

ANEMIE EMOLITICHE RARE E DI DIFFICILE DIAGNOSI

DIAGNOSTICA DI III LIVELLO

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Milano.**



1° PATIENT'S DAY 13 NOVEMBRE 2010

CARATTERIZZAZIONE MOLECOLARE

BASI MOLECOLARI DELLA PATOLOGIA

PROGNOSI



COUNSELING GENETICO



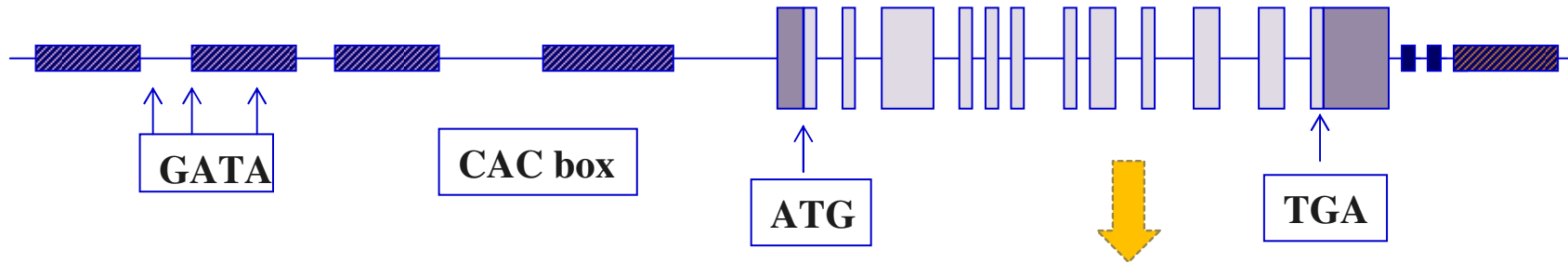
CARATTERIZZAZIONE MOLECOLARE

- **Impossibilita' di ricevere sangue fresco**
- **Scarsita' del campione**
- **Recenti trasfusioni**
- **Cause di falsa negativita' dei test enzimatici**

ACGTGTGCGTCGACTGCTAAAACTGACTGATTCGAGTACTGTGTCATCGTATATAGGGGGAGA

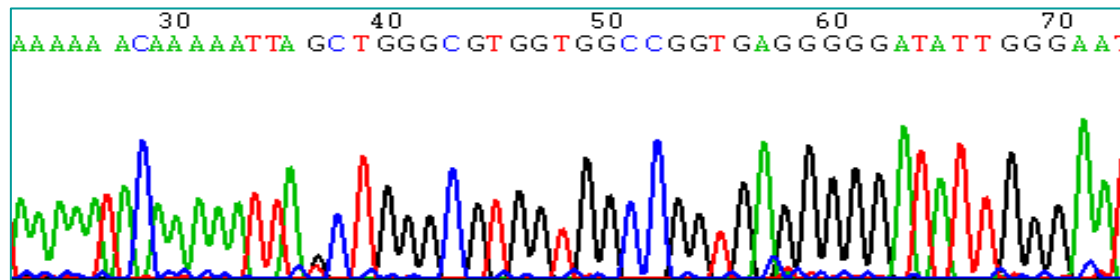
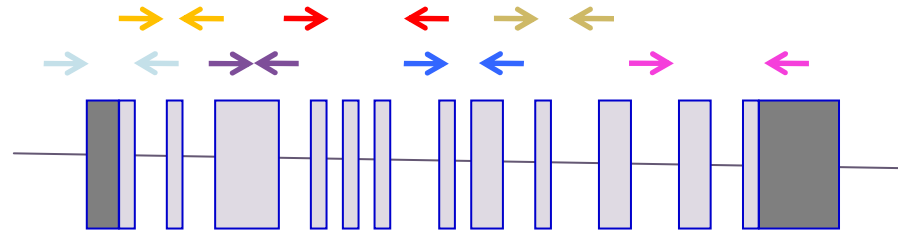
Regione regolatrice PROMOTORE

