INTRIGUING COMBINATION OF MUTATIONS IN WAS PATIENT

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Wiskott-Aldrich syndrome

X - LINKED

- eczema
- thrombocytopenia
- immunodeficiency

- Wiskott A, Monatsschr Kinderheilkd, 1937
- Aldrich RA et al., Pediatrics, 1954

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- autoimmunity and/or malignancy

Scoring system to define phenotype of WAS

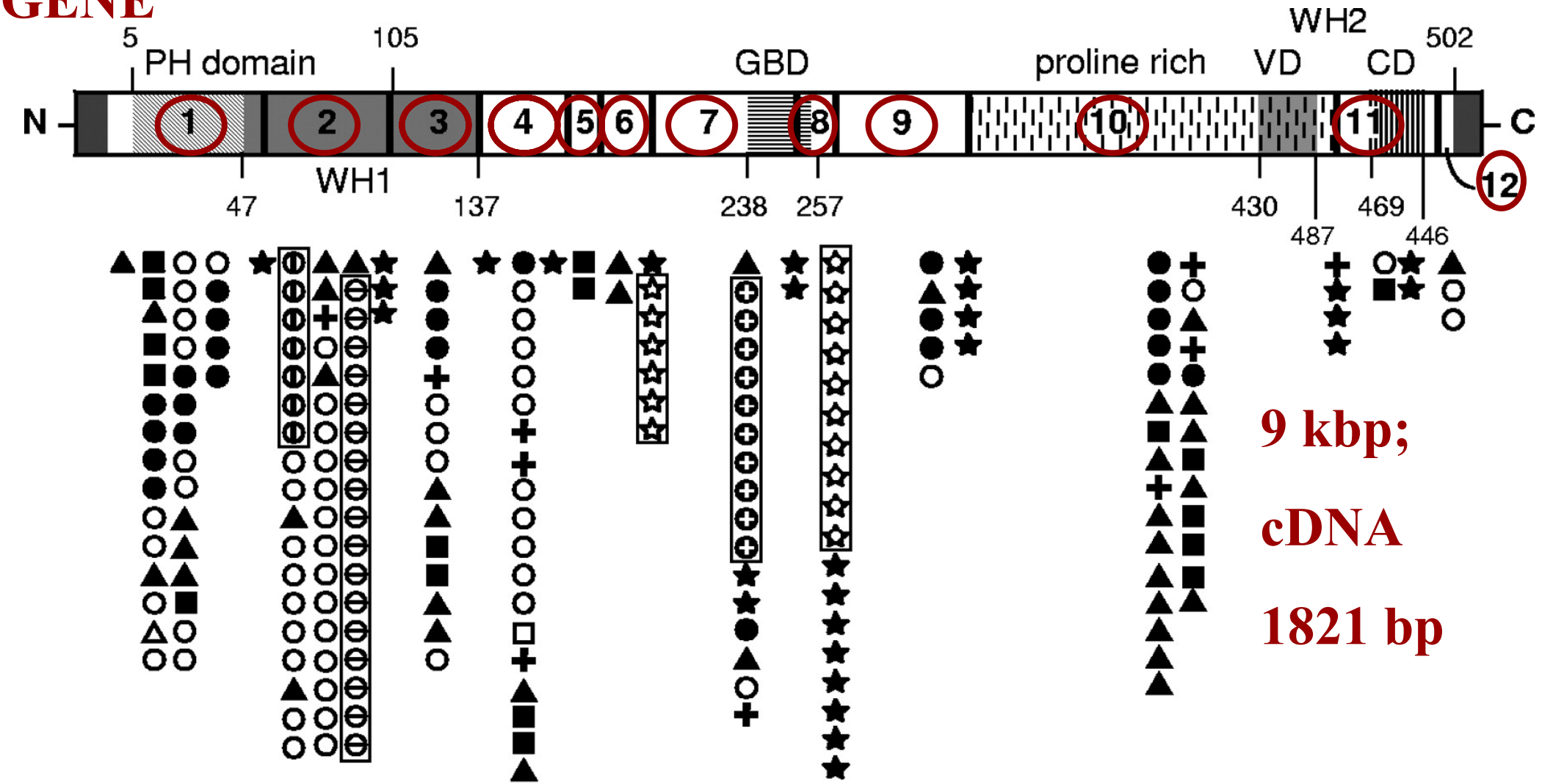
Disease	XLT		Classic WAS		
Score	1	2	3	4	5
thrombocytopenia	+	+	+	+	+
small platelets	+	+	+	+	+
eczema	-	(+)	+	++	+/++
immunodeficiency	-/(+)	(+)	+	+	+
infections	-	(+)	+	+/++	+/++
autoimmunity and/or malignancy	-	-	-	-	+

Genetic background

- **gene *WASP***
  - WASP - key regulator of lymphocyte and platelet function
    - critical role in signal transduction
    - regulation of the cytoskeleton reorganization

