

RHCE*Ce329C: A novel RHCE allele associated with a weakened C antigen expression and genetically linked to RHD*DVII allele

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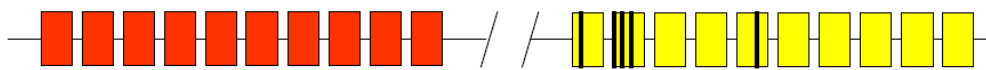
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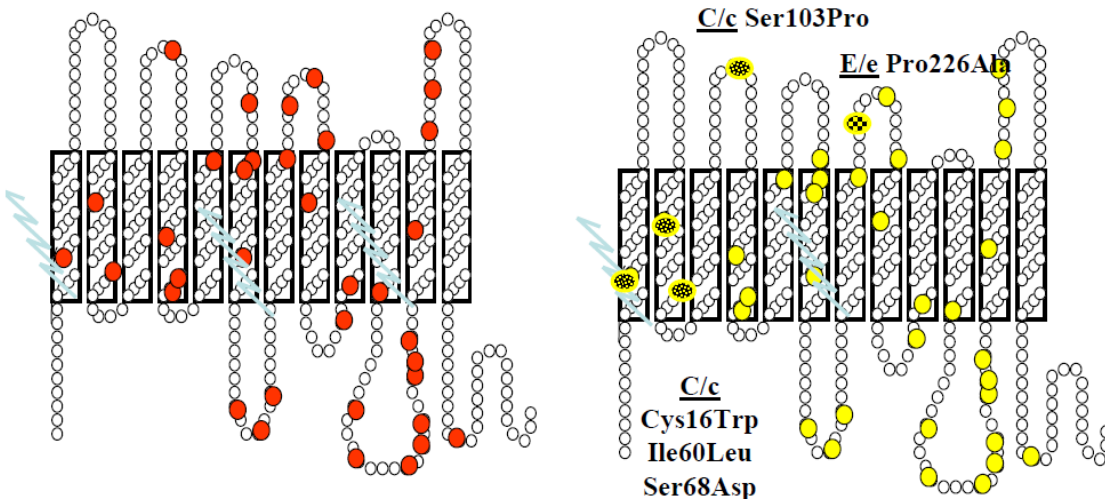
The Rh blood group system (ISBT 004)

- 55 antigens
 - *Most known: D, C, E, c, e ...*
- 2 genes

RHD & RHCE



⇒ 2 proteins with 93% of homology



- More than 500 allelic variants

Laboratory investigation of Rh variants

- One of the activities of our reference center (CNRGS)
 - Discrepant and/or weakened reactivity of one/several Rh antigens
 - Tools
 - ✓ serology : monoclonal antibodies
 - ✓ molecular biology : DNA chips, gDNA or cDNA sequencing
- Samples from donors and patients

Study background

24 blood samples referred for a weak or discrepant C antigen

- 3 patients (pregnant women) and 21 donors
- From North Africa (N=21) or West Europe (N=3)

Donor serology (on automated Olympus/Beckman PK7300 system)

- ✓ Positive with clones P3X255-13G8 + MS24
- ✓ Negative with clone DGC02

With a set of monoclonal reagents

✓ For C antigen

Clones	MS-24 (IgM, millipore)	MS-273 (IgM, millipore)	DGCO2 (IgM, Diagast)	P3x255- 13G8 (IgM, Diagast)	MS-23 (IgG, Millipore)
Reactivity	2 to 4+	0 to 4+	0+	0+	3+