Regione CODIFICANTE



Met Val Trp Ala Phe Lue Phe Cys Gly Gln Glu Trp

SEQUENZIAMENTO DIRETTO DNA



MUTAZIONI MISSENSE

Puntiformi: c.48T Arg19Trp

MUTAZIONI NONSENSE

Stop codon (c.2152 C-T Arg718X)

Mutazioni di splicing (Ivs6-1g)

Inserzioni

Delezioni

MUTAZIONI OMOZIGOTI

Arg19Trp/Arg19Trp

DOPPI ETEROZIGOTI

Arg19Trp/DelAla21

MUTAZIONI ETEROZIGOTI

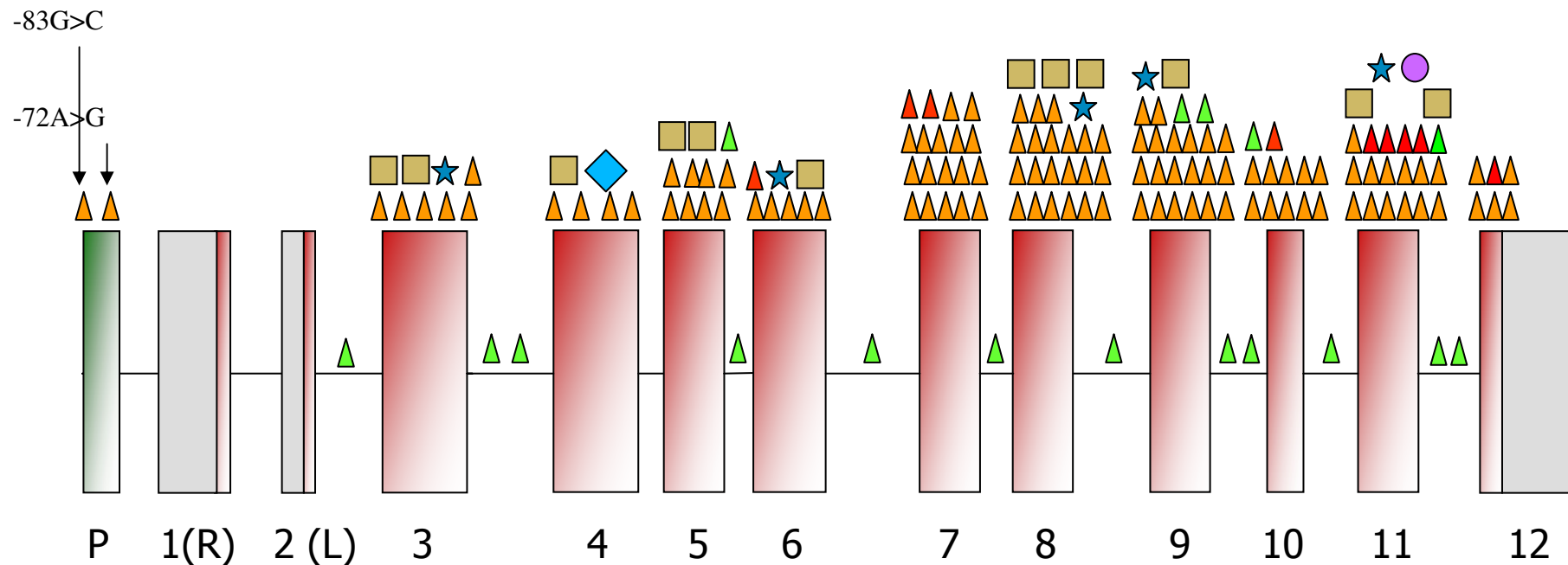
Arg19Trp/WT

- **Eritroenzimopatie**
 - Deficit di piruvato chinasi 125
 - Glucosio fosfato isomerasi 6
 - Trioso fosfato isomerasi 6
 - Pirimidin 5' nucleotidasi 8
 - Adenilato chinasi 3
 - Metaemoglobinemia 8
 - Fosfofrutto chinasi 2