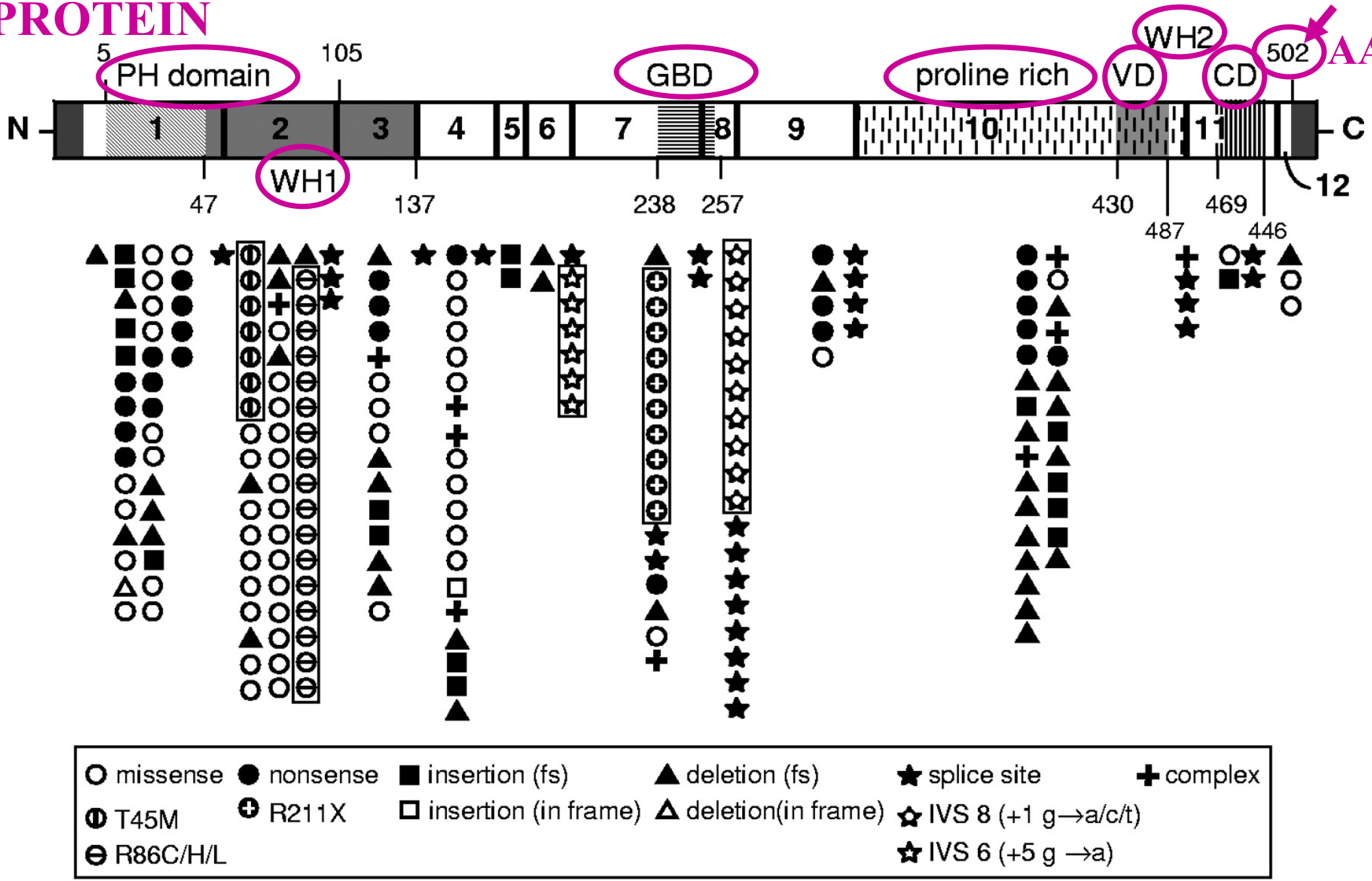
Derry JM et al, Cell, 1994

GENE

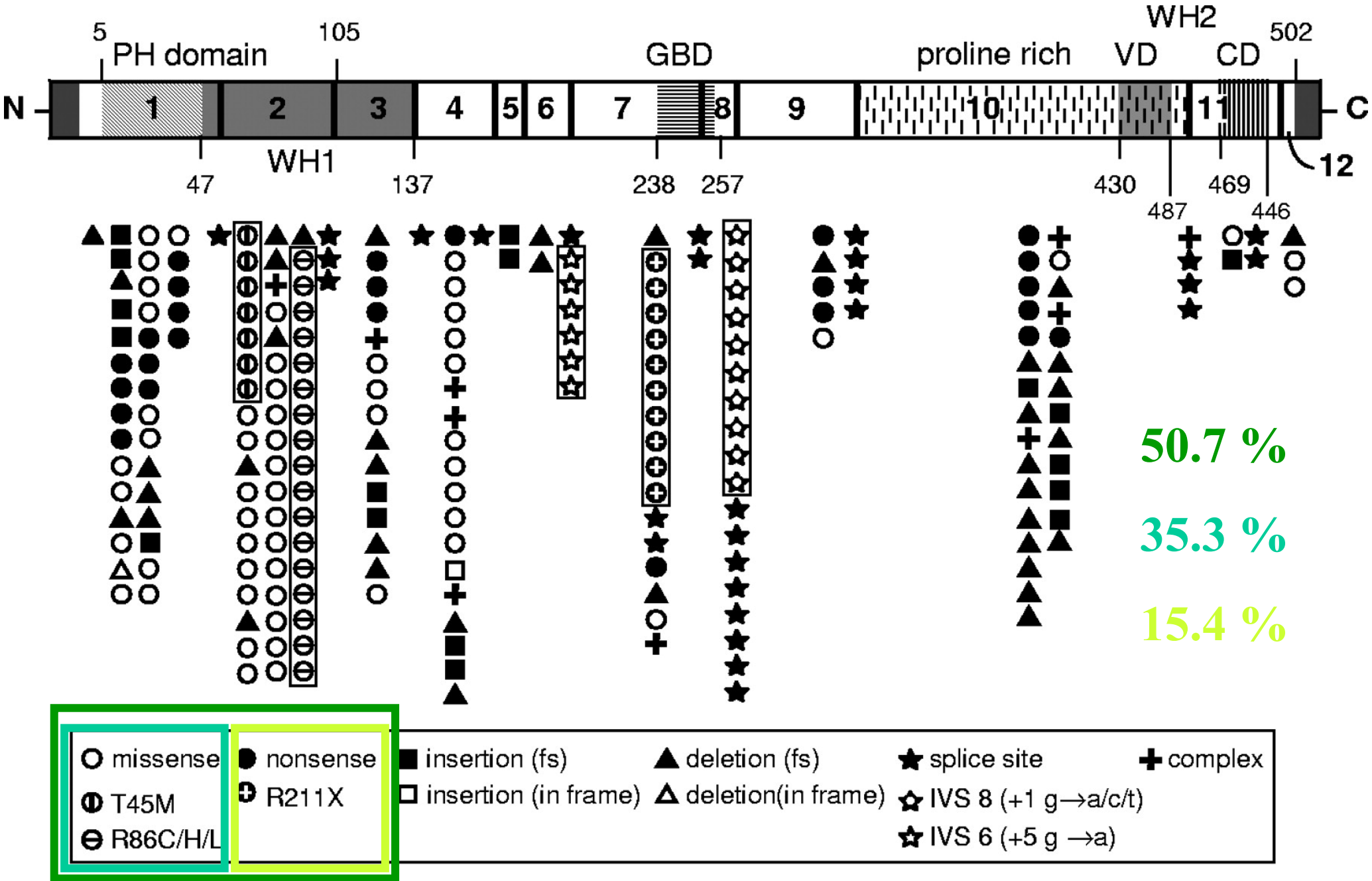


Jin Y et al, Blood, 2004

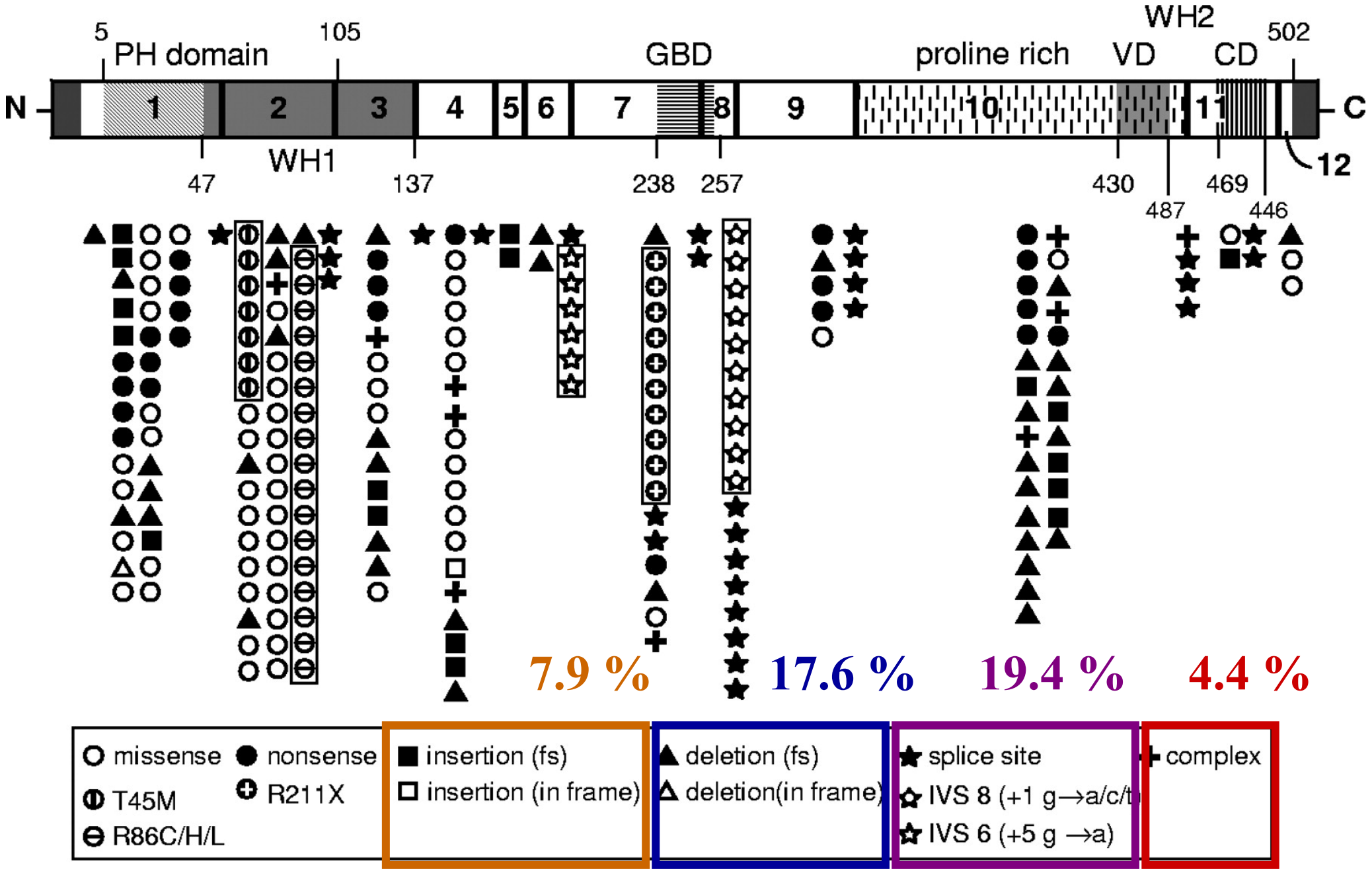
PROTEIN



Jin Y et al, Blood, 2004



Jin Y et al, Blood, 2004



Jin Y et al, Blood, 2004

Molecular genetic diagnostics of WAS

in Centre for PID, Brno

- PCR of all coding regions followed by direct sequencing
  - exons 1, 2, 3+4, 5+6, 7, 8+9, 10, 11, 12

