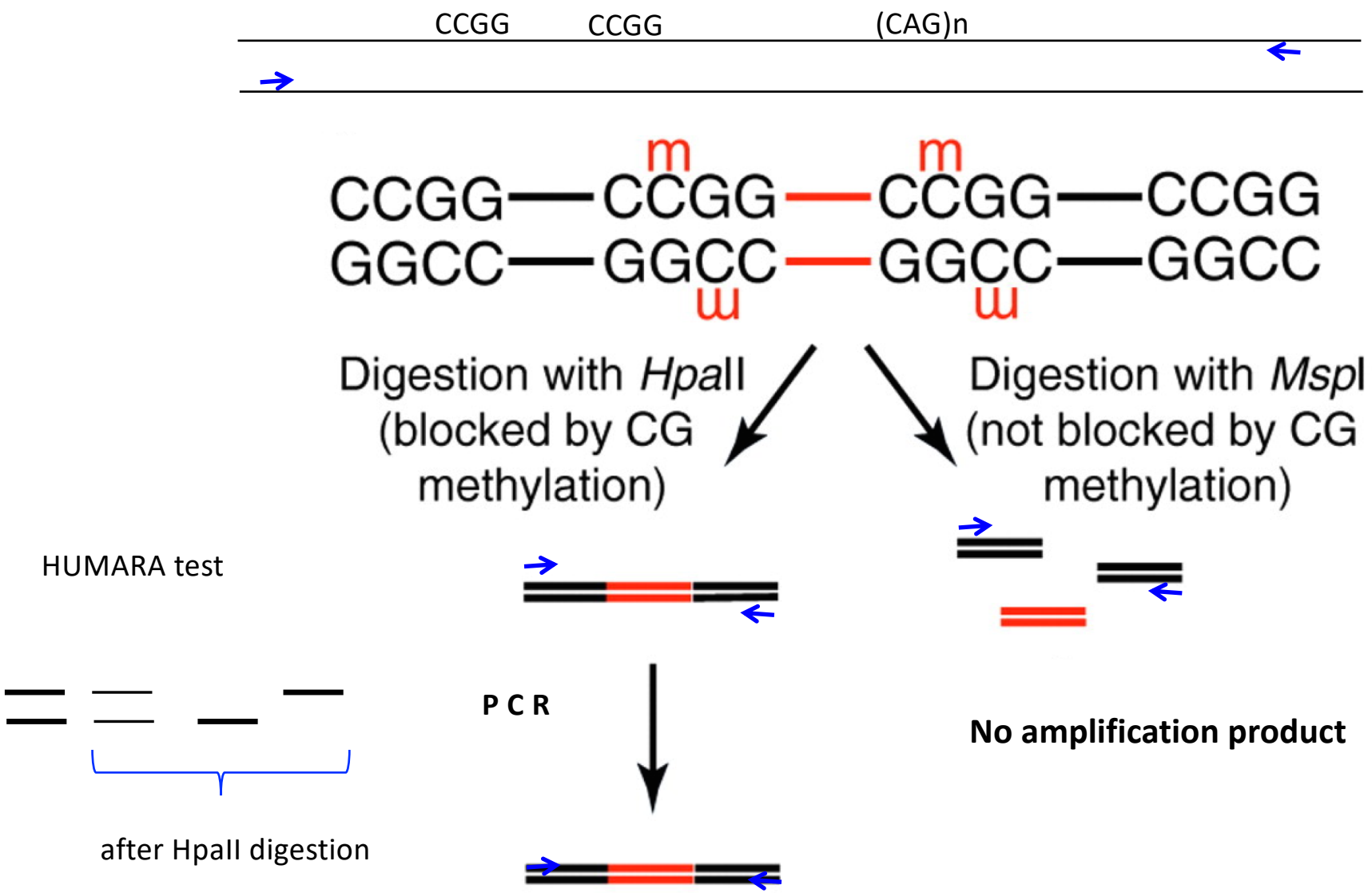
Hpa II and Msp I recognize the same restriction site : they are isoschizomeres.

However there is one important difference :

Hpa II is **sensitive** to DNA methylation

Msp I is **insensitive** to DNA methylation


CCGG
CCGG
(CAG)11
GGCC
GGCC
The active X
is not methylated
microsatellite
CmC GG
CmC GG
(CAG)14
G GmCC
GGmCC
The inactive X
is methylated
HUMARA test (PCR)
14
11
1
random
X inactivation
2
non random
X inactivation
Only the DNA from the inactive X
can be amplified by PRC.