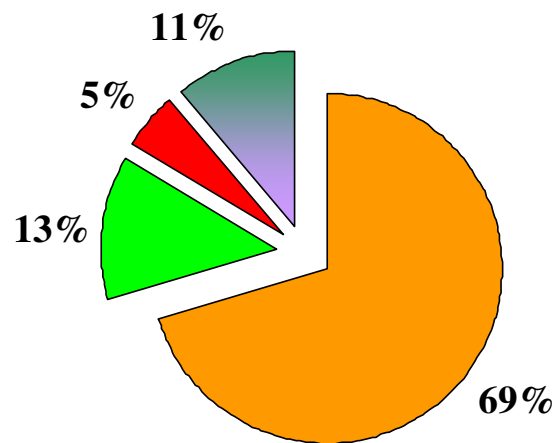
- **Difetti di membrana**
 - Sferocitosi Ereditaria
 - Ellissocitosi ereditaria

- **Difetti di glicosilazione**
 - Anemia diseritropoietica congenita di tipo II (CDAII) 40

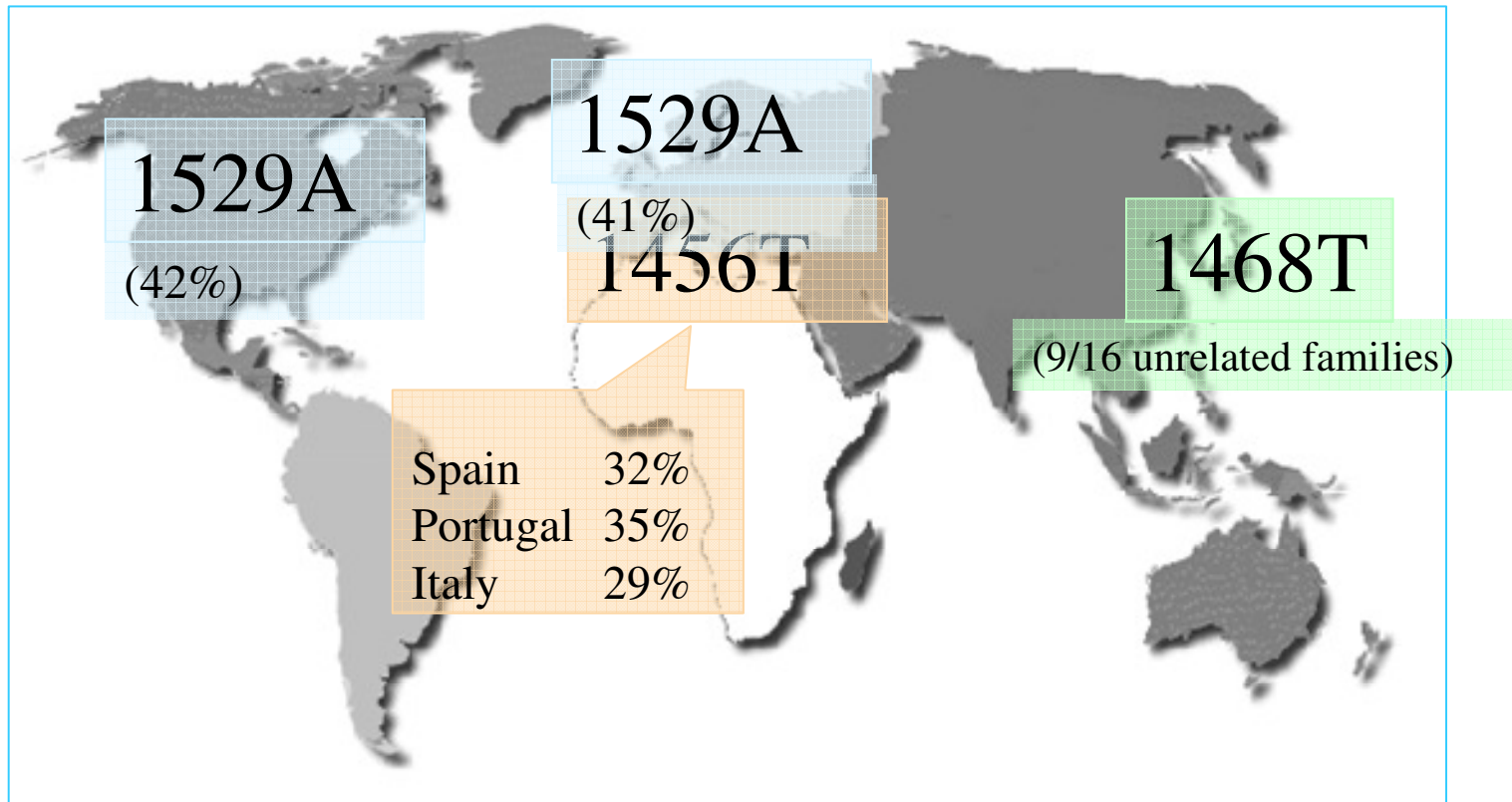
Deficit di piruvato chinasi



- MISSENSE
- SPLICE SITE
- NON SENSE
- INSERTION
- DELETION
- INS/DEL
- DUPLICATION



Distribuzione geografica delle mutazioni nel deficit di piruvato chinasi eritrocitaria(1529A, 1456T, 1468T)



DATI CLINICI

NUMEROSITA'