(Jones LN et al., Blood Cells, Molecules and Diseases, 2002)

- confirmation by independent PCR (PCR-SSP or PCR+restriction or PCR+sequencing)

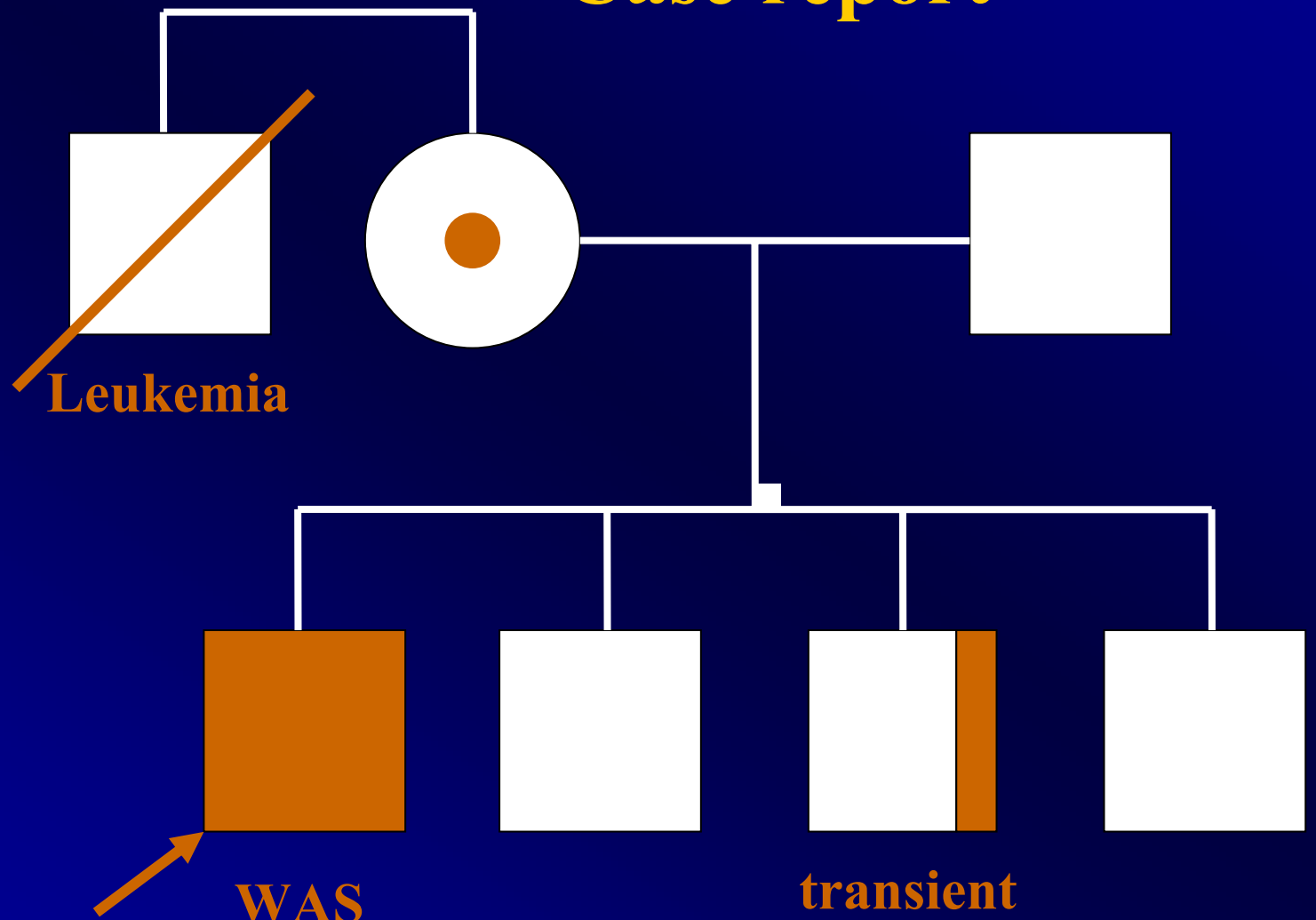
Case report

- boy
- petechial exanthema, thrombocytopenia early after birth
- eczema
- recurrent purulent otitis, pneumonia (Str. pneumoniae, Staph. aureus, Moraxella catarrhalis)

- splenomegaly

- Leu 5600/ul, Ly 2100/ul, T ly 1290/ul (61 %), B ly 11 %;
  - CD4+ 30 %, CD8+ 31 %
- IgG 10,5 g/l N, IgA 3,96 g/l ↑, IgM 0,17 g/l ↓, IgE 1331 IU/ml ↑

