AABB Annual Meeting Education Program 2014

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Presentation Handouts

(9104-TC) Blood Group Systems Update: JR, LAN, VEL

October 25, 2014 ✧ 10:30 AM - 12:00 PM

Advancing Transfusion and Cellular Therapies Worldwide

Event Faculty List

Event Title: **(9104-TC)** Blood Group Systems Update: JR, LAN, VEL
Event Date: October 25, 2014
Event Time: 10:30 AM - 12:00 PM

Director/Moderator
Gregory Halverson, BS, MT(ASCP)SBB, DLM
halvergy@ucmail.uc.edu
Disclosure: No

Speaker
Lilian Castilho, PhD
Professor and researcher
Hemocentro, Unicamp
castilho@unicamp.br
Disclosure: No

Speaker
Thierry Peyrard, PhD, PharmD, EurClinChem
Director of the French National Immunohematology Reference Laboratory
National Institute of Blood Transfusion
tpeyrard@ints.fr
Disclosure: No

Speaker
Jill Storry, PhD, FIBMS
Region Skne
Jill.Storry@med.lu.se
Disclosure: No

Blood Group Systems Update: JR, Lan, Vel

JR Blood Group System

Lilian Castilho, PhD

Disclosures

Relevant Financial Relationship

None

Lilian Castilho
Hemocentro-UNICAMP-Campinas-Brazil

JR Blood group system

Overview

- History and terminology
- Antigens and antibodies
- Molecular basis
- Gene and alleles
- Structure of ABCG2 protein and function

JR Blood group system

History and terminology

Year 1st antigen reported	**1970 (Stroup and MacIlroy, 23rd AABB Annual Meeting)**
How 1st antigen was found	An antibody to a new high prevalence antigen
Naming of 1st antigen	After the first maker of anti-Jr^a, Rose Jacobs 1990: ISBT 901.005
Naming of the system	Jr^a was promoted to a system in 2012 when it was shown that *ABCG2* null alleles define the Jr(a–) phenotype
ISBT System name, symbol, number	JR, JR, 032

JR Blood group system

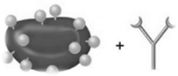
JR antigens 

Number of antigens	**1**
Common (all populations: >99%)	Jr^a
Basis of antigen expression	Not reported
Cord blood cells	Fully developed
Effect of proteases	Resistant
Effect of thiol compounds	Resistant

Rare JR phenotypes

Jr(a-) and Jr(a+^{w}/–)
Human monoclonal anti-Jr^a , clone HMR0921 (Miyazaki and colleagues , 1994): Jr(a–) occurs in 0.07% of Japanese donors
Jr (a–): Various populations (Northern European extraction, Bedouin Arabs and one Mexican) but is found most often in Japaneses and other Asians. No data are available on the frequency of Jr(a–) phenotype in African blacks
Atypical: Jr(a+^{w}/–)

JR Blood group system

Jr^a antibody 

Immunoglobulin class	**IgG more common than IgM**
Optimal technique for detection	IAT
Complement binding in vitro	Some
Autoantibodies	Not reported

JR Blood group system

Clinical relevance of anti-Jr^a

Transfusion reaction	Rare because antibody is rare
Type & severity	Anti-Jr^a may cause moderate hemolytic transfusion reactions, mostly as DHTR
Matching RBCs for transfusion	Hemagglutination and DNA predictions
HDFN	DAT positive but rarely HDFN
Type	Immune destruction
Severity	Mild to severe; rare fatal cases of HDFN

JR Blood group system

Attempts to define the Jr^a antigen
For many years, numerous laboratories, using various techniques have failed to characterize Jr^a
Attempts to immunoprecipitate and immunoblot the antigen using human anti-Jr^a were unsuccessful until 2012

JR Blood group system

JR is carried on ABCG2 transporter and encoded by ABCG2 gene

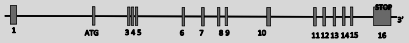
Zelinski and coworkers (Nature genetics 2012; 44:131-2)	Saison and coworkers (Nature genetics 2012; 44:174-7)
Genetic approach	**Biochemical approach**
Homozygosity-by-descent mapping study to identify the chromosomal region containing the gene responsible for Jr^a expression	**HMR0921 MoAb weakly reactive with human RBCs but strongly reactive with cat RBCs** 
Four candidate genes were found but only the product of *ABCG2* was known to be expressed on RBCs	**Immunoprecipitation of cat RBCs with HMR0921 enabled the identification of a protein, identified by mass spectrometry as abcg2, encoded by the cat ortholog (*abcg2*) of the human *ABCG2* gene**
6 Jr(a–): 3 different mutations in *ABCG2*	**18 Jr(a–): 8 different mutations in *ABCG2***
	c.376C>T (Asians); c.706C>T (Gypsies)

42 years later: Jr^a is carried on the ABCG2 transporter and encoded by *ABCG2*

Promoted from ISBT 901.005 to a blood group system (JR or ISBT 032)

JR Blood group system

JR gene and alleles

Gene name	JR (ABCG2)
Entrez Gene ID; GenBank #s	9429; NM_004827.2 (DNA)
Chromosome location	4q22.1 
Number of exons and size	16 exons spread over approximately 68.6 kbp of gDNA 
Type of null alleles (in order of decreasing frequency)	Nonsense, deletions, insertions
Type of mod alleles	Missense

JR Blood group system

23 different null alleles of *ABCG2* have been reported to encode the rare **Jr(a–)**

Alleles	Nucleotide change (exon/intron)	Amino acid	Ethnicity	Report
ABCG2*01N.01	**c.376C>T (4)**	**Gln126X**	**Asian**	Zelinski et al, 2012
ABCG2*01N.02.01	**c.706C>T (7)**	**Arg236X**	**Caucasian**	Zelinski et al, 2012
ABCG2*01N.02.02	**c.34G>A(2), 706C>T (7)**	**Val12Met, Arg236X**	**Asian**	Zelinski et al, 2012
ABCG2*01N.03	**c.736C>T (7)**	**Arg246X**	**Caucasian**	Zelinski et al, 2012
ABCG2*01N.04	c.337C>T (4)	Arg113X	Caucasian	Tobita et al, 2013
ABCG2*01N.05	c.784G>T (7)	Gly262X	Caucasian	Hue-Roye et al, 2013
ABCG2*01N.06	c.34G>A (2), 1591C>T (13)	Val12Met, Gln531X	Caucasian	Hue-Roye et al, 2013
ABCG2*01N.07	**c.187_197delATATTATCGAA (2)**	**Ile63TyrfsX**	**Caucasian**	Saison et al, 2012
ABCG2*01N.08	**C.542_543insA (6)**	**Phe182ValfsX**	**Caucasian**	Saison et al, 2012
ABCG2*01N.09	**c.730C>T (7)**	**Gln244X**	**Caucasian**	Zelinski et al, 2012
ABCG2*01N.10	**c.791_792delTT (7)**	**Leu264HisfsX**	**Caucasian**	Saison et al, 2012
ABCG2*01N.11	**c.875_878dupACTT (8)**	**Phe293LeufsX**	**Caucasian**	Saison et al, 2012
ABCG2*01N.12	**c.1111_1112delAC (9)**	**Thr371LeufsX**	**Asian**	Saison et al, 2012
ABCG2*01N.13	**c.34G>A(2), c.244_245insC (3)**	**Val12Met, Thr82HisfsX**	**Asian**	Zelinski et al, 2012
ABCG2	c.27T>C (2)	Met1Thr	Asian	Tanaka et al, 2014
ABCG2	c.263+1G>A (Intron 3)	r.spl?	Asian	Tobita et al, 2013
ABCG2	c.289A>T (4)	Lys97Ter	Asian	Tobita et al, 2013
ABCG2	c.565_566del (6)	Gly189fs	Asian	Tobita et al, 2013
ABCG2	c.1515delC (13)	Gln141Lys, Ala505fs	Asian	Tobita et al, 2013
ABCG2	c.421C>A (5), c.1515delC (13)	Gln141Lys, Ala505fs	Asian	Tanaka et al, 2014
ABCG2	c.1723C>T (14)	Arg575X	Asian	Tobita et al, 2013
ABCG2	c.1789_1790insT (15)	Ala597fs	Asian	Tobita et al, 2013
ABCG2	27-kb deletion in the promoter region om a *ABCG2*01W.01*		Asian	Ogasawara et al, 2014

JR Blood group system

Alleles encoding Jr(a+^{w}/–) or unclear Jr^{a} status

4 different alleles of *ABCG2* have been reported to encode the rare Jr(a+^{w}/–) phenotypes

9 different alleles of *ABCG2* have been reported to encode unclear Jr^{a} status

Phenotype	Alleles	Nucleotide change (exon/intron)	Amino acid	Ethnicity
Jr(a+^{w}/–)	*ABCG2*01W.01*	c.421C>A (5)	Gln141Lys	Caucasian[1,2]
Jr(a+^{w}/–)	*ABCG2*01W.02*	c.1858G>A (16)	Asp620Asn	Caucasian[1]
Jr(a+^{w}/–)		c.383A>T (5)	Asp128Val	Asian[2]
Jr(a+^{w}/–)		c.1859G>A (16)	Asp620Gly	Asian[2]
Unclear		c.421C>A; 440G>A(5)	Gln141Lys; Arg147Gln	Asian[2]
Unclear		c.421C>A; 458C>T(5)	Gln141Lys; Thr153Met	Asian[2]
Unclear		c.455T>C(5); 1819T>C(16)	Met152Thr; Cys608Arg	Asian[2]
Unclear	*ABCG2*01N.14*	c.1017_1019delCTC (9)	Ser340del	Caucasian[1]
Unclear		c.1384G>A (12)	Gly462Arg	Asian[2]
Unclear		c.1714A>C (14)	Ser572Arg	Caucasian[1]
Unclear		c.1819T>C (16)	Cys608Arg	Asian[2]
Unclear		c.1820+1G>A (Intron 15)	r.spl	Asian[2]
Unclear		c.1841T>G (16)	Leu614Trp	Asian[2]

[1]Hue-Roye, 2013; [2]Tobita et al, 2013

JR Blood group system

Summary
JR alleles encoding Jr(a–), Jr(a+^{w}/–) or unclear Jr^{a} status

Mutations in *ABCG2*

2012: 14 *ABCG2* null alleles: (11 in Caucasians and 3 in Asians)
Zelinski et al, 2012; Saison et al, 2012; Hue-Roye et al, 2012