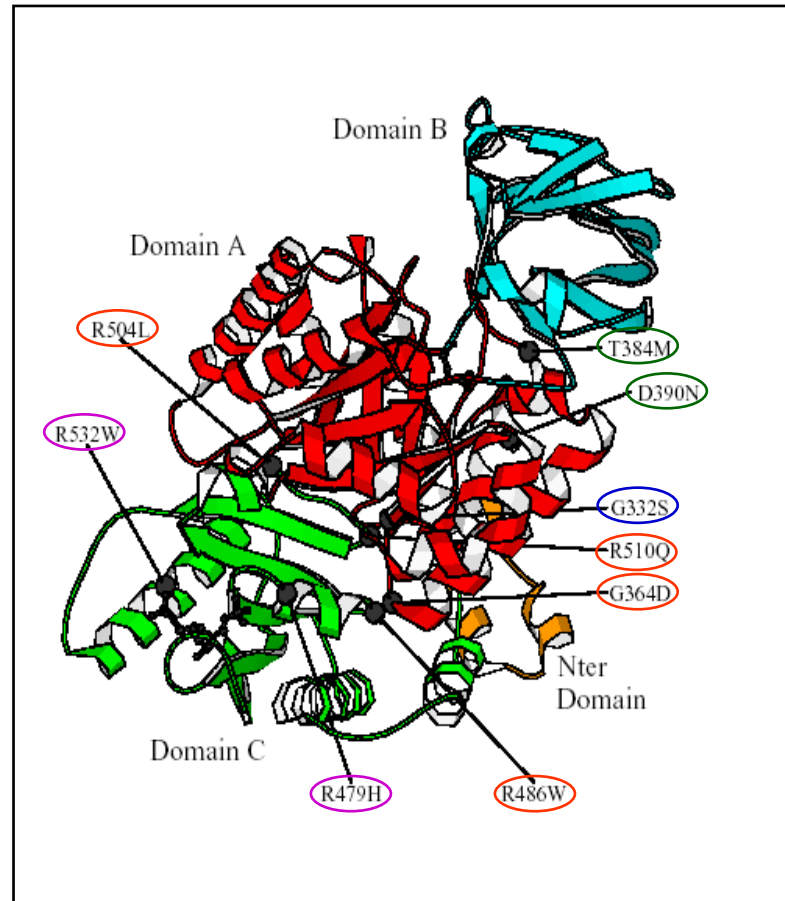
DATI MOLECOLARI



CORRELAZIONE GENOTIPO- FENOTIPO

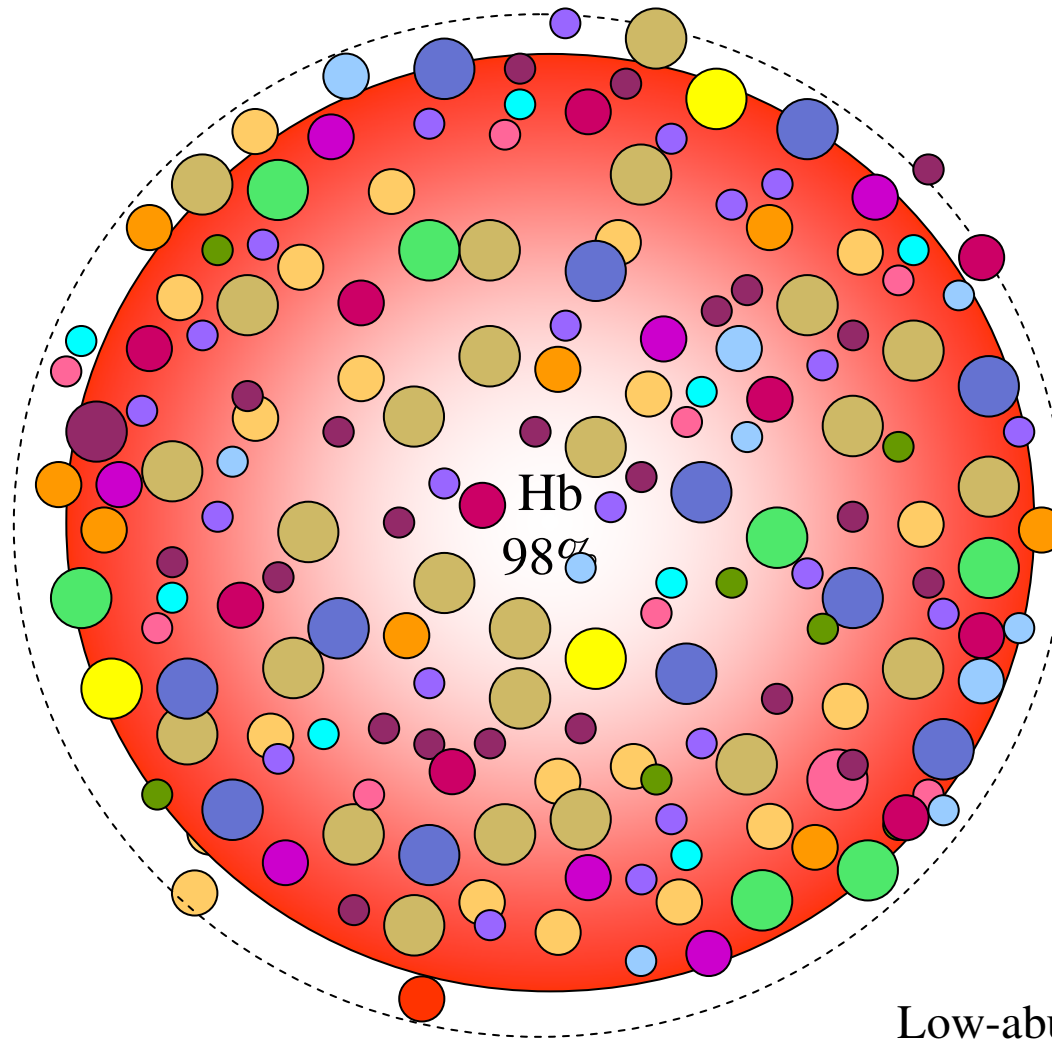
- **1456T/ 1456T Arg486-Trp/Arg486-Trp** **Anemia lieve**
- **1529A/1529A Arg510-Gln/Arg510-Gln** **Anemia piu' severa**
- **994A/994A Gly332-Ser/Gly332-Ser**
- **Mutazioni drastiche non sense**