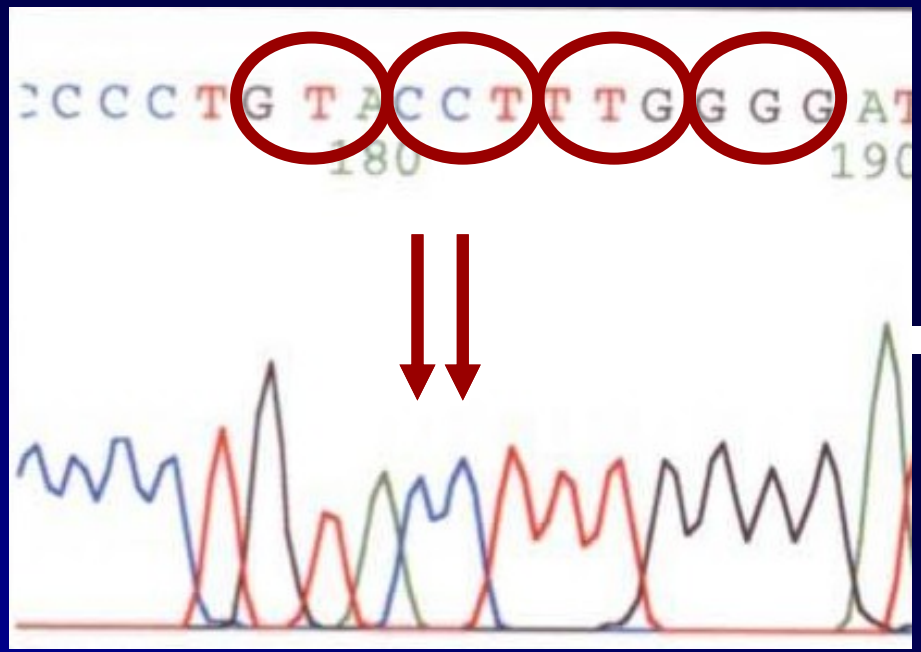
Case report



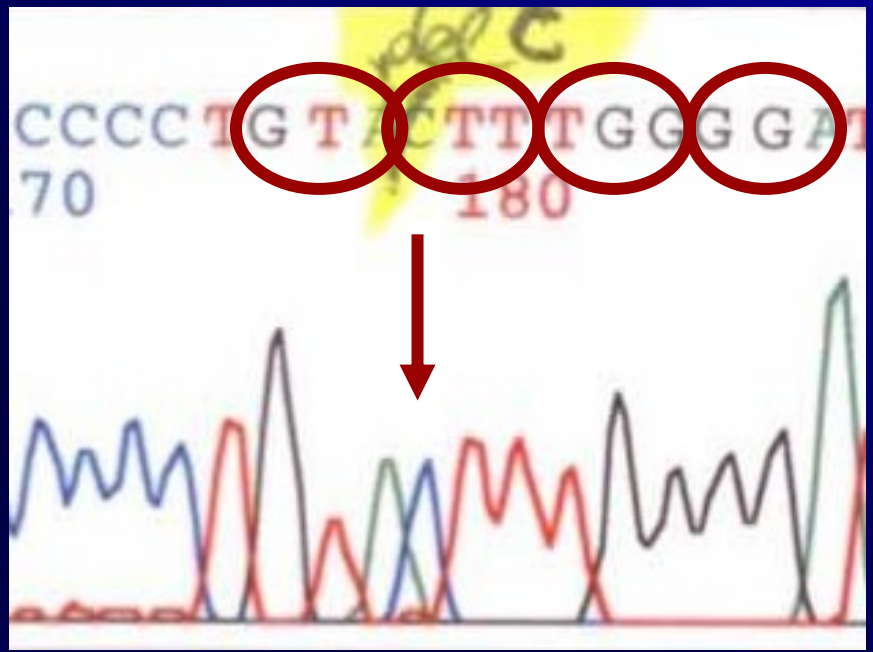
Case report

- IVIG, antibiotics
- HSCT from HLA identical brother at 11 years of age
- in a good shape 2 years after HSCT