2013: 6 *ABCG2* null alleles in Asians, 4 *ABCG2* "weak" alleles (2 in Caucasians and 2 in Asians) and 9 *ABCG2* alleles with unclear status: (2 in Caucasians and 7 in Asians)
Tobita et al, 2013; Hue-Roye et al, 2013

2014: 3 *ABCG2* null alleles in Asians
Tanaka et al, 2014; Ogasawara et al, 2014

JR Blood group system

Predominant alleles encoding Jr(a–) and Jr(a+^{w}/–) phenotypes

Mutations in *ABCG2*

c.376C>T nonsense mutation in *ABCG2* is the most frequently detected mutation in Jr(a–) individuals
1.7% of Japaneses: "high"-incidence of the Jr(a–) phenotype

c.706C>T and c.736C>T nonsense mutations in *ABCG2* are more frequently detected in Caucasian Jr(a–) individuals

c.421C>A, c,1714A>C and c.1858G>A) in *ABCG2* are linked to weakned expression of the Jr^{a} antigen

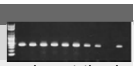
The majority of Jr(a–) individuals is homozygous for a single mutation

JR Blood group system

DNA-based genotyping to identify Jr(a–) individuals

DNA-based genotyping

PCR assay for c.376C>T: 90% of Japaneses Jr(a–)



High-throughput genotyping assay targeting frequently occurring mutations in *ABCG2* (c.376T, c.706T and c.736T) that cause the Jr(a–) phenotype

Haer-Wigman et al, Transfusion 2014, 54:1836-46

The extended heterogeneity of mutations causing Jr(a–) phenotype in most populations makes genetic screening for the Jr(a–) phenotype inefficient
Genetic screening for a specific mutation that causes the Jr(a–) in specific ethinicities can be more efficient

JR Blood group system

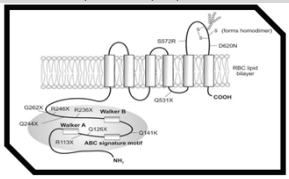
Haer-Wigman L, Ait Soussan A, Ligthart P, de Haas M, van der Schoot CE. **Molecular analysis of immunized Jr(a–) or Lan– patients and validation of a high-throughput genotyping assay to screen blood donors for Jr(a–) and Lan- phenotypes.** Transfusion 2014;54:1836-46.

Investigation of copy number variation in *ABCG2* in Dutch Jr(a–) individuals
No copy number variation was detected in *ABCG2*
Negativity is not due to loss of heterozygosity of *ABCG2*, but to the presence of two affected alleles of *ABCG2*

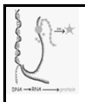
JR Blood group system

JR Protein (ABCG2)

Name(s)	Jr glycoprotein, ATP-binding cassette, sub-family G, member 2 [ABCG2]; breast cancer resistance protein [BCRP]
Membrane orientation	Multipass protein with 6 predicted passes
Structure	Glycoprotein of 655 amino acids with one nucleotide binding domain with Walker A, Walker B, and Signature motifs oriented to the cytoplasmic surface followed by one membrane spanning domain. The functional molecule is likely a homodimer in the membrane
M, on SDS-PAGE	72,000 reduced; 180,000 non-reduced



Predicted topology of ABCG2 protein with location of amino acid changes encoded by nonsense and missense alleles



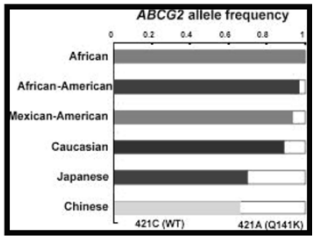
JR Blood group system

ABCG2 Protein

In other tissues	High expression in placenta Low expression in epithelial cells of small and large intestines, liver ducts, colon, lobules of the breast, endothelial cells of veins and capillaries, and brain microvessel endothelium, stem cells, lung and in the apical membrane of proximal tubules of the kidney Unregulated in breast and brain tumors
Function	An ATP-dependent transport protein of a broad range of substrates Involved in multidrug resistance in tumor cells, particularly in breast cancer Function in the defense of normal cells against toxic agents A role in folate homeostasis
Disease association	The Gln126Stop and Gln141Lys variants of ABCG2 are associated with an increased risk for gout

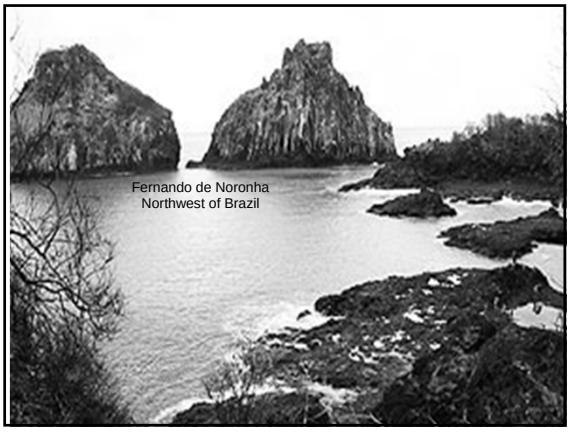
JR Blood group system

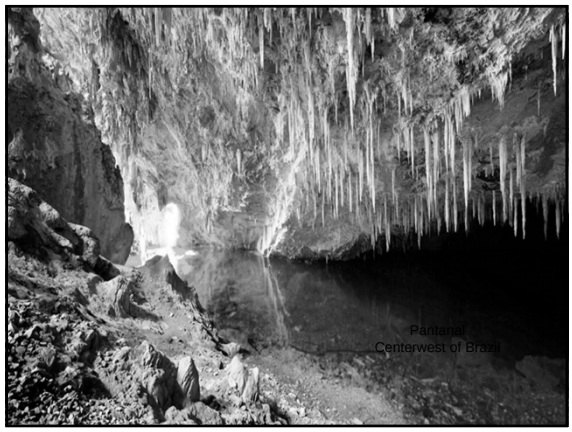
c.421C>A Gln141Lys



JR Blood group system

About the alleles	To date, more than 1,000 synonymous and non-synonymous single-nucleotide polymorphisms (SNPs) in the gene sequence of *ABCG2* have been described (http://www.ncbi.nlm.nih.gov/snp) Additional diversity within the JR blood group system is still expected
About the function	Jr(a–) individuals provide a large cohort of "natural knockouts" for *ABCG2* (*ABCG2*$^{-/-}$) Opportunity to study the exact role and function of ABCG2 in humans under normal physiology and pathologic conditions





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Thierry PEYRARD
PharmD, PhD
European Specialist in Clinical Chemistry and Laboratory Medicine

National Institute of Blood Transfusion
Director, National Immunohematology Reference Laboratory
Paris, France

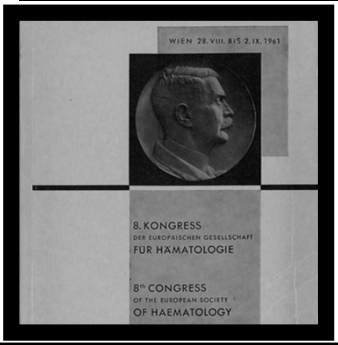
AABB – Philadelphia
October 25th 2014

INSTITUT NATIONAL DE LA TRANSFUSION SANGUINE

The LAN blood group system

A review and 2014 update

The Lan antigen: a 50-year old mystery



van der Hart & al 1961

Lan for « Langereis », name of the first proband (Dutch origin)

The Lan antigen

The 901 series of high incidence antigens

Lan ANTIGEN

Terminology

ISBT symbol (Number)	Lan (901.002)
Other names	Langereis, Gn², Gonsowski, So, 900.003
History	Reported in 1961, named after the first antigen-negative proband to make anti-Lan

Occurrence

All populations: > 99%.
The Lan− phenotype occurs in about 1 in 20 000 people, found in Blacks,[1,2] Caucasians and Japanese.

Effect of enzymes/chemicals on Lan antigen on intact RBCs

Ficin/papain	Resistant
Trypsin	Resistant
α-Chymotrypsin	Resistant
Pronase	Resistant
Sialidase	Resistant
DTT 200 mM	Resistant
Acid	Resistant

The Lan antigen used to belong to the 901 series

Lan- is a rare blood type worldwide

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What is the 901 series?

- **Antigens with a prevalence >90% in most populations, that do not fit into any system or collection**
- **In 2012, this series included 8 antigens officially recognized by the ISBT *Working Party on Red Cell Immunogenetics and Blood Group Terminology***

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The 901 series of RBC antigens in 2012

N°	Name	Symbol	Prevalence (%)
901002	Langereis	**Lan**	**> 99**
901003	August	**At^a**	> 99
901005		**Jr^a**	> 99
901008		**Emm**	> 99
901009	Anton	**AnWj**	> 99
901011	Sid	**Sd^a**	90
901014		**PEL**	> 99
901016		**MAM**	> 99

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Why studying Lan?

- **30 Lan- people in the French National Registry of People with a Rare Blood Type, most originating from the Maghreb area (North Africa)**

- **25/30 with anti-Lan**
- **Anti-Lan considered a clinically significant alloantibody**

7

Clinical significance of anti-Lan

1. Hemolytic disease of the newborn caused by anti-Lan, anti-Jka, and anti-c.
   Shertz WT, Carty L, Wolford F.
   Transfusion. 1987 Jan-Feb;27(1):117. No abstract available.
   PMID: 3101246 [PubMed - indexed for MEDLINE]
   Related citations Remove from clipboard

2. Clinical significance of anti-Lan.
   Judd WJ, Oberman HA, Silenieks A, Steiner EA.
   Transfusion. 1984 Mar-Apr;24(2):181. No abstract available.
   PMID: 6580590 [PubMed - indexed for MEDLINE]
   Related citations Remove from clipboard

3. Hemolytic disease of the newborn due to anti-Lan.
   Page PL.
   Transfusion. 1983 May-Jun;23(3):256-7. No abstract available.
   PMID: 6679383 [PubMed - indexed for MEDLINE]
   Related citations Remove from clipboard

4. Haemolytic disease of the newborn caused by anti-Lan antibody.
   Smith DS, Stratton F, Johnson T, Brown R, Howell P, Riches R.
   Br Med J. 1969 Jul 12;3(5662):90-2.
   PMID: 5790273 [PubMed - indexed for MEDLINE] Free PMC Article
   Related citations Remove from clipboard

5. [Lan antigen of erythrocytes and clinical significance of anti-Lan antibody].
   Kuśnierz-Alejska G, Wiecek B.
   Acta Haematol Pol. 1993;24(2):169-75. Polish.
   PMID: 8372617 [PubMed - indexed for MEDLINE]
   Related citations Remove from clipboard

Mild to moderate HDFN

Mild to moderate hemolytic transfusion reactions

How did we find out the molecular basis of Lan?