Espressione di mutanti in vitro e caratterizzazione biochimica delle varianti

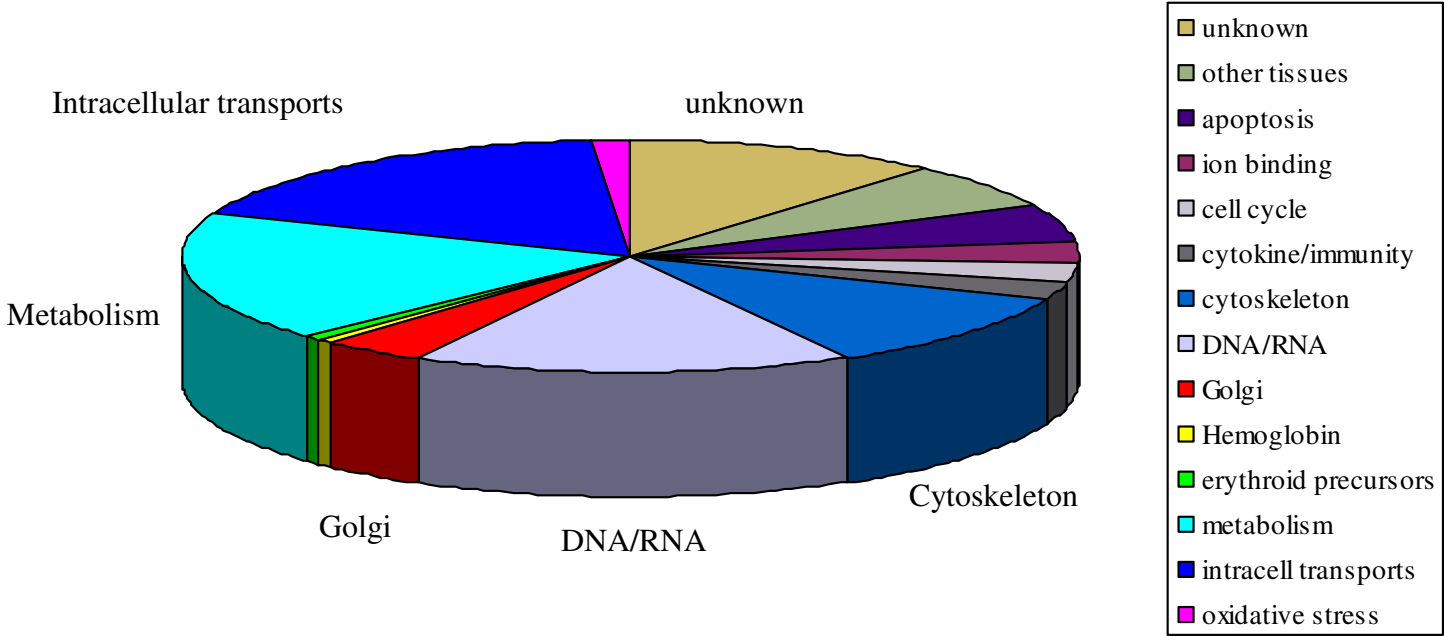


Aiuta a definire gli effetti di una sostituzione aminoacidica sulle proprietà funzionali dell'enzima e a correlare il genotipo al fenotipo clinico

Anemia diseritropoietica congenita (CDAII): dalla ricerca alla diagnosi



Protein Equalizer Technology



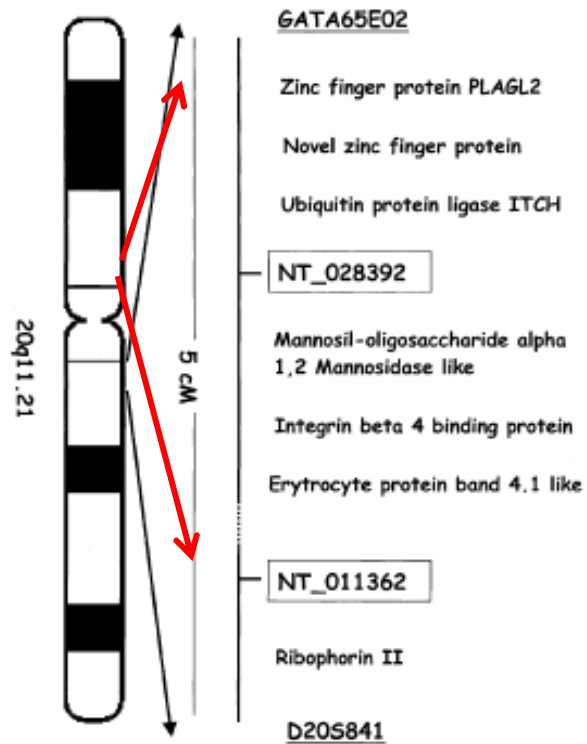
1578 proteine

Localization of the Congenital Dyserythropoietic Anemia II Locus to Chromosome 20q11.2 by Genomewide Search