WASP gene, exon 10, direct sequencing

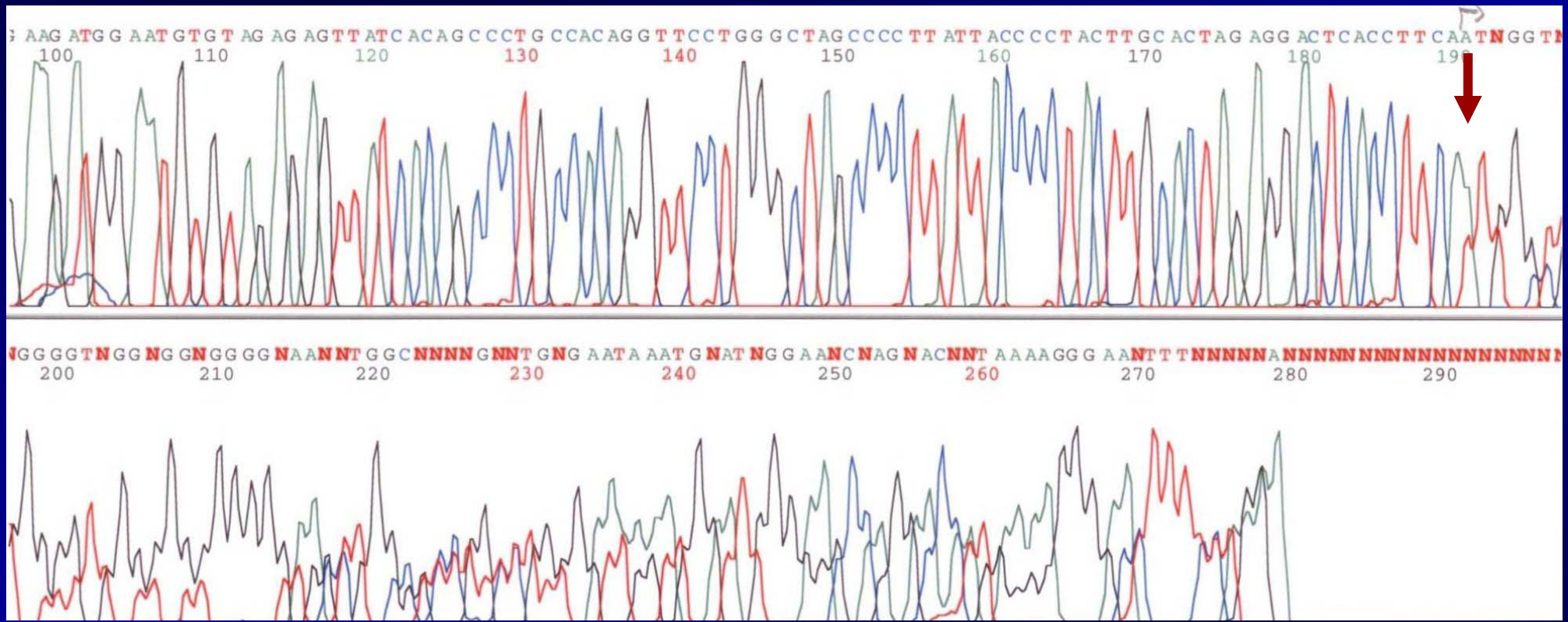


10. exon
wild type

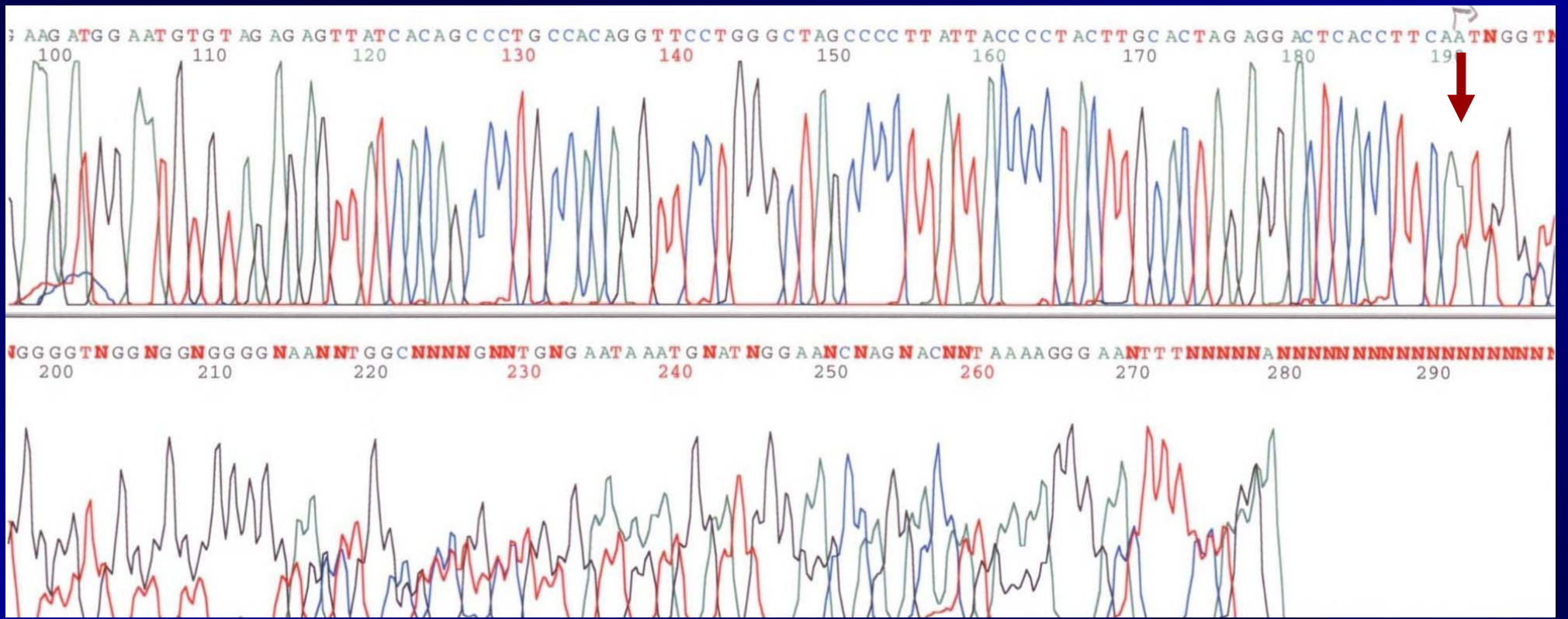


10. exon
c.1071delC
p.P346fsX444

WASP gene, exon 6+5, direct sequencing



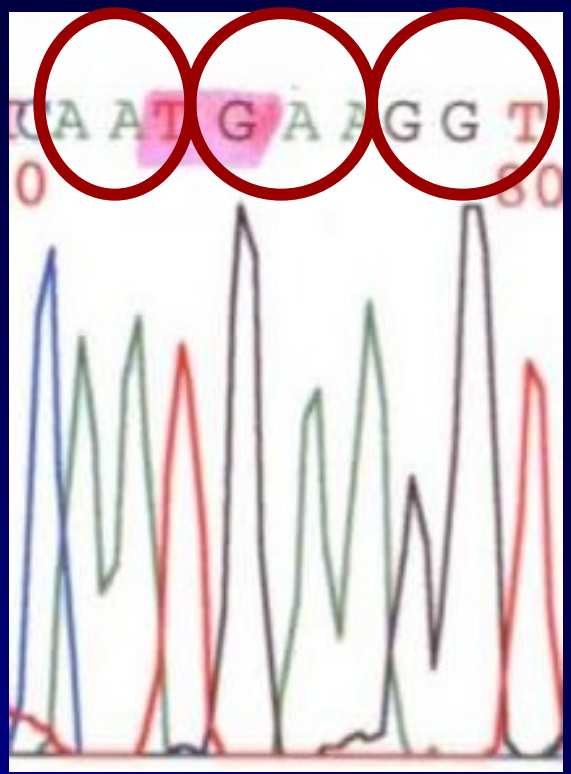
WASP gene, exon 6+5, direct sequencing



artefact?