9

OSK43 monoclonal anti-Lan: the key to elucidate the Lan mystery?

XVIIIth Regional Congress Asia - ISBT, Hanoi, Vietnam, 2007 P-071
ESTABLISHMENT AND CHARACTERIZATION OF HUMAN MONOCLONAL ANTI-LAN
Tani *et al.*
Japanese Red Cross Osaka Blood Center, Osaka, Japan.

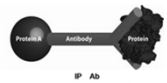
Monoclonal anti-Lan OSK43 (human IgG1κ)

Agglutination | **Flow cytometry** | ~~**Western blot**~~

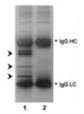
10

Immunoprecipitation with monoclonal anti-Lan OSK43

- **Immunoprecipitation** of the Lan carrier with OSK43 from a RBC lysate



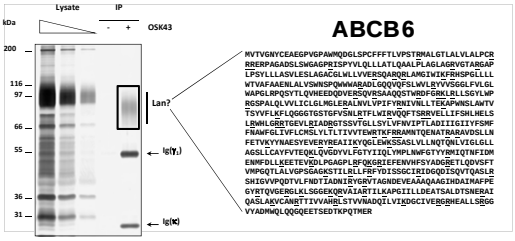
- Analysis of the immunoprecipitate by **electrophoresis** and **protein silver staining**



- **Band cutting** on the gel => identification of candidate carriers by **mass spectrometry**, after trypsin digestion

11

OSK43 immunoprecipitates the ABCB6 human transporter of human RBCs



Silver-stained gel

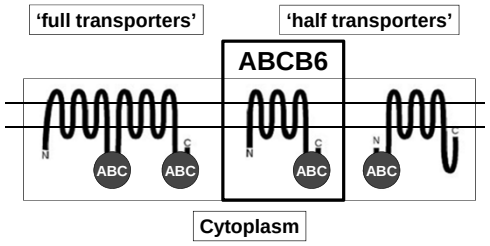
ABCB6

MVTVGNYCEAEGPVGPAWMQDGLSPCFFFTLVPSTRMALGTLALVLALPCR
RRERPAGADSLSWGAGPRISPYVLQLLLATLQAALPLAGLAGRVGTARGAP
LPSYLLLASVLESLAGACGLWLLVVERSQARQRLAMGIWIKFRHSPGLLLL
WTVAFAAENLALVSWNSPQWWWAADLGQQVQFSLWVLEYVSGGLFVLGL
WAPGLRPQSYTLQVHEEDQDVERSQVRSAAQQSTWRDFGRKLRLLSGYLWP
RGSPALQLVVLICLGLMGLERAINVLVPIFYRNIVRLLTEKAPWNSLAWTV
TSVVFLKFLQGGGTGSTGFVSNLRTFLWIRVVQQFTSRRVELLIFSHLHELS
LRWHLGRRTGEVLRIAQRGTSSVTGLLSYLVFNVIPTLADIIIGIIYFSMF
FRAWFGLIVFLCMSLYLTLTIVVTEWRTKFRRAMNTQENATRARAVDSLLN
FETVKYYNAESYEVERYREAIIKYQGLEWKSSASLVLLNQTQNLVIGLGLL
AGSLCAVFVTEQKLQVGDYVLFGTYIIQLVMPLNWFGTYYRMIQTNFIDM
ENMFDLLKEETEVKDLPGAGPLRFQKGRIEFENVHFSYADGRETLQDVSFT
VMPGQTLALVGPSGAGKSTILRLLFRFYDISSGCIRIDGQDISQVTQASLR
SHIGVVPQDTVLFNDTIADNIEYGRVTAGNDEVEAAAQAAGIHDAIMAFPE
GYRTQVGERGLKLSGGEKQRVAIARTILKAPGIILLDEATSALDTSNERAI
QASLAKVCANRTTIVVAHRLSTVVNADQILVIKDGCIVERGRHEALLSRGG
VYADMWQLQQGQEETSEDTKPQTMER

Mass spectrometry analysis
High confidence level: 17 peptides

12

The ATP-binding cassette (ABC) transporter family



ATP-binding cassette transporters actively facilitate the transmembrane flux of numerous substances

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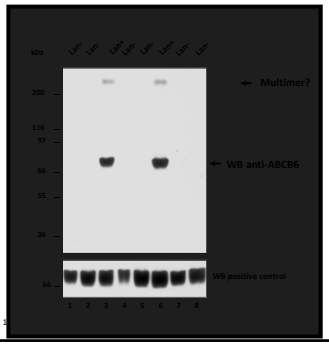
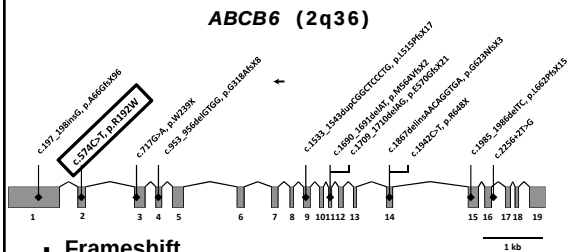
ABCB6 is absent in the membrane of all tested Lan- RBCs



Lan- are « Lan null » and can be considered « human knockouts » for *ABCB6*

Examples of null alleles of *ABCB6* identified by genomic DNA sequencing



- **Frameshift**
- **Nonsense mutations (stop codons)**
- **Missense mutation (point mutation)**

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Missense c.574C>T mutation in *ABCB6*

VoxSanguinis Vox Sang 2013;104:159-65
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2012)

ORIGINAL PAPER

DOI: 10.1111/j.1423-04 10.2012.01650.x

The *ABCB6* mutation p.Arg192Trp is a recessive mutation causing the Lan− blood type

C. Saison,[1,4] V. Helias,[1,4] T. Peyrard,[1,2] L. Merad,[3] J.-P. Cartron[1] & L. Arnaud[1]
[1]National Institute of Blood Transfusion (INTS), Paris, France
[2]National Reference Center for Blood Groups (CNRGS), Paris, France
[3]Centre Europe Le Palatin, Hyères, France

- **Accelerated degradation of ABCB6?**
- **Occurred in several ethnic backgrounds (France, North Africa, Poland, Italy…)**

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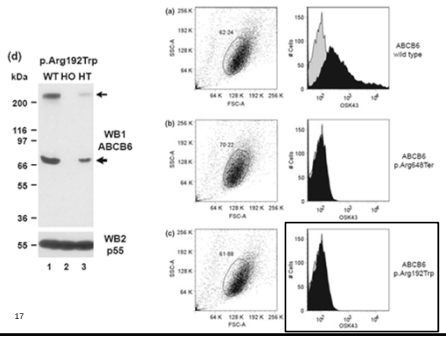
Missense c.574C>T mutation in *ABCB6*



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Variant alleles of *ABCB6* responsible for Lan- and Lan weak phenotypes


The LAN blood group system: a review
T. Peyrard

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Variant *ABCB6* alleles

Alleles officially recognized by the ISBT *Working Party on Red Cell Immunogenetics and Blood Group Terminology*

- **15 null alleles** responsible for a **Lan-** phenotype *ABCB6*01N.01* to *ABCB6*01N.15* => **difficult to implement Lan testing on the current genotyping platforms (which one(s) should we choose)**
- **4 alleles** responsible for a **Lan weak** phenotype *ABCB6*01W.01* to *ABCB6*01W.04*

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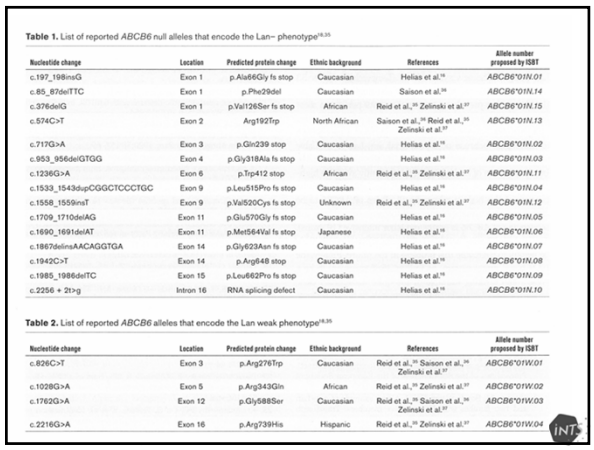

Table 1. List of reported *ABCB6* null alleles that encode the Lan– phenotype[18,35]

Nucleotide change	Location	Predicted protein change	Ethnic background	References	Allele number proposed by ISBT
c.197_198insG	Exon 1	p.Ala66Gly fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.01*
c.85_87delTTC	Exon 1	p.Phe29del	Caucasian	Saison et al.[34]	*ABCB6*01N.14*
c.376delG	Exon 1	p.Val126Ser fs stop	African	Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01N.15*
c.574C>T	Exon 2	Arg192Trp	North African	Saison et al.,[34] Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01N.13*
c.717G>A	Exon 3	p.Gln239 stop	Caucasian	Helias et al.[18]	*ABCB6*01N.02*
c.953_956delGTGG	Exon 4	p.Gly318Ala fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.03*
c.1236G>A	Exon 6	p.Trp412 stop	African	Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01N.11*
c.1533_1543dupCGGCTCCCTGC	Exon 9	p.Leu515Pro fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.04*
c.1558_1559insT	Exon 9	p.Val520Cys fs stop	Unknown	Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01N.12*
c.1709_1710delAG	Exon 11	p.Glu570Gly fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.05*
c.1690_1691delAT	Exon 11	p.Met564Val fs stop	Japanese	Helias et al.[18]	*ABCB6*01N.06*
c.1867delinsAACAGGTGA	Exon 14	p.Gly623Asn fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.07*
c.1942C>T	Exon 14	p.Arg648 stop	Caucasian	Helias et al.[18]	*ABCB6*01N.08*
c.1985_1986delTC	Exon 15	p.Leu662Pro fs stop	Caucasian	Helias et al.[18]	*ABCB6*01N.09*
c.2256 + 2t>g	Intron 16	RNA splicing defect	Caucasian	Helias et al.[18]	*ABCB6*01N.10*