P. Gasparini,¹ E. Miraglia del Giudice,² J. Delaunay,³ A. Totaro,¹ M. Granatiero,¹ S. Melchionda,¹ L. Zelante,¹ and A. Iolascon⁴

¹Servizio di Genetica Medica, IRCCS-CSS, San Giovanni Rotondo (Fg), Italy; ²Dipartimento di Pediatria, II Università Napoli, Naples; ³Service d'Hématologie/U299, Le Kremlin-Bicêtre, France; and ⁴Dipartimento di Biomedicina dell'Età Evolutiva, Università di Bari, Bari, Italy

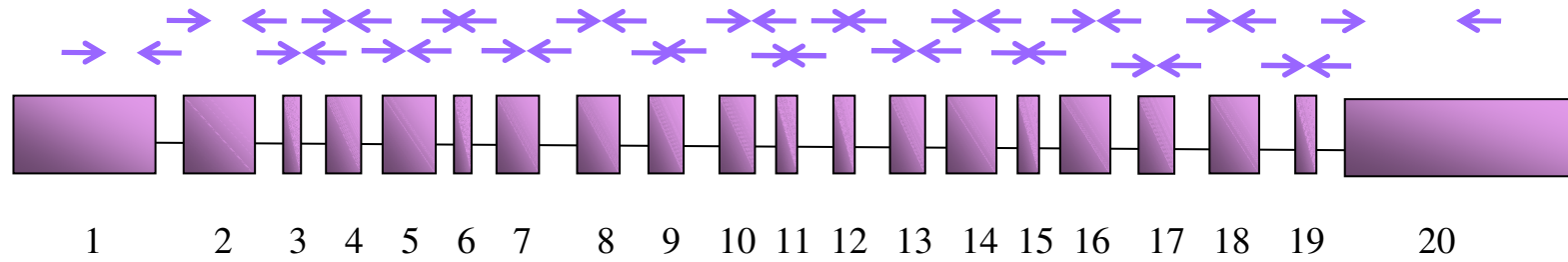
CDAN2 locus 5 cM, 20 q11.2



Lanzara et al, BCMD 2003

	SNX5	20p11	Sorting nexin 5
	SEC23B	20p11.23	Sec23 homolog B (<i>S. cerevisiae</i>)
	DTD1	20p11.23	D-tyrosyl-tRNA deacylase 1 homolog (<i>S. cerevisiae</i>)
	NAT5	20p11.23	N-acetyltransferase 5
	GINS1	20p11.21	GINS complex subunit 1 (Psf1 homolog)
20p13			
20p12	BCL2L1	20q11.21	BCL2-like 1
	MAPRE1	20q11.1-q11.23	Microtubule-associated protein, RP/EB family,
20p11.2	CHMP4B	20q11.22	Chromatin modifying protein 4B
20p11.1	EIF2S2	20pter-q12	eukaryotic translation initiation factor 2, subunit 2 β
20q11.1			
20q11.2	AHCY	20cen-q13.1	S-adenosylhomocysteine hydrolase
20q12	ACSS2	20q11.22	acyl-CoA synthetase short-chain family member 2
20q13.1	GSS	20q11.2	glutathione synthetase
20q13.2	EIF6	20q12	eukaryotic translation initiation factor 6
20q13.3	CPNE1	20q11.22	copine I
	EPB41L1	20q11.2-q12	erythrocyte membrane protein band 4.1-like 1
	C20orf77	20q11.21-q12	chromosome 20 open reading frame 77
	TGM2	20q12	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)

c.40 C-T Arg14Trp



c. 325 G-A Glu109Lys

CORRELAZIONE GENOTIPO- FENOTIPO



86 casi appartenenti a 77 famiglie

FINE

DATI CLINICI

NUMEROSITA'