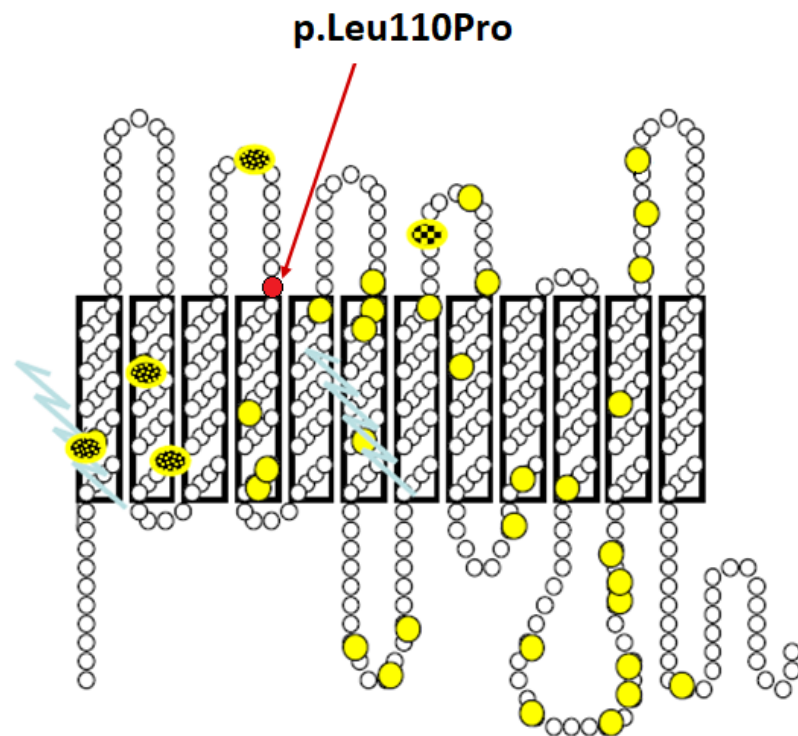
✓ For e antigen

Clones (IgM from Millipore)	MS-16	MS-21	MS-62	MS-63
Reactivity	3 to 4+	2 to 3+	4+	4+

Molecular study of the *RHCE* gene

The 1st of these cases was investigated in 2007 (pregnant woman), but only exon 4 and exons 7 to 9 sequencing was available at that time