CCGG
CCGG
(CAG)n
m
m
CCGG—CCGG—CCGG—CCGG
GGCC—GGCC—GGCC—GGCC
Digestion with HpaII (blocked by CG methylation)
Digestion with MspI (not blocked by CG methylation)
HUMARA test
P C R
No amplification product
after HpaII digestion

Example of testing

Mother shows non random X inactivation

Patient shows non random X inactivation

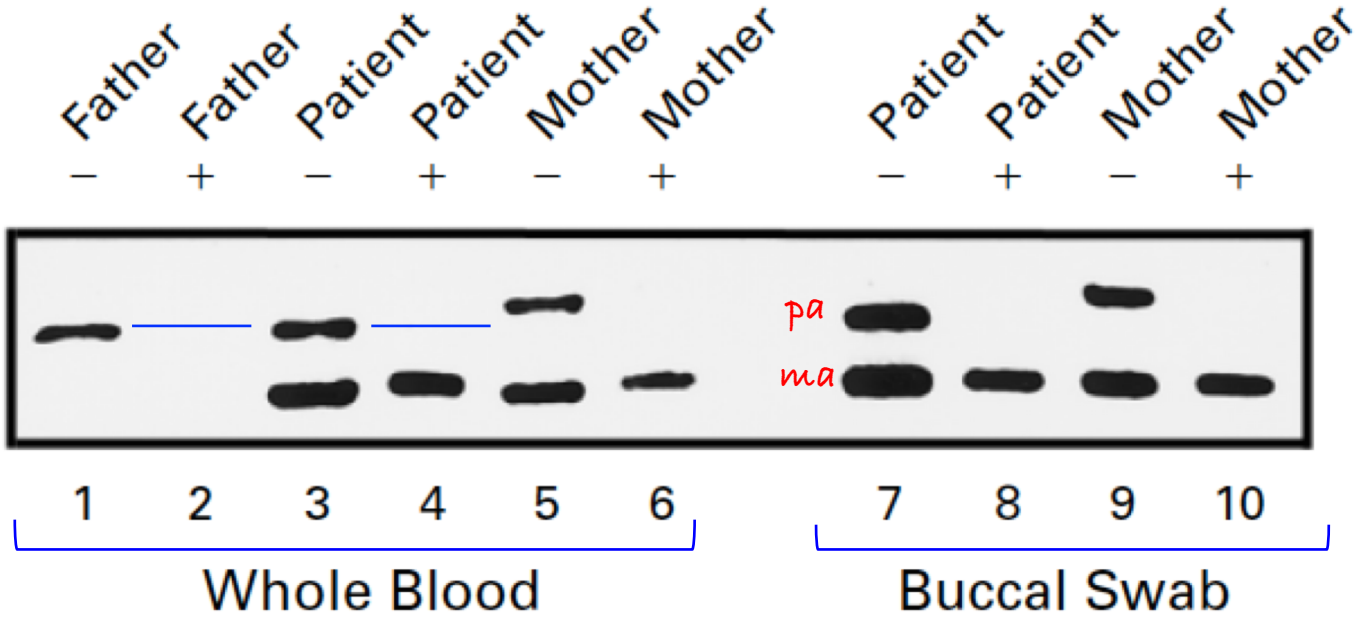
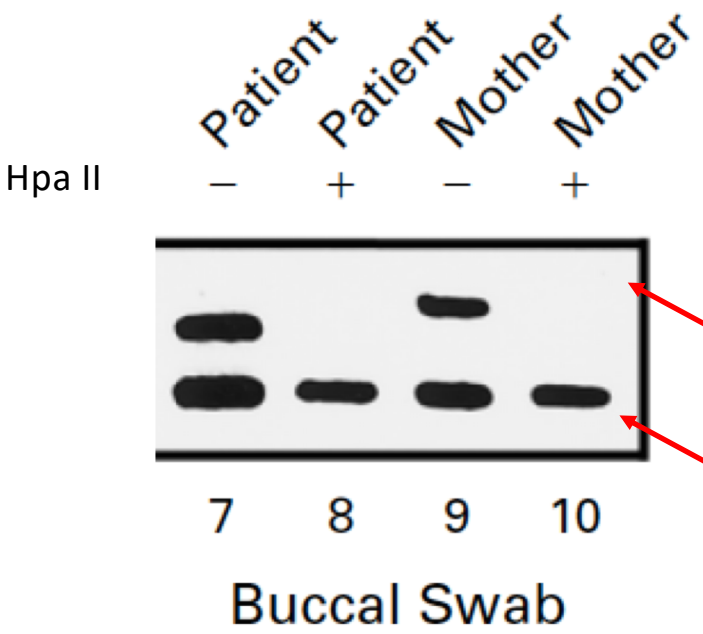


Figure 1. Analysis of the Pattern of X-Chromosome Inactivation in the Patient and Her Parents.