Table 2. List of reported *ABCB6* alleles that encode the Lan weak phenotype[18,35]

Nucleotide change	Location	Predicted protein change	Ethnic background	References	Allele number proposed by ISBT
c.826C>T	Exon 3	p.Arg276Trp	Caucasian	Reid et al.,[35] Saison et al.,[34] Zelinski et al.[37]	*ABCB6*01W.01*
c.1028G>A	Exon 5	p.Arg343Gln	African	Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01W.02*
c.1762G>A	Exon 12	p.Gly588Ser	Caucasian	Reid et al.,[35] Saison et al.,[34] Zelinski et al.[37]	*ABCB6*01W.03*
c.2216G>A	Exon 16	p.Arg739His	Hispanic	Reid et al.,[35] Zelinski et al.[37]	*ABCB6*01W.04*

Variant *ABCB6* alleles: Update 2014

Novel alleles to be considered by the ISBT *Working Party on Red Cell Immunogenetics and Blood Group Terminology*

- **13 null alleles** responsible for a **Lan-** phenotype reported in 2013 and 2014
- **3 alleles** responsible for a **Lan weak** phenotype reported in 2014
- **6 alleles with unclear status** reported in 2013 and 2014

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13 novel *ABCB6* null alleles (1)

Exon 1	c.301_302ins G	p.Ala101fs
Exon 1	*c.459del*	p.Leu154fs
Exon 1	c.459del	p.Leu154fs
Exon 3	c.718C > T	p.Arg240ter
Exon 4	c.881_884del	p.Thr294fs
Exon 10	c.1617delG	p.Gly539del

Yamamuro Y, *Vox Sang 2013;105(Suppl 1):230-231*

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13 novel *ABCB6* null alleles (2)

Exon 1	c.1A > C	p.0
Exon 3	c.827G > A	p.Arg276Glu
Intron 4	c.971-1G > A	r.spl?
Exon 13	c.1825G > A	p.Val609Met
Exon 14	c.1912C > T	p.Arg638Cys
Exon 16	c.2155C > T	p.Glu719Ter
Intron 17	c.2351 + 1g > a	r.spl?

Lonneke Haer-Wigman et al. *Transfusion* 2014

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3 novel *ABCB6* alleles encoding a Lan weak phenotype

Exon 1	c.317A > G	p.Tyr106Cys
Exon 15	c.2206G > C	p.Ala736Pro
Exon 1	c.403C > A	p.Arg135Ser

Yamamuro Y, *Vox Sang 2014;107(Suppl 1):186-87*

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6 novel *ABCB6* alleles with unclear status with regard to Lan expression

Exon 1	c.20A > G	p.Tyr7Cys
Intron 3	c.869-2A > G	r.spl?
Exon 6	c.1199_1210del	p.Ile400_Tyr404del
Intron 16	c.2256 + 1G > A	r.spl?
Exon18	c.2383_2385del	p.Leu795del

Yamamuro Y, *Vox Sang* 2013;*105(Suppl 1):230-231*

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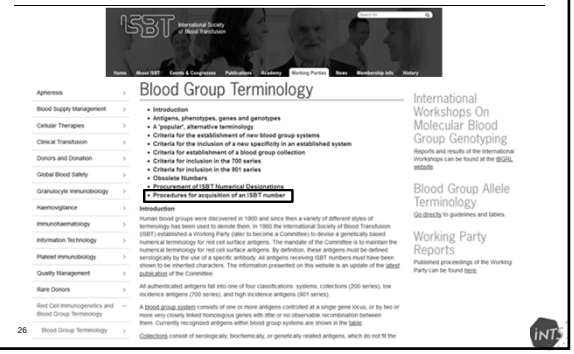
What to do if you find out a novel *ABCB6* allele? (1)



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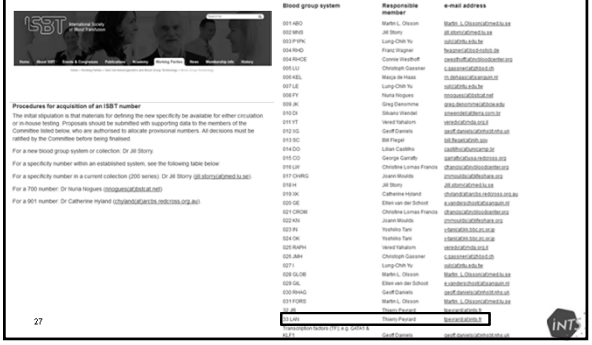
What to do if you find out a novel *ABCB6* allele? (2)



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What was unexpected with the discovery of the molecular basis of Lan!

- **ABCB6 was not considered to be present on RBCs (only described on outer mitochondrial membrane and Golgi apparatus)**
- **ABCB6 reported to be an essential protein in erythropoiesis, especially through mitochondrial porphyrin uptake**

However, Lan- people, who may be considered human "knockouts" for *ABCB6,* appear to be healthy!

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Biological data in Lan- people

- **Four Lan- subjects were tested for porphyrin levels**
  - ✓ RBCs 2.1 µmol ± 0.2 N: 0.1 – 1.9
  - ✓ Plasma < 5 nmol/l N: 6.5 – 20.0
  - **Slight increase in RBCs but not comparable at all to porphyria!**
    => Probable compensatory mechanism
- **Four Lan- subjects were tested for blood count, since Lan was described to be essential for erythropoiesis: nothing abnormal**

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Biological data in Lan- people

Parameters	Units	Sex Age	Lan- subject #1 Female 40 years	Lan- subject #2 Female 27 years	Lan- subject #3 Female 87 years	Lan- subject #4 Male 76 years
RBC	10^{12}/L		4,37	4,79	4,07	4,52
HGB	g/dL		12,3	13,9	12,9	12,9
HCT	%		36,0	40,1	40,9	39,6
MCV	fL		82,4	84,0	100,5	87,6
MCH	pg		28,1	29,0	31,7	28,5
MCHC	g/dL		34.0	34,7	32,0	32,6
Reticulocytes	10^9/L		45	49	59	56
Platelets	10^9/L		415	289	282	226
WBC	10^9/L		6,84	7,10	5,75	7,83
Neutrophils	10^9/L		3,73	4,00	3,77	5,21
Eosinophils	10^9/L		0,31	0,10	0,34	0,28
Basophils	10^9/L		0,03	0,00	0,02	0,02
Lymphocytes	10^9/L		2,34	2,70	1,20	1,66
Monocytes	10^9/L		0,43	0,30	0,42	0,66
Neutrophils	%		54,6	56,0	65,6	66,5
Eosinophils	%		4,5	2,0	5,9	3,6
Basophils	%		0,4	0,0	0,3	0,3
Lymphocytes	%		34,2	38,0	20,9	21,2
Monocytes	%		6,3	4,0	7,3	8,4

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Publication of the work

LETTERS

nature genetics

Received 6 May 2011; accepted 9 December 2011; published online 15 January 2012; doi:10.1038/ng.1069

ABCB6 is dispensable for erythropoiesis and specifies the new blood group system Langereis

Virginie Helias[1], Carole Saison[1], Bryan A Ballif[2], Thierry Peyrard[1,3], Junko Takahashi[4], Hideo Takahashi[4], Mitsunobu Tanaka[4], Jean-Charles Deybach[5], Hervé Puy[5], Maude Le Gall[6], Camille Sureau[1], Bach-Nga Pham[1,3], Pierre-Yves Le Pennec[1,3], Yoshihiko Tani[4], Jean-Pierre Cartron[1] & Lionel Arnaud[1]

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Lan is the 33rd blood group system

(Lan) LAN was officially elevated to the status of 33rd human blood group system by the ISBT *Working Party on Red Cell Immunogenetics and Blood Group Terminology* in July 2012

(ISBT Meeting, Cancun, Mexico)

901 series before July 2012

N°	Name	Symbol	Prevalence (%)
901002	Langereis	Lan	> 99
901003	August	At^a	> 99
901005		Jr^a	> 99
901008		Emm	> 99
901009	Anton	AnWj	> 99
901011	Sid	Sd^a	90
901014		PEL	> 99
901016		MAM	> 99

→ **33rd blood group system**

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Variant *ABCB6* functional alleles and diseases (1)

ARTICLE

ABCB6 Mutations Cause Ocular Coloboma **Leu811Val mutation in ABCB6**

Lejing Wang,[1,14,*] Fei He,[2,3,14] Juan Bu,[1,14] Xiaqi Liu,[2,3] Wei Du,[1,13] Jiamei Dong,[1,4] Jeffrey D. Cooney,[5,6] Sushil Kumar Dubey,[7] Yi Shi,[2,3] Bo Gong,[2,3] Jing Li,[1] Paul F. McBride,[5,6] Yanlei Jia,[8] Fang Lu,[2,3] Kathleen A. Soltis,[5,6] Ying Lin,[2,3] Prasanthi Namburi,[7] Chen Liang,[1] Periasamy Sundaresan,[7] Barry H. Paw,[5,6] Dean Y. Li,[9,10,11] John D. Phillips,[12] and Zhenglin Yang[2,3,*]

Ocular coloboma is a developmental defect of the eye and is due to abnormal or incomplete closure of the optic fissure. This disorder displays genetic and clinical heterogeneity. Using a positional cloning approach, we identified a mutation in the ATP-binding cassette (ABC) transporter *ABCB6* in a Chinese family affected by autosomal-dominant coloboma. The Leu811Val mutation was identified in seven affected members of the family and was absent in six unaffected members from three generations. A LOD score of 3.2 at $\theta = 0$ was calculated for the mutation identified in this family. Sequence analysis was performed on the *ABCB6* exons from 116 sporadic cases of microphthalmia with coloboma (MAC), isolated coloboma, and aniridia, and an additional mutation (A57T) was identified in three patients with MAC. These two mutations were not present in the ethnically matched control populations. Immunostaining of transiently transfected, Myc-tagged *ABCB6* in retinal pigment epithelial (RPE) cells showed that it localized to the endoplasmic reticulum and Golgi apparatus of RPE cells. RT-PCR of *ABCB6* mRNA in human cell lines and tissue indicated that ABCB6 is expressed in the retinae and RPE cells. Using zebrafish, we show that *abcb6* is expressed in the eye and CNS. Morpholino knockdown of *abcb6* in zebrafish produces a phenotype characteristic of coloboma and replicates the clinical phenotype observed in our index cases. The knockdown phenotype can be corrected with coinjection of the wild-type, but not mutant, *ABCB6* mRNA, suggesting that the phenotypes observed in zebrafish are due to insufficient *abcb6* function. Our results demonstrate that *ABCB6* mutations cause ocular coloboma.