=> cDNA sequencing
=> discovery of a novel
mutation: **c.329T>C in *RHCE***

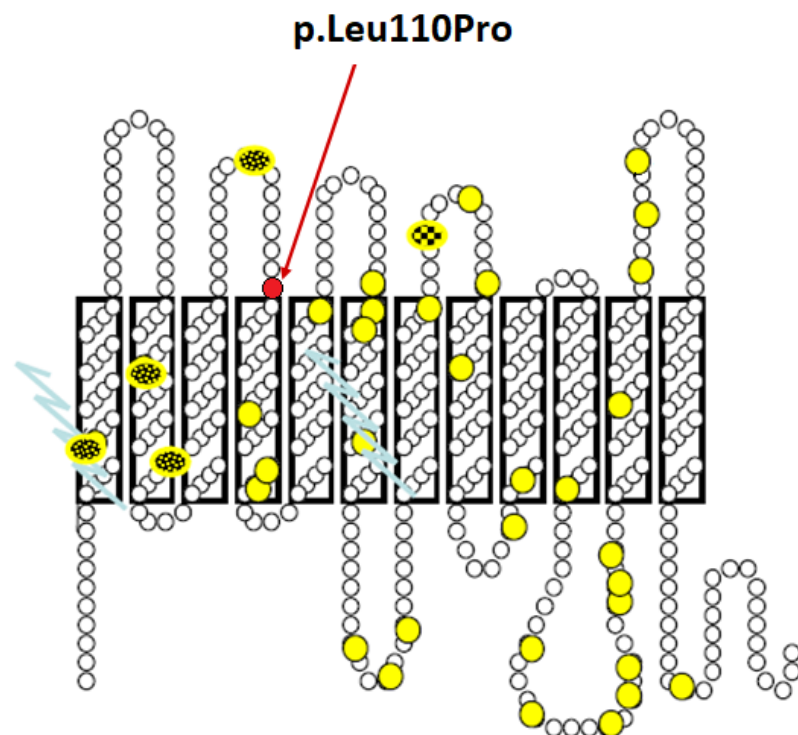


Molecular study of the *RHCE* gene

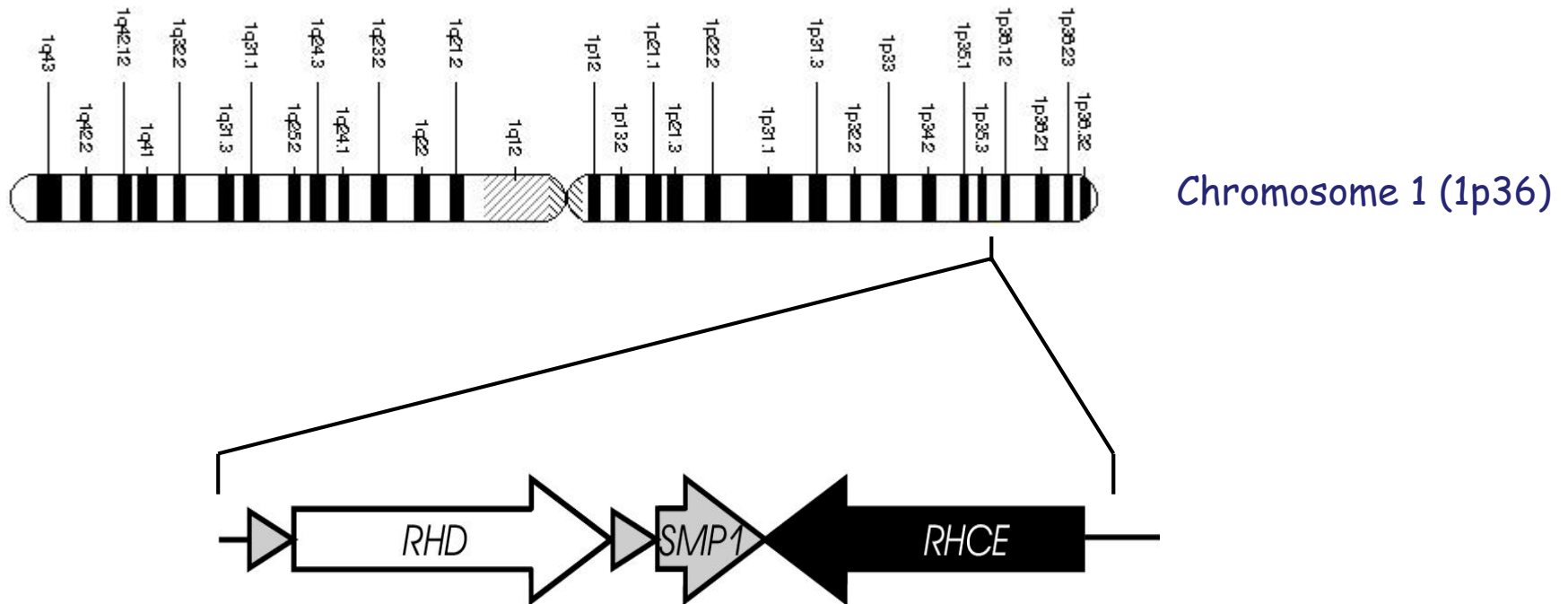
All others cases were explored and characterized by gDNA sequencing

One case was homozygous for the *Ce(329C)* allele

No anti-C alloimmunisation has been observed



The RH Haplotype



⇒ *RHD/RHCE* genes on a same chromosome transmitted together to the offspring

RH « genetic association »

When a *RH* allele very often segregates with a given *RH* allele (*in cis* position).

Example of the R^N haplotype (from Sec- subject)