DNA was extracted from whole blood or oral mucosal cells from the patient and her parents and amplified by PCR with specific primers that flank the *HUMARA* locus (lanes 1, 3, 5, 7, and 9; labeled with a minus sign). In addition, the DNA was digested with the methylation-sensitive enzyme *Hpa*II before PCR amplification (lanes 2, 4, 6, 8, and 10; labeled with a plus sign). The samples were analyzed on 3 percent agarose gel and stained with ethidium bromide.

Hpa II digeston ***before*** PCR



Mother shows
non random
X inactivation

Why ? XIC is mutated

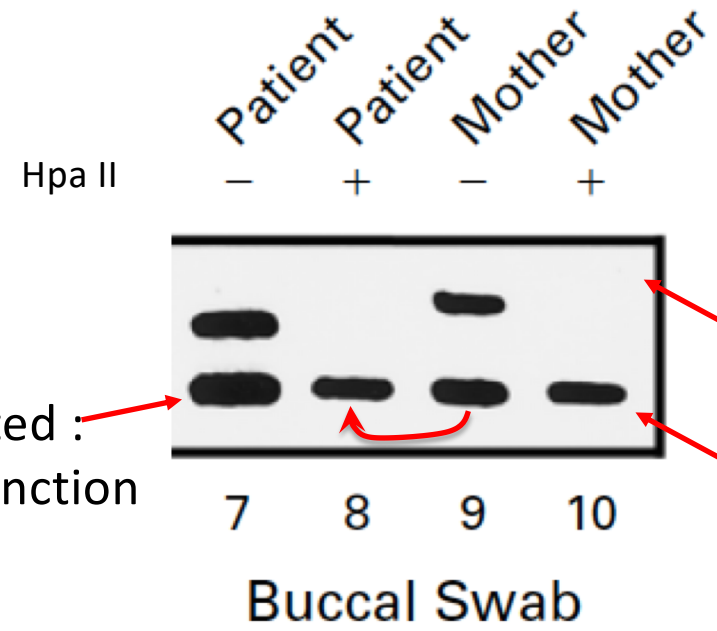
never inactivated

always inactivated

A priori 2 possibilities :