The American Journal of Human Genetics *90*, 40–48, January 13, 2012

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Variant *ABCB6* functional alleles and diseases (2)

Research Article

Missense mutations in the ABCB6 transporter cause dominant familial pseudohyperkalemia

Immacolata Andolfo,[1,2] Seth L. Alper,[3,4,5] Jean Delaunay,[6] Carla Auriemma,[1,2] Roberta Russo,[1,2] Roberta Asci,[1] Maria Rosaria Esposito,[1] Alok K. Sharma,[3,4,5] Boris E. Shmukler,[3,4,5] Carlo Brugnara,[7] Lucia De Franceschi,[8] and Achille Iolascon[1,2]*

Am. J. Hematol. 88:66–72, 2013.

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Variant *ABCB6* functional alleles and diseases (3)

« Previous Article | Journal of Dermatological Science | Articles in Press | Next Article »

Article in Press

Novel missense mutations of *ABCB6* in two chinese families with dyschromatosis universalis hereditaria

Chaoxia Lu[1], Jie Liu[1], Fang Liu, Yaping Liu, Donglai Ma[✉], Xue Zhang[✉]
[1] These authors contributed equally to this work.

Received: May 28, 2014; Published Online: September 13, 2014

DOI: http://dx.doi.org/10.1016/j.jdermsci.2014.08.015

Publication stage: In Press Corrected Proof

Abstract | Full Text | Images | References | Supplemental Materials

Dyschromatosis universalis hereditaria (DUH) is a very rare genodermatosis characterized by generalized hypopigmented and hyperpigmented macules of varying sizes and shapes occurring over almost all the body. It was first described in 1933 by Ichikawa and Higari. It is most commonly reported in Japan [1], but has also been reported in other parts of Asia, Europe, America, and Africa. The skin lesions in DUH show no seasonal change or spontaneous regression with age [2]. Systemic abnormalities, including short stature, high-frequency deafness, erythrocyte, platelet and tryptophan metabolism abnormalities, insulin-dependent diabetes mellitus, bilateral glaucoma and unilateral cataract, photosensitivity along with neurosensory hearing defects, and grand-mal seizures, are rare [3].

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This illustrates the "gain-of function mutation" concept

It sometimes appears to be much "better" not to have the protein at all than having an altered form of the protein!

=> This defines the so-called "gain-of-function mutations", that change the gene product such that the protein gains a new and abnormal function (also called "neomorphic mutations). Such mutations usually demonstrate a dominant trait.

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ABCB6 and anticancer drug resistance

ANTICANCER RESEARCH
International Journal of Cancer Research and Treatment

Expression of ABCB6 Is Related to Resistance to 5-FU, SN-38 and Vincristine

KENTARO MINAMI[1,2*], YOUHEI KAMIDO[1,4*], YUKIHIKO NISHIZAWA[1,2], SHO TABATA[1,5], FUMITO HORIKUCHI[1,4], MASATATSU YAMAMOTO[1], KOICHI KAWAHARA[1], YOSHINARI SHINSATO[1], TOKUSHI TACHIWADA[1,2], ZHE-SHENG CHEN[6], KAZUTAKE TSUJIKAWA[7], MASAYUKI NAKAGAWA[3], NAOHIKO SEKI[8], SHIN-ICHI AKIYAMA[1,9], KAZUNARI ARIMA[4], YASUO TAKEDA[2] and TATSUHIKO FURUKAWA[1]

Departments of [1]Molecular Oncology, [2]Clinical Pharmacy and Pharmacology and [3]Urology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; [4]Department of Organic and Biological Chemistry, Graduate School of Science and Engineering, Kagoshima University, Kagoshima, Japan; [5]Institute for Advanced Biosciences, Keio University, Yamagata, Japan; [6]College of Pharmacy and Allied Health Professions, Department of Pharmaceutical Sciences, St. John's University, Queens, NY, U.S.A.; [7]Graduate School of Pharmaceutical Science, Osaka University, Osaka, Japan; [8]Department of Functional Genomics, Chiba University Graduate School of Medicine, Chiba, Japan; [9]Clinical Research Center, National Kyushu Cancer Center, Fukuoka, Japan

ANTICANCER RESEARCH *34*: 4767-4774 (2014)

Abstract. *A previously established arsenite-resistant cell line, KAS, is also resistant to a variety of anticancer drugs. In order to understand responsible molecules for the multidrug resistance phenotype of KAS cells, we examined the expressions of ATP-binding cassette (ABC) transporters and found that the ABCB6 and ABCC1/ multidrug resistance protein 1 (ABCC1/MRP1) were increased. ABCC1/MRP1 was not completely responsible for the drug resistance spectrum of KAS cells and several reports have suggested that ABCB6 is related to anticancer drug and metal resistance. We, therefore, established and examined ABCB6-expressing KB cells and ABCB6-knockdown KAS cells. ABCB6 expression enhanced resistance to 5-fluorouracil (5-FU), SN-38 and vincristine (Vcr) but not to arsenite. Conversely, down-regulation of ABCB6 in KAS cells increased the sensitivity of KAS cells to 5-FU, SN-38 and*

Are the Lan- people more sensitive to 5-FU anticancer drug?
Could the usual dose of 5-FU be toxic in Lan- patients (overdosage)?

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INSTITUT NATIONAL DE LA TRANSFUSION SANGUINE

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Lionel Arnaud, Carole Saison, Virginie Helias, Jean-Pierre Cartron, and all the staff of the French National IRL
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Japanese Red Cross, Hokkaido Blood Center, Japan

Yoshihiko Tani
Japanese Red Cross, Kinki Block Blood Center, Japan

All Lan- patients/donors who kindly accepted to participate to our research works

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THANK YOU FOR YOUR ATTENTION

Blood group system update: The Vel blood group system

Jill R. Storry, Ph.D.
Associate Professor
Chair, ISBT Working Party on Red Cell Immunogenetics and Blood Group nomenclature

AABB meeting
25 October 2014
Philadelphia

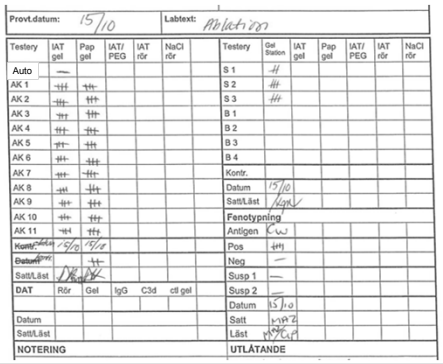
Case study 2014-10-15

- Female; born 1944, admitted for cardiac ablation
  - In OP, "low risk" for bleeding
- Tx 2006 with 2 x B+ RBCs, 2 x Plts
  - Negative type and screen
- Tx 2004 with 2 x Plts
- 15/10: screening cells 3+ IAT-gel
- Blood grouping:

Anti-A	Anti-B	Anti-D	Anti-D	A1c	Bc
0	4+	4+	4+	4+	4+

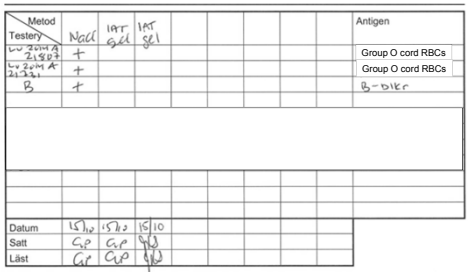
Vel update
25 October 2014
Philadelphia

Acute antibody investigation



Vel update
25 October 2014
Philadelphia

Antibody investigation contd.



Vel update 5 October 2014 Philadelphia

Further testing showed:

- Patient typed Vel-, E-, s-, Fy(a-)
- Other antibodies excluded
- Antibody was completely inactivated with 10 mM DTT: IgM!!!
- Titre: 2 with untreated RBCs, 64 with papain-treated RBCs

Vel update 5 October 2014 Philadelphia

Case #2

- Positive antibody screen prior to elective surgery on a group B RhD– 72 year-old woman
- Investigated 8 years previously at another regional hospital 8 years when treated for a hip fracture
- Previous investigation looked like an autoantibody:
  - DAT-positive
  - Positive auto control
  - Hemolysis with papain-treated RBCs
- Untransfused, pregnancy history unknown

Vel update 5 October 2014 Philadelphia

Case #2 Serology

Current investigation:

- Strong reactivity with papain-treated RBCs in gel
  - Negative autocontrol
- 8 av 9 panel RBCs reactive with gel-IAT
  - Reactivity from +^{w} to 2+
  - Negative autocontrol
- Rouleaux in saline 4ºC and RT
- DAT negative
- **6** av 10 RBC concentrates compatible by gel-IAT

Antibody ID panel

	D	C	E	c	e	M	N	S	s	P	K 1	k	Fy^{a}	Fy^{b}	Jk^{a}	Jk^{b}	Gel IAT	Pap-37C
1	+	+	+	+	+	+	0	+	0	0	0	+	0	+	+	+	2+	3+
2	0	0	0	+	+	+	0	0	+	+	+	0	+	+	0	+	2+	3+
3	0	+	0	+	+	+	0	0	+	0	0	+	+	0	+	+	1+	3+
4	0	0	+	+	+	+	0	+	+	+	0	+	0	+	+	0	2+	3+
5	+	+	0	0	+	+	+	0	+	+	0	+	+	0	+	0	2+	3+
6	+	0	+	+	0	0	+	0	+	+	0	+	+	+	0	+	1+^{sv}	3+
7	0	0	0	+	+	+	0	+	+	0	+	+	0	+	+	+	1+^{sv}	3+
8	+	0	0	+	+	0	+	0	+	+	0	+	+	+	0	+	0	3+
9	0	0	0	+	+	0	+	0	+	+	+	+	+	0	+	0	1+^{sv}	3+
Egna																	0	0

Additional testing

- Papain autoadsorption was ineffective
  - Performed based on previous report
- Not inhibited by pooled plasma
  - AK8 known to be Rg- and Kn(a-)
- Surgery started despite low Hb
  - Deemed low bleeding risk

Investigation cont.d

Enzyme- and DTT-treated RBCs:

	Gel-IAT
Papain	3+
Trypsin	3+
α-chymotrypsin	2+
Pronase	4+
200mM DTT	1+^{w}

?