$RHCE^*Ce-D(4)-Ce$
allele

systematically
associated with



RHD^*D allele

<i>RHD</i> allele(s)		<i>RHCE</i> allele(s)
<i>RHD</i> *DAU0	←	<i>RHCE</i> *ceMO (Westhoff et al, 2013)
<i>RHD</i> *DAU0 or <i>RHW1</i> type 4.0	←	<i>RHCE</i> *ceCF (Martin-Blanc et al, 2013)
<i>RHD</i> *DAR	←	<i>RHCE</i> *ceAR or <i>RHCE</i> *ceEK (Hemker et al, 1999 ; Noizat-Pirenne et al, 2002)
<i>RHD</i> *DIVa-2	←	<i>RHCE</i> *ceTI (Westhoff et al, 2013)
<i>RHD</i> *DOL1 or DOL2	←	<i>RHCE</i> *ceBI or <i>RHCE</i> *ceSM (Roussel et al, 2013)
<i>RHD</i> *DIIIa or <i>RHD</i> *DIIIa-CEVS(4-7)-D or <i>RHD</i> *D-CEVS(4-7)-D	←	<i>RHCE</i> *ce(733G,1006T) (Faas et al, 1997 ; Pham et al, 2009)

For every new *RH* allelic variant discovered ->
Search for a potential association *in cis*

Our 24 samples were all found to show a *RHD*DVII* allele

*RHD*DVII* allele

- partial D (lack of epD8)
- *c.329T>C* mutation (Rouillac *et al*, 1995)
- more frequent than DVI in France (Hennion *et al*, Transfus Clin Biol 2013 (Suppl))
- Usually undetected by routine anti-D typing reagents for patients/donors

Our 24 samples were all found to show a *RHD*DVII* allele

- 9 at heterozygous state
- 15 at homozygous or most probably hemizygous state. Partial D confirmed by serological data
- The homozygous Ce(329C) sample was tested for the Rhesus box (Innotrain) and confirmed to be homozygous for the *RHD*DVII* allele

systematically
associated with
?

*RHCE*Ce329C* allele



*RHD*DVII* allele

22 DVII samples were available in our blood library

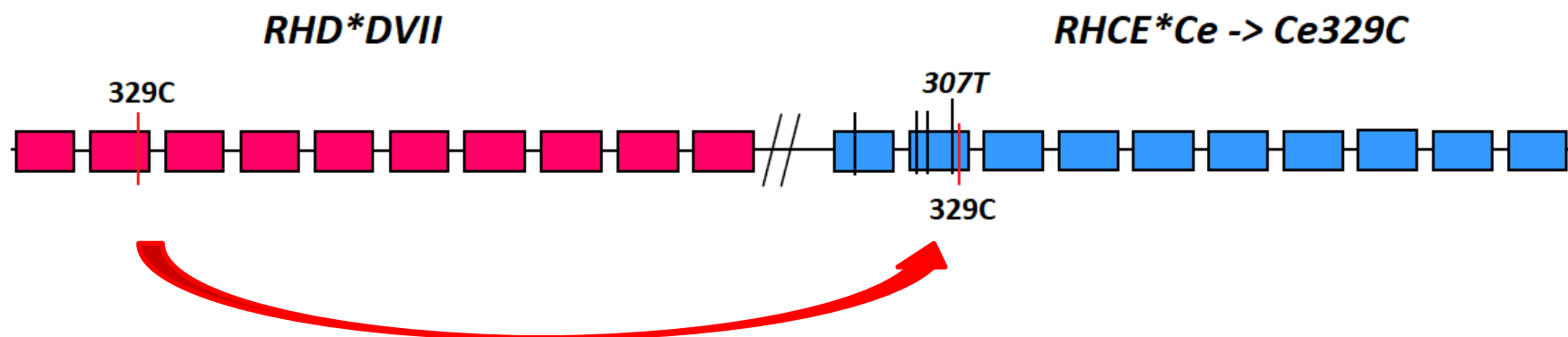
- ✓ 16 referred for a discrepant D reactivity
- ✓ 6 referred for anti-D immunisation in a D+ subject

All showed a conventional *RHCE*Ce* allele
(no c.329T>C mutation in exon 2)

CONCLUSIONS

Novel *RHCE*Ce329C* allele \longrightarrow genetically associated with *RHD*DVII* allele