1. Top allele has a **loss-of-function** mutation
2. Bottom allele has a **gain-of-function** mutation

Hpa II digeston ***before*** PCR



XIC mutated :
gain-of-function

never inactivated

always inactivated

Patientr shows
non random
X inactivation

Why ? XIC is mutated

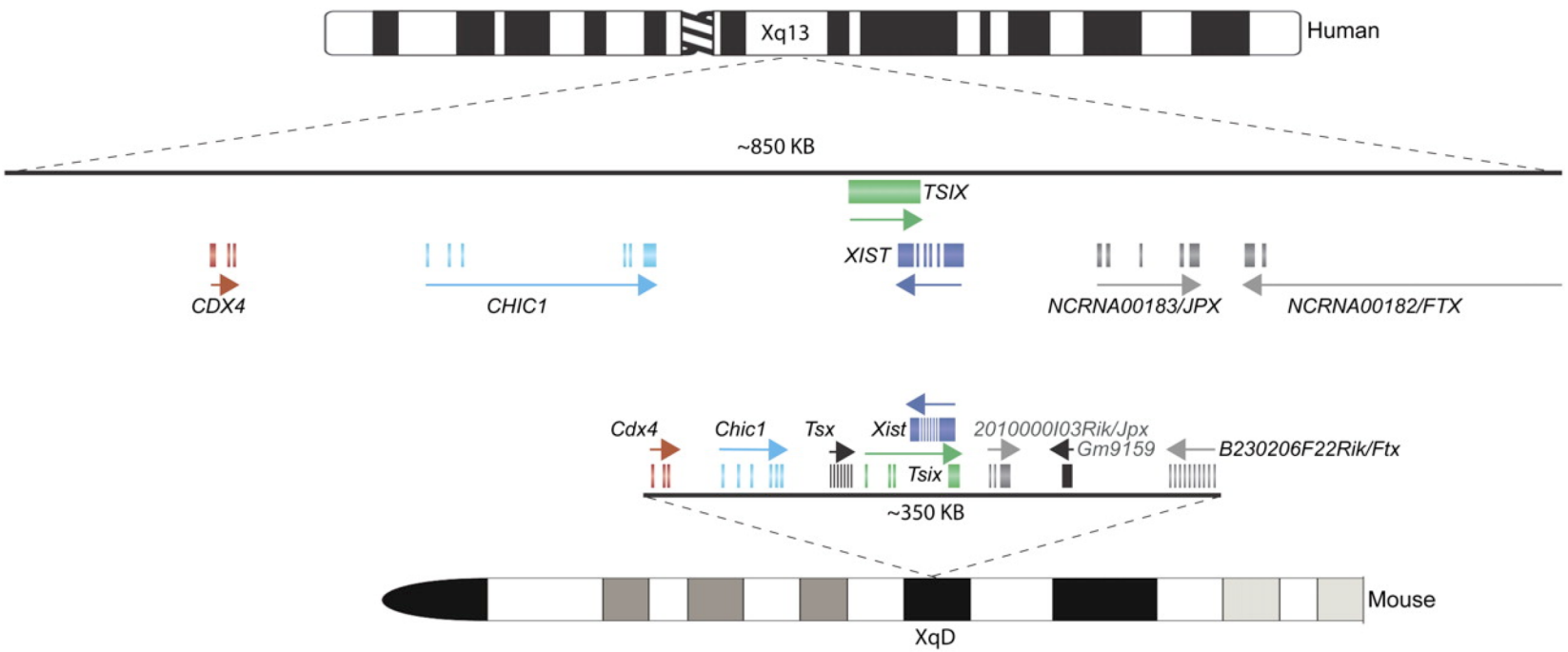
Observation in the patient shows that the mother has a gain-of-function mutation

2 possibilities :

1. Top allele has a **loss-of-function** mutation
2. Bottom allele has a **gain-of-function** mutation

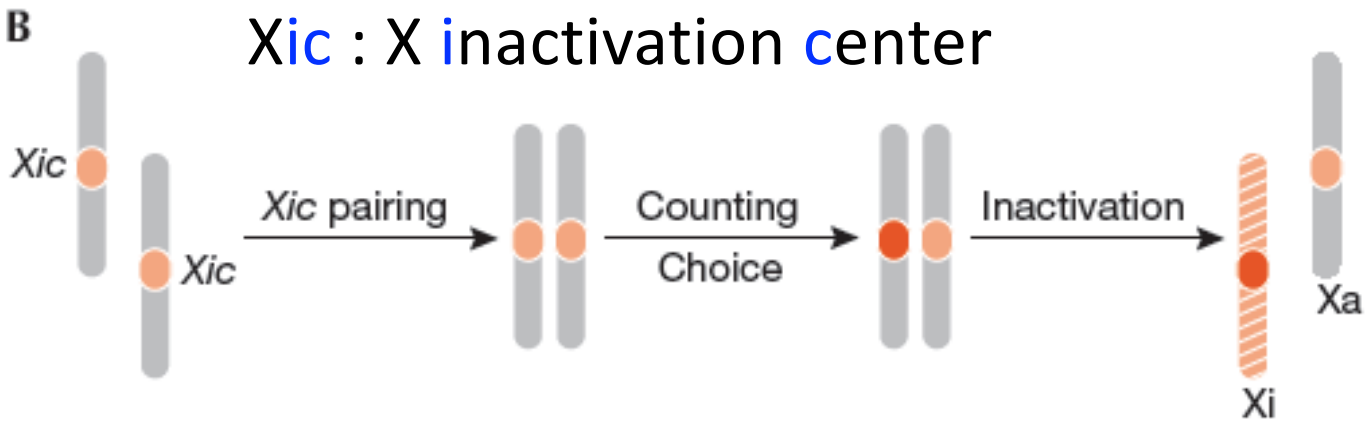
XIC = X Inactivation Center

TSIX and XIST are non coding RNA



Mutation of XIC can be
- loss-of-function
- gain-of-function

Xic : X inactivation center



Xic is required for X inactivation.
Loss of function of Xic makes the X chromosome unable to inactivate.
Consequence :
The X inactivation is not random.