Interpretation

Papain	Trypsin	α-ct	Pronase	DTT	Probable specificity
0	0	0	0	+	Ch/Rg, Xg^{a}
0	0	0	0	0	In, JMH
0	0	+	0	+	MN, En^{a}TS, Ge2, Ge4
0/+	+	0	0	+	'N', Ss, Fy^{a}/Fy^{b}, Fy6
0/+	+	0	0	0	Yt^{a}
0	+	+	0	+	En^{a}FS
+	0	0	0	+^{w}	Lu, MER2
+	0	0	+	+^{w}	Knops
+	+	+	+	0	Kell
+	+	+	+^{w}/0	0	Sc
+	0	+^{w}	0	0	Do, Ge3
+	+	0	0	0	Cromer,
+	+	+^{w}	0	0	LW
+	+	+	+	+	Jk3, Fy3, Di^{b} ,Co^{a}, Ge3; Ok^{a}, P, LKE, At^{a}, Cs^{a}, Emm, Er^{a}, Jr^{a}, Lan, Vel, PEL
+	+	+	+	++	Kx

Reid, Lomas Francis & Olsson: The blood group antigen factsbook 3rd ed. 2012 Academic Press

The patient was transfused…

- Postop day 1: the patient suffered from heart complications due to her anemia
  - Transfused with one of the compatible units
- After ~50 mL, the patient suffered from chills and vomiting
- Transfusion stopped and samples sent to the lab

➔ The serological picture combined with the clinical reaction made us suspect anti-Vel…

Why anti-Vel…

- The patient's plasma reacted more weakly with DTT-treated RBCs
- Rainer T. et al. The effects of dithiothreitol-treated red blood cells with anti-Vel. Transfusion 2004;44:122A (Suppl.)
  - 4/10 anti-Vel showed weaker reactivity with DTT-treated RBCs

Confirmed in Lund

	Untreated			DTT-treated		
Antibody	Vel+, K-	Vel-, K+	Vel-, K-	Vel+, K-	Vel-, K+	Vel-, K-
1.Vel + K	3+	2+	0	3+	0	0
2.Vel	3+	0	0	2+	0	0
3.Vel + K	1+	3+	0	0	1+	0
4.Vel	2+	0	0	2+	0	0
5.Vel	3+	0	0	1+^{w}	0	0

Antibody confirmation

- Patient's RBCs typed Vel-neg
- Plasma was compatible with 3 examples of 3 Vel– RBCs
- Patient also had HLA antibodies

The patient refused further transfusion

Vel antigen varies considerably on normal RBCs

	D	C	E	c	e	M	N	S	s	P	K 1	k	Fy^a	Fy^b	Jk^a	Jk^b	Pt	Känd anti-Vel
1	+	+	+	+	+	+	0	+	0	0	0	+	0	+	+	+	2+	3+
2	0	0	0	+	+	+	0	0	+	+	+	0	+	+	0	+	2+	3+
3	0	+	0	+	+	+	0	0	+	0	0	+	+	0	+	+	1+	2+
4	0	0	+	+	+	+	0	+	+	+	0	+	0	+	+	0	2+	2+st
5	+	+	0	0	+	+	+	0	+	+	0	+	+	0	+	0	2+	2+
6	+	0	+	+	0	0	+	0	+	+	0	+	+	+	0	+	1+^{sv}	2+
7	0	0	0	+	+	+	0	+	+	0	+	+	0	+	+	+	1+^{sv}	2+st
8	+	0	0	+	+	+	0	+	0	+	+	0	+	+	+	0	+	0	1+
9	0	0	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0	1+^{sv}	2+

Vel update 28 October 2014 Philadelphia

Bakground
Vel: a 60-year-old puzzle

- Anti-Vel described in 1952 by Sussman and Miller
  - Patient with antibodies to an unknown high-prevalence antigen who suffered a severe hemolytic transfusion reaction
- Anti-Vel are often a mixture of IgG and IgM
  - Hemolytic *in vivo* and *in vitro*
  - Evidence that perhaps it was a carbohydrate antigen
- Clinically important in transfusion and in hemolytic disease of the fetus and newborn
- In Sweden, 1 in ~1700 individuals lack the Vel antigen
  - Prevalence in other European populations is 1 in ~5000

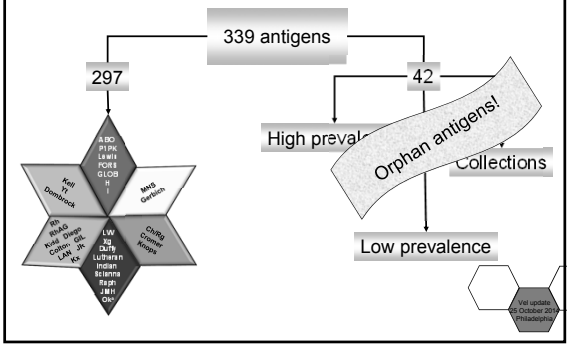
Vel update 28 October 2014 Philadelphia

A clinical problem that has long awaited a solution

- Difficult to perform Vel phenotyping:
  - No commercial reagents
  - No monoclonal antibodies
  - Well-known variability in Vel expression
- Global shortage of Vel-negative blood
  - In southern Sweden, we had only one active Vel-negative donor…
  - Umeå, in Northern Sweden has provided Vel-negative blood world-wide
- We have searched for the Vel molecule and its gene for a long time…

Vel update 28 October 2014 Philadelphia

Classification of blood group antigens - 2012



”Orphan” blood group antigens

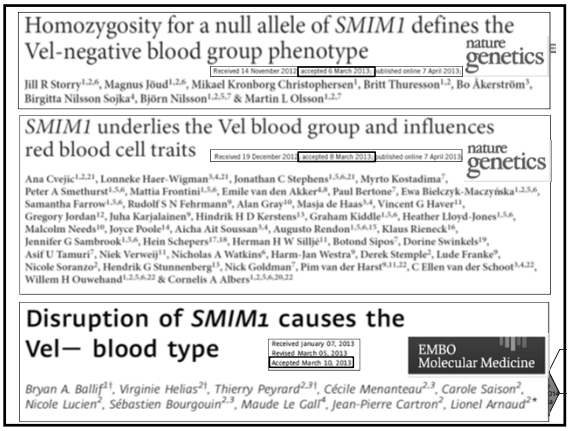
- 42 of 339 known blood group antigens do not belong to a system because the genetic background (locus) is unknown
  - Genotyping is not possible
  - Cannot be recombinantly produced
- These antigens are divided between:
  - Collections (200-series) n=18
  - LFA-series (700-series) n=18
  - HFA-series (901-series) n=6

2004

Vel collection = 200212
Vel
ABTI

- In most cases, the carrier molecule is unknown
- Vel is the most clinically important antigen of these “orphans”

Vel update 28 October 2014 Philadelphia



Homozygosity for a null allele of *SMIM1* defines the Vel-negative blood group phenotype

nature genetics

Received 14 November 2012; accepted 6 March 2013; published online 7 April 2013

Jill R Storry[1,2,6], Magnus Jöud[1,2,6], Mikael Kronborg Christophersen[1], Britt Thuresson[1,2], Bo Åkerström[3], Birgitta Nilsson Sojka[4], Björn Nilsson[1,2,5,7] & Martin L Olsson[1,2,7]

SMIM1 underlies the Vel blood group and influences red blood cell traits

nature genetics

Received 19 December 2012; accepted 8 March 2013; published online 7 April 2013

Ana Cvejic[1,2,21], Lonneke Haer-Wigman[3,4,21], Jonathan C Stephens[1,5,6,21], Myrto Kostadima[7], Peter A Smethurst[1,5,6], Mattia Frontini[1,5,6], Emile van den Akker[4,8], Paul Bertone[7], Ewa Bielczyk-Maczyńska[1,2,5,6], Samantha Farrow[1,5,6], Rudolf S N Fehrmann[9], Alan Gray[10], Masja de Haas[3,4], Vincent G Haver[11], Gregory Jordan[12], Juha Karjalainen[9], Hindrik H D Kerstens[13], Graham Kiddle[1,5,6], Heather Lloyd-Jones[1,5,6], Malcolm Needs[10], Joyce Poole[14], Aicha Ait Soussan[3,4], Augusto Rendon[1,5,6,15], Klaus Rieneck[16], Jennifer G Sambrook[1,5,6], Hein Schepers[17,18], Herman H W Silljé[11], Botond Sipos[7], Dorine Swinkels[19], Asif U Tamuri[7], Niek Verweij[11], Nicholas A Watkins[6], Harm-Jan Westra[9], Derek Stemple[2], Lude Franke[9], Nicole Soranzo[2], Hendrik G Stunnenberg[13], Nick Goldman[7], Pim van der Harst[9,11,22], C Ellen van der Schoot[3,4,22], Willem H Ouwehand[1,2,5,6,22] & Cornelis A Albers[1,2,5,6,20,22]

Disruption of *SMIM1* causes the Vel— blood type

Received January 07, 2013; Revised March 05, 2013; Accepted March 10, 2013

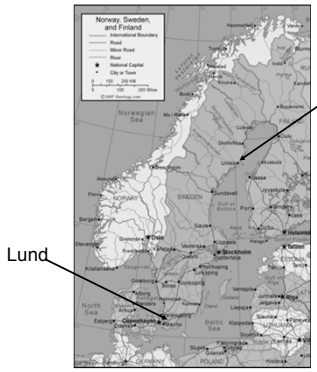
EMBO Molecular Medicine

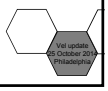
Bryan A. Ballif[21], Virginie Helias[21], Thierry Peyrard[2,31], Cécile Menanteau[2,3], Carole Saison[2], Nicole Lucien[2], Sébastien Bourgouin[2,3], Maude Le Gall[4], Jean-Pierre Cartron[2], Lionel Arnaud[2]*

Turned to SNP arrays….