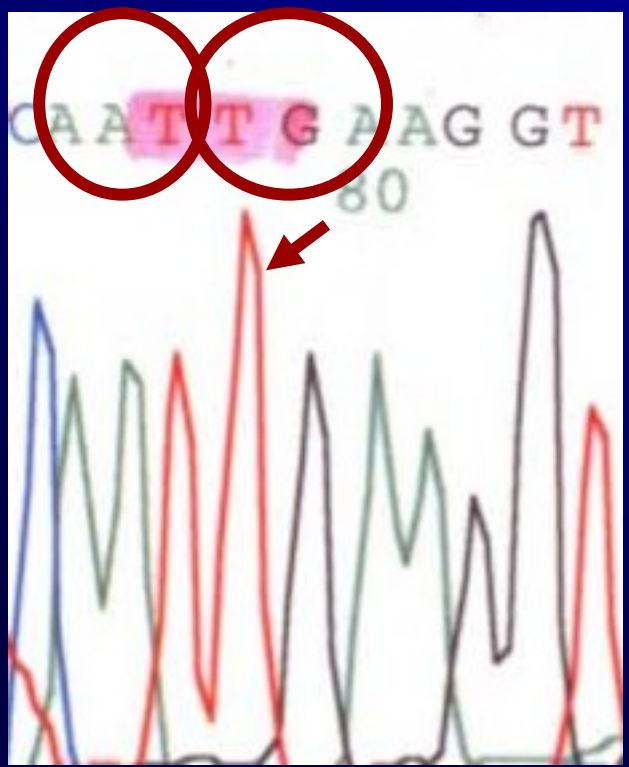
somatic mosaicism?