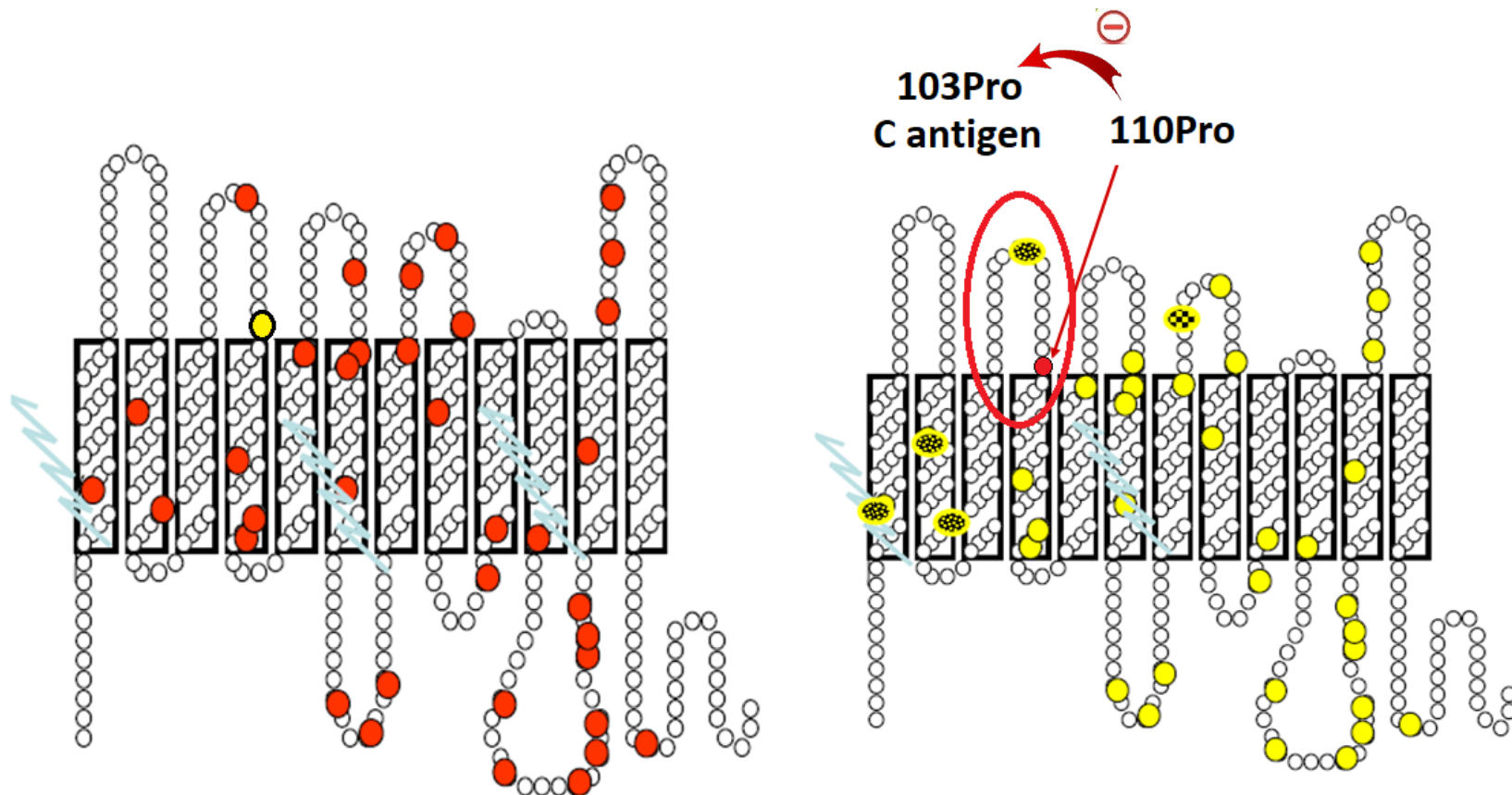
Another example of RHCE=>RHD genetic linkage



Ancestral gene conversion event?

*RHD*DVII* : detectable by only very few antibodies

*RHCE*Ce329C* : easily detectable by anti-C Ab



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ÉTABLISSEMENT FRANÇAIS DU SANG

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