Hypothesis

The Vel-negative phenotype is caused by a homozygous founder mutation surrounded by an identifiable SNP signature





Sample cohort for SNP analysis with Illumina HumanOmni 2.5M BeadChip

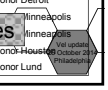
Array 1

Sample nr.	Vel phenotype	Source
1	Vel–	Lund donor
2	Vel–	brother 1
3	Vel+ weak	son
4	Vel+ weak	daughter
5	Vel–	Umeå sibling donor
6	Vel–	Umeå sibling donor
7	Vel–	Umeå sibling donor
8	Vel+ weak	Umeå sibling donor
9	Vel–	patient with anti-Vel
10	Vel–	patient with anti-Vel
11	Vel–	Unrelated donor Lund
12	Vel+	unrelated donor Umeå
13	Vel–	unrelated donor Minneapolis
14	Vel–	unrelated donor Minneapolis
15	Vel–	unrelated donor Umeå
16	Vel–	unrelated donor Umeå

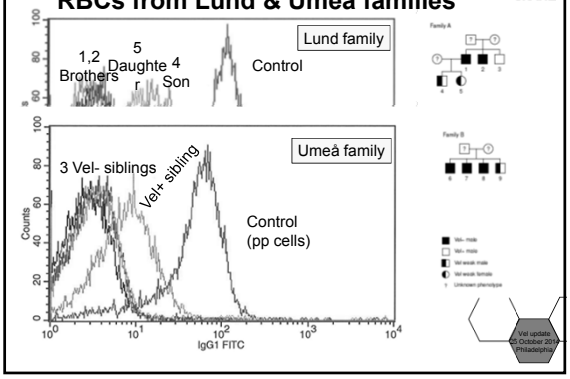
Array 2

Sample nr.	Vel phenotype	Source
17	Vel–	unrelated donor Umeå
18	Vel–	unrelated donor Umeå
19	Vel–	unrelated donor Umeå
20	Vel–	unrelated donor Umeå
21	Vel–	unrelated donor Lund
22	Vel+	unrelated donor Umeå
23	Vel–	Lund donor
24	Vel–	brother 1
25	Vel+	brother 2
26	Vel+ weak	son
27	Vel+ weak	daughter
28	Vel–	unrelated donor Detroit
31	Vel+ weak	unrelated donor Houston
32	Vel+ weak	unrelated donor Lund

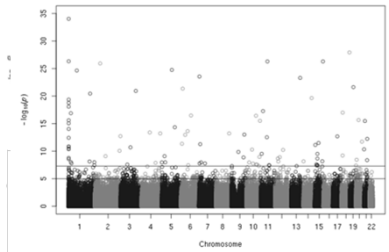
DNA from 20 Vel– individuals; 2 families



Vel expression by flow cytometry on RBCs from Lund & Umeå families

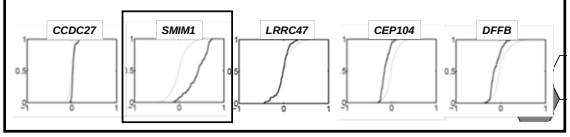


Results of SNP analysis with Illumina HumanOmni 2.5M BeadChip

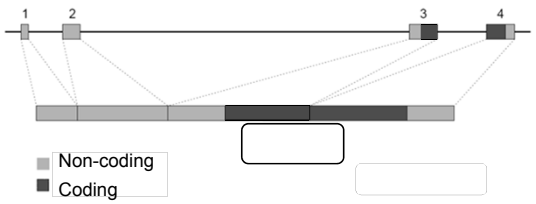


Candidate genes

Candidate	AA (kDa)	Protein product	Function/localisation
CCDC27	656 (75.4)	Coiled-coil domain-containing protein 27	Unknown
SMIM1	78 (8.7)	Uncharacterised protein	Type 1 transmembrane protein
LRRC47	583 (63.4)	Leucine-rich repeat-containing protein 47	RNA-binding, phenylalanine-tRNA ligase
CEP104	925 (104.4)	Centrosomal protein 104kDa	Centriole; cytoskeleton
DFFB	338 (39.1)	DNA fragmentation factor, 40 kDa, beta polypeptide	Nucleus, apoptosis factor



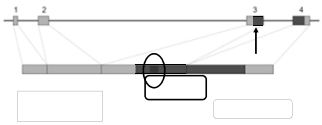
SMIM1 consists of four exons with an open reading frame contained within exons 3 & 4



SMIM1 mRNA found in:

- peripheral blood
- erythroleukemia cell lines of CML origin (e.g. K562, HEL)
- Primary bone marrow from healthy volunteers cultured towards erythroid cells

Sequence analysis of *SMIM1* in Vel-negative individuals



- All Vel– samples have the same 17-bp deletion (c.64-80) in exon 3!
  - Gene sequenced in 43 Vel– individuals
  - Predominantly Swedish but also from USA, UK, Switzerland, Israel
  - The mutation disturbs the open reading frame and causes a frameshift
- No mutation identified in 2 ABTI– samples (from the same family)

What does *SMIM1* encode?

- NCBI = uncharacterised protein
  - Ensembl = No gene product
- No homology with other human proteins!
  - No known functional motifs ⇨ no known function
- However – high homology with all other mammals
  - 56% homology i zebrafish

SMIM1

- 78 amino acids
- Type 1 transmembrane protein

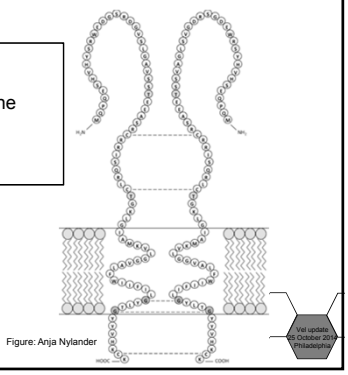
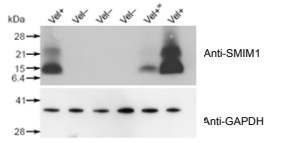
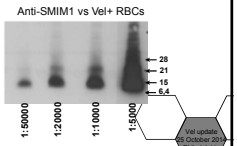


Figure: Anja Nylander

SMIM1 can be detected by med Western blot

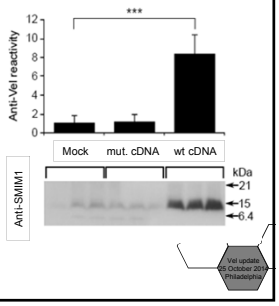
- Four overlapping peptides synthesised to the extracellular domain
  - Inhibition studies showed partial inhibition with some anti-Vel
- Polyclonal antibodies raised in rabbits to peptides 1 & 3
  - High specificity by Western blotting
  - Very high titre, especially antibody to SMIM1 peptide 1





Transfection of K562 cells show Vel expression is dependent on *SMIM1*

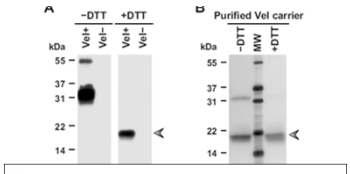
- cDNA from Vel+ or Vel– individuals was cloned into the pEF1α-IRES-ZsGreen1 expression vector
- Transfected into K562 cells
- Analysed after 48 hours with flow cytometry with human anti-Vel
- Analysed by Western blot using anti-SMIM1

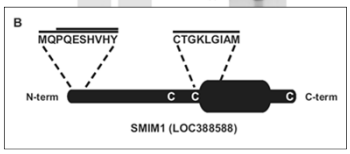


Disruption of *SMIM1* causes the Vel− blood type

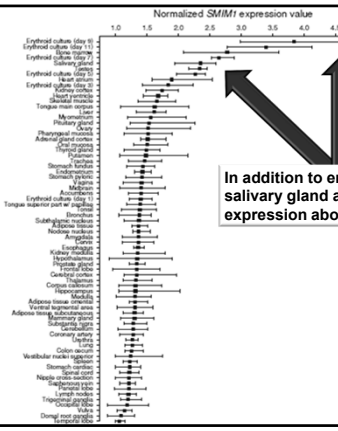
EMBO Molecular Medicine

Bryan A. Ballif[†1], Virginie Helias[†2], Thierry Peyrard[2,3†], Cécile Menanteau[2,3], Carole Saison[2], Nicole Lucien[2], Sébastien Bourgoin[2,3], Maude Le Gall[4], Jean-Pierre Cartron[2], Lionel Arnaud[2*]



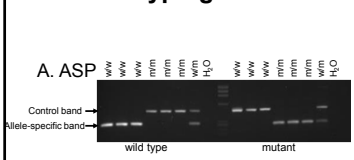


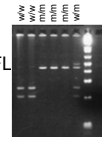
Tiss

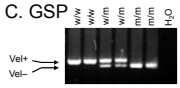


In addition to erythroid cells, salivary gland and testis showe expression above background

Genotyping for the *SMIM1* deletion



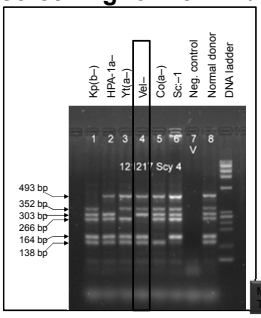




520 southern Swedish donors screened:
30 heterozygotes (1/17)
0 homozygous mutants

Calculated frequency of Vel− ~1/1200

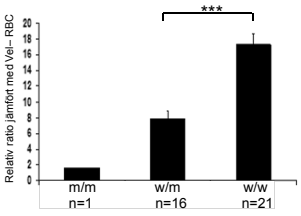
Genetic screening for Vel– individuals



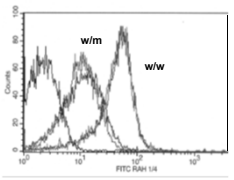
Modified after Wagner et al. Transfusion 2008;48:1169-73

Multiplex PCR for alleles to high prevalence blood group antigens identifies Vel– donors (2/1800)