18/20 clones

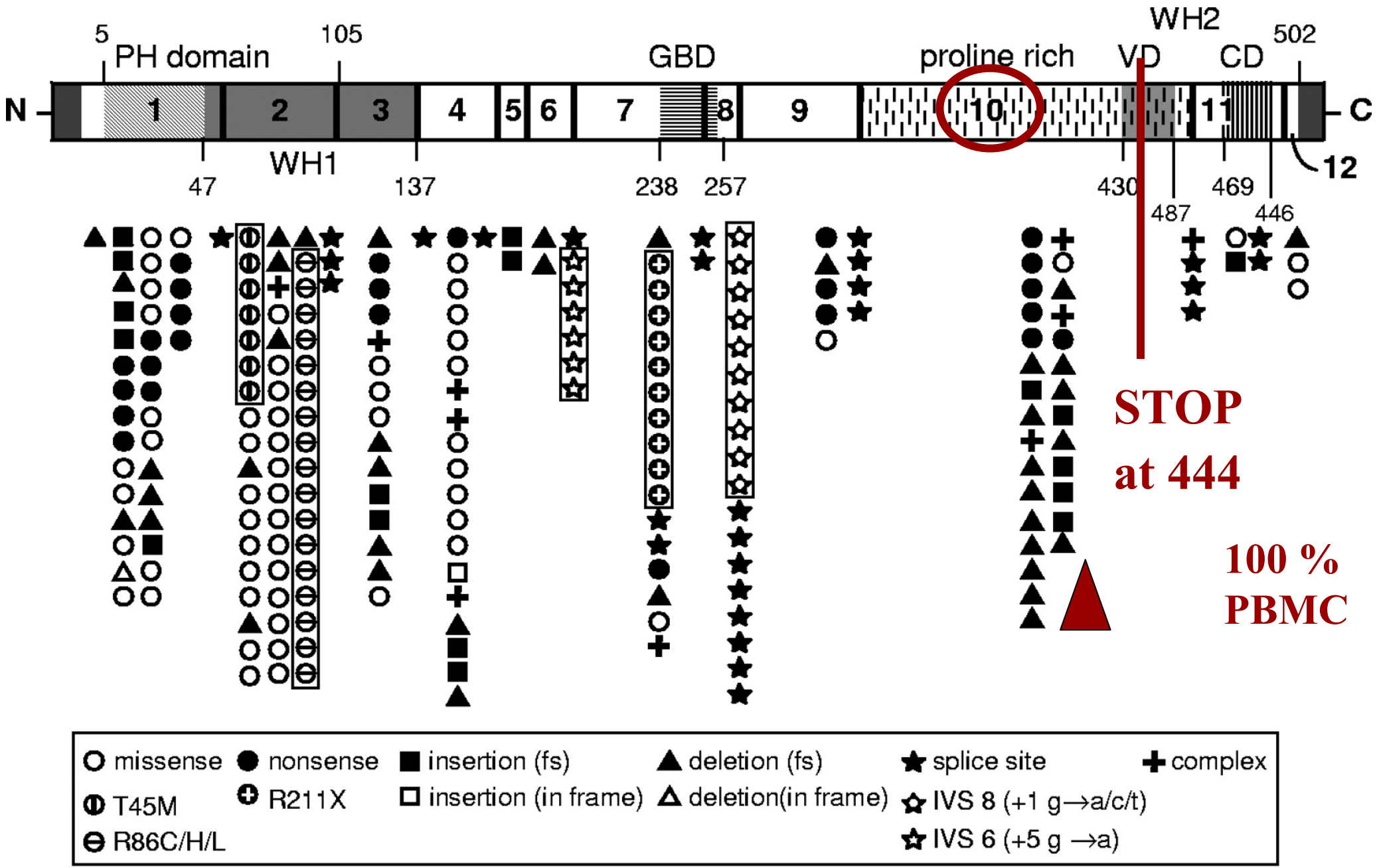


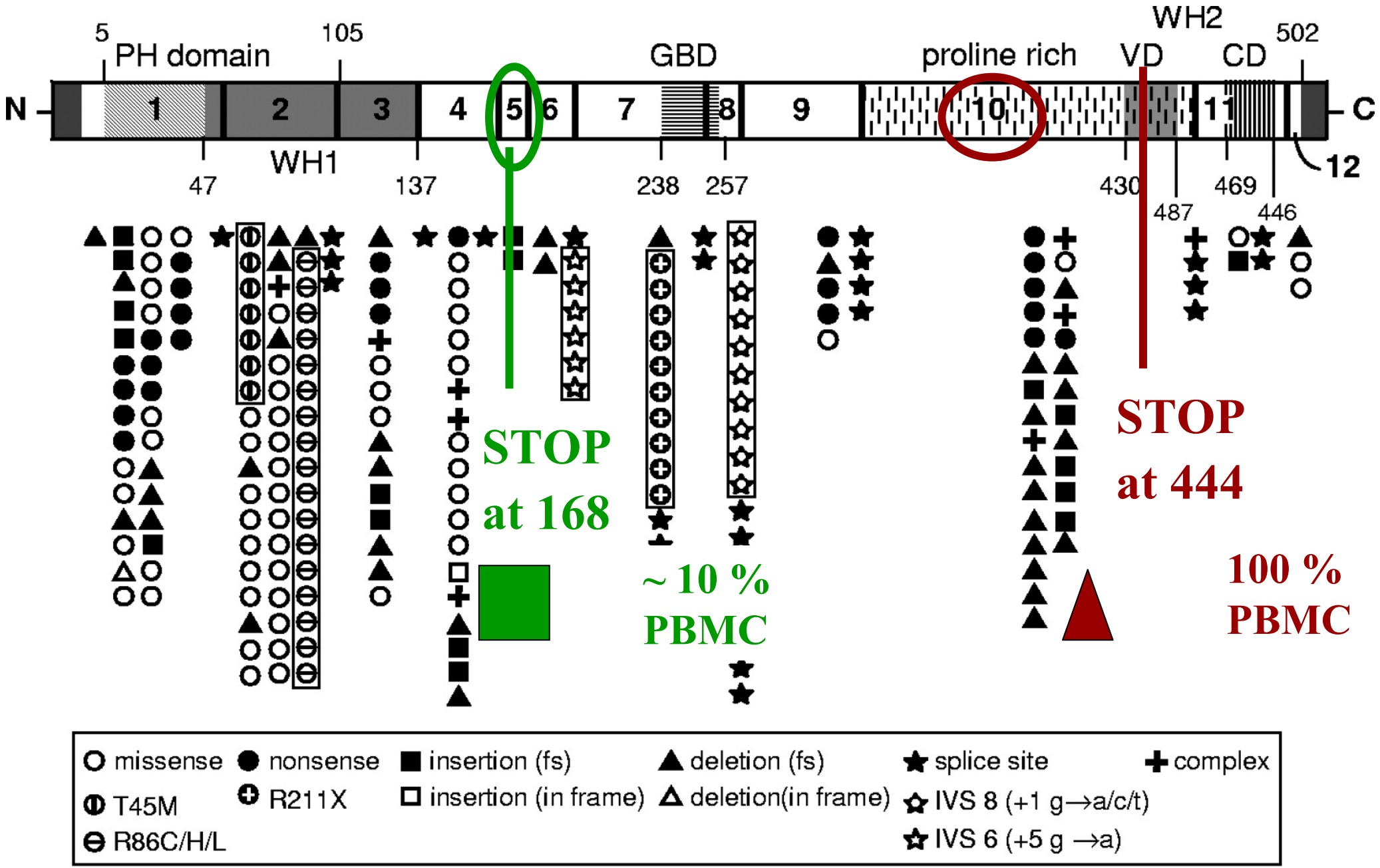
5. exon
wild type

2/20 clones



5. exon
c.535_536insT
p.E168fsX168


5
PH domain
105
GBD
proline rich
WH2
VD
CD
502
N
1
2
3
4
5
6
7
8
9
10
11
C
12
WH1
47
137
238
257
430
487
469
446
STOP
at 444
100 %
PBMC
missense
nonsense
insertion (fs)
deletion (fs)
splice site
complex
T45M
R211X
insertion (in frame)
deletion(in frame)
IVS 8 (+1 g→a/c/t)
R86C/H/L
IVS 6 (+5 g →a)


5
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4
5
6
7
8
9
10
11
C
12
WH1
47
137
238
257
430
469
487
446
STOP
at 168
~ 10 %
PBMC
STOP
at 444
100 %
PBMC
missense
nonsense
insertion (fs)
deletion (fs)
splice site
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Summary

In WAS patient detected:

- **new 1 bp deletion resulting in stop codon at 444 in all PBMC**
- **new 1 bp insertion resulting in stop codon at 168 in about 10 % of PBMC**

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In WAS patient detected:

- **new 1 bp deletion resulting in stop codon at 444 in all PBMC … primary (?)**
- **new 1 bp insertion resulting in stop codon at 168 in about 10 % of PBMC … (secondary (?), selective advantage (?))**

Significance of the second site mutation

UNCLEAR

IN OUR CASE

Significance of the second site mutation

- ?? spontaneous reversion of mutation ??
  - nature of mutations … rather against
  - mutations far from each other in different functional domains … rather against

  - tertiary structure?

… insufficient information at present time …

- separated cell populations before HSCT … not available for examination
- examination of patient's dry blood spot
  - mutation status at the time of the birth
- examination of brother suffering from transient XLT (if available)
- examination of parents (particularly mother) (if available)

… will give us additional pieces of information …