Zygosity for the 17-bp deletion correlates with Vel antigen expression



Anti-Vel



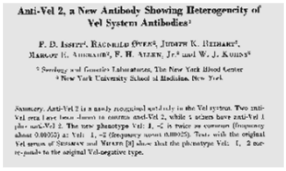
Anti-Vel

Other nucleotide variations that affect Vel antigen expression

- 152 T>A/G Met51Lys/Arg
  Cvejic et al. Nat. Genet 2013;45:542-5
  Storry et al. Transfusion 2013;53:40A
  van der Schoot et al. Vox Sang 2014;107 (Suppl):16
- Weakens Vel antigen expression, confirmed by transfection studies in HEK-293T cells (van der Schoot)
- Rs1175550, located in *SMIM1* intron 2

Testing at the NYBC

- to determine if the same 17-bp deletion is associated with the Vel– phenotype in a multi-ethnic population
  - African-American - Caucasian
  - Hispanics
- 40 samples selected from 77 in NYBC archives
  - 11 patients with anti-Vel - 7 SCARF
  - 15 donors (7 Black, 2 Hispanic, 6 Caucasian) - 7 referred from other centers
- 11 originally identified as VEL:–1,–2 or VEL:–1, 2 at NYBC



Anti-Vel 2, a New Antibody Showing Heterogeneity of Vel System Antibodies[1]

F. D. Issitt[2], Rachelle Oyen, Judith K. Hemmer[2], Margot R. Anstee, P. H. Allen, Jr.[3] and W. J. Adams[4]

[1] Serology and Genetics Laboratories, The New York Blood Center [2] New York University School of Medicine, New York

Summary: Anti-Vel 2 is a newly recognized antibody in the Vel system. Two anti-Vel sera have been shown to contain anti-Vel 2, while 5 others have anti-Vel 1 plus anti-Vel 2. The new phenotype Vel: 1, -2 is twice as common (frequency about 0.00202) as Vel: -1, -2 (frequency about 0.00102). Tests with the original Vel sera of Stratton and Vernon [6] show that the phenotype Vel: 1, 2 most resembles the original Vel-negative type.

1968, Issitt, Oyen, et al.
***Vox Sang* 15:125**

Results

number	phenotype	Genotype	AS-PCR
22	Vel -1 -2	homozygous 17 bp deletion	del/del
14	Vel -1 -2 ? variant?	heterozygous 17 bp deletion	WT/ del
4	Vel -1 -2 ? variant?	no deletion	WT/WT

- The so-called VEL:–1,–2 samples associated with homozygous (del/del)
- The VEL:–1, variant ? samples were heterozygous carriers (WT/del)
  - have weak expression of Vel antigen
- 4 samples without deletion WT/WT
  - 1- heterozygous for Met51Arg change
  - 3 - no changes by sequencing exon 3, 4. Under investigation
- 17-bp deletion present in samples from African-American and Hispanics
- Molecular background is the same as that reported in Europeans
- 10/11 patients with anti-Vel were homozygous for the deletion (del/del)
  - One with no changes in exon 3, 4 is under investigation

Results

Serology	Molecular Result	predicted phenotype	Anti-Vel samples			
			V.S. 2006	Paul. 1970	N. Q. 1982	D.W. 1996
Vel+	**wt/wt +control**	**Vel+**	**4+**	**2+**	**2+**	**2+**
VEL:–1,–2	**del/del (RE)**	**Vel–**	**0**	**0**	**0**	**0**
VEL:–1,–2	**del/del (CW)**	**Vel–**	**0**			
VEL:–1,2	**del/wt (RY)**	Vel+weak	3+	2+	1+	0
VEL:–1,2	**del/wt (TK)**	Vel+weak	3+	2+	1+	0
VEL:–1,2	**del/wt (JM)**	Vel+weak	3+	2+	1+	0

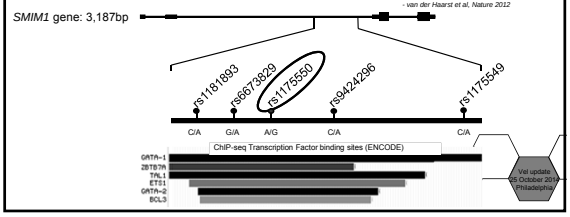
- Performed cross-testing of 4 anti-Vel with genotyped samples
- RBCs from samples with two copies of the deletion (del/del) were non-reactive
- RBCs from samples with one copy (del/wt) were positive

The *SMIM1* gene and SNPs in intron 2

Table 1 | Genomic loci associated with red blood cell phenotypes

Region	Sentinel SNP	Position (B36)	Alleles (EA/OA)	EAF	Phenotype	Effect (SE)	P	Candidate genes
1p36	rs1175550	8,681,388	G/A	0.22	MCHC	0.008 (0.013)	8.6×10^{-15}	*CCDC27*[n], *LRRC48*[n]
1p34	rs3916764	39,842,526	G/A	0.71	MCH	0.008 (0.004)	3.1×10^{-10}	*HEYL*[n]
1p32	rs741959	47,448,820	G/A	0.57	MCV	0.157 (0.025)	6.0×10^{-10}	*TAL1*[n]
1q23	rs857684	156,842,353	C/T	0.74	MCHC	−0.006 (0.011)	3.5×10^{-16}	*OR6Y1*[n], *OR10Z1*[ns], *SPTA1*[ns]

- van der Harst et al, Nature 2012



SNP rs1175550

- Major rs1175550A and minor rs1175550G alleles
- Situated within a non-canonical binding motif of GATA-1:

  -TAG**A**TTGG-
  -TAG**G**TTGG-

- GATA-1 binding motif from JASPAR database

- Presumably, the G allele disrupts this GATA-1 site
  ⇒ less *SMIM1* expression?

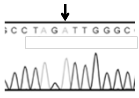
Material & Methods

- 150 samples from Swedish blood donors
  - Screened for the 17 bp deletion to include only samples that were homozyous wild type
- Prepared nuclear extracts from the erythroid HEL cell line for EMSA

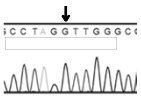
Tissue/material	Application
Genomic DNA	Sequencing
Extracted mRNA	RT-qPCR
Peripheral blood	Flow cytometry
RBC membranes	Western blot
Nuclear extract	Electrophoretic Mobility Shift Assay (EMSA)

Sequencing

- rs1175550 allelic distribution, n=150:

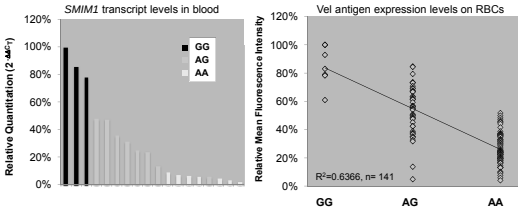


rs1175550	
AA	60,7%
AG	33,6%
GG	5,7%



- Comparable to the frequencies reported by 1000Genomes
  - 76% A and 24% G

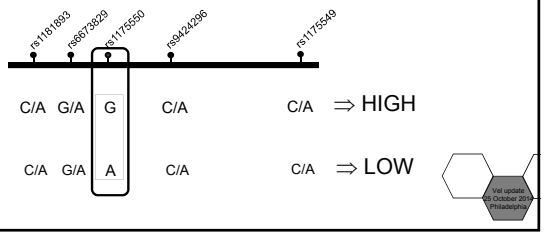
qPCR and flow cytometry show high expression linked to rs1175550G



- rs1175550G => highest transcript level and anti-Vel reactivity
- rs1175550A => lowest transcript level and anti-Vel reactivity
- 63% of variation in anti-Vel reactivity can be contributed to rs1175550G (in the absence of the 17-bp deletion)

rs1175550 as a candidate regulator of *SMIM1* expression

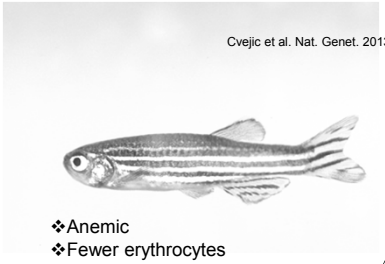
- None of the other SNPs showed any correlation between *SMIM1* expression and genotype



Function?

SMIM1 knockout zebrafish

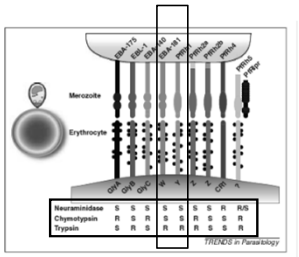
Cvejic et al. Nat. Genet. 2013;45:542-5



- Anemic
- Fewer erythrocytes

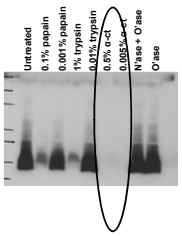
Role in Hb/heme/Fe metabolism ?

Malaria ligands and RBC receptors



Tham WH, Healer J, Cowman AF. *Trends in Parasitology*, 2012;*28, 23-30*

Is SMIM1 the chymotrypsin-sensitive *Plasmodium falciparum* receptor?

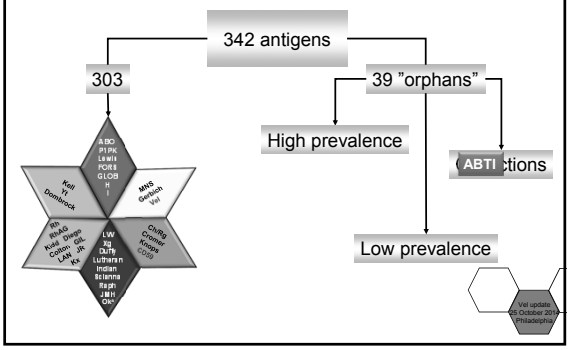


Anti-SMIM1 vs. protease-treated erythrocytes

Conclusions

- Expression of the Vel blood group antigen is controlled by the erythroid gene, *SMIM1*
- The majority of Vel– individuals are homozygous for a 17-bp deletion in exon 3
  - Vel– individuals are human "knock-outs" and lack SMIM1
- SMIM1 is expressed at the RBC surface although the function is not yet known
- The sequence is conserved throughout evolution, which implies that it is a functionally relevant protein
- We can screen for Vel-negative donors using a simple DNA-based method
- These data form the basis of evidence that elevates Vel to a new blood group system, number 34

Classification of blood group antigens 2014



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