DATI MOLECOLARI



CORRELAZIONE GENOTIPO- FENOTIPO

Case	Sex	Origin	Age at diagnosis (yr)	Neonatal jaundice	ExTx	Tx (units)	Spleen	Hb (g/dL)	Retics (10 ⁹ /L)	Unconj bil (U/L)	SF (µg/L)	PK Activity (IU/gHb)	Mutation	Effect
Severe														
1	M	Northern Italy	3mo	Yes	No	>100	Out	6.1	249	1.9	828	9.5	721T/507A	Glu241End/splicing
2	F	Central Italy	10mo	No	No	10	In	5.3	116	42.7	nd	8.2	1456T/278T	Arg486Trp/Thr93Ile
3	F	Gypsy	7	Yes	Yes	>50	Out	8.4	1347	nd	nd	5.4	Del 1149bp "Gypsy"/ Del 1149bp "Gypsy"	Del ex11/ Del ex11
4	M	Belgium	11	nd	nd	>50	In	8.2	305	128.2	25	2.0	1484T/TVS9-1c	Ala495Val/splicing
5	F	Northern Italy	2	Yes	Yes	5	In	4.3	138	37.6	nd	12.1	994A/994A	Gly332Ser/Gly332Ser
6	M	Australia	Birth	Yes	Yes	0	In	8.9	nd	46.2	>4000	nd	409A/ del 5006bp (IVS3 → nt 1431)	Ala137Thr/ del ex 4-11
7	F	Central Italy	40	No	No	0	Out	10.5	87	22.2	3530	10.7	1706A/?	Arg569Gln/?
8	M	Central Italy	33	Yes	Yes	>50	Out	9	1166	130	2290	5.3	661A/1209A	Asp221Asn/Met403Ile
9	M	Central Italy	35	Yes	No	>50	Out	9	990	71.8	3210	5.5	661A/1209A	Asp221Asn/Met403Ile
10	F	Central Italy	65	nd	nd	nd	Out	12	139	12.5	540	5.5	1456T/1209A	Arg486Trp /Met403Ile
11	F	England	2 w	Yes	No	8	In	2.2	101	39	nd	6.4	1369 G/?	Ile457Val/?
12	M	Oman	Birth	No	No	>30	In	6.7	36	35	2310	5.3	DelC244 /DelC244	Frameshift/frameshift
13	F	Pakistan	Birth	No	No	15	In	8	112	28	nd	3.4	403T/1190T	Arg135Trp/Asp397Val
Mild-moderate														
14	F	Southern Italy	8	Yes	Yes	2	In	10.3	146	39.3	nd	5.9	1465T/1232C	Arg486Trp/Gly411Ala
15	M	Northern Italy	16	Yes	Yes	0	In	12.4	54	27.4	109	3.2	1456T/721T	Arg486Trp/Glu241End
16	F	Southern Italy	19	No	No	3	In	8.3	203	112.9	212	5.6	1529A/859C	Arg510Gln/Phe287Leu
17	F	Belgium	44	No	No	0	In	8	200	47.9	600	2.4	1456T/1178G	Arg486Trp/ Asn393Ser
18	M	Northern Italy	10mo	No	No	0	In	9.5	122	34.2	107	13.6	1456T/958A	Arg486Trp/Val320Met
19	M	Central Italy	18	No	No	0	In	15.1	139	59.8	143	10.6	107G/?	Ala36Gly/?
20	F	Southern Italy	14	Yes	Yes	2	In	10.1	187	104.3	146	3.9	1465T/994A	Arg486Trp/Gly332Ser
21	F	Southern Italy	9	No	No	3	In	9.5	377	63.3	192	3.4	994A/1492T	Gly332Ser/Arg496Cys
22	F	Northern Italy	9	No	No	0	In	13.2	140	39.3	33	8	A1094T/?	Lys365Met/?
23	F	Guinea	1 mo	Yes	No	0	In	14.4	nd	312.9	nd	4.3	1269A/TVS9+43t	Splicing/splicing
Ref. values								12.2-16.7	24-84	<12	19-238	11.1-15.5		

Studi clinici: pazienti con anemia grave

Mutation

Effect

1529A/1529A

1529A/994A

994A/994A

721T/507A

IVS3(-2)c/721T

1269C/ 787A

del 227-231/?

1483A/ Ivs6 (-2)t

1456T/1003A

1456T/1160G

1456T/1181T

1456T/1181A

Arg510-Gln/Arg510-Gln

Arg510-Gln/Gly332-Ser

Gly332-Ser/Gly332-Ser

Glu241-End/splice site

splice site/Glu241-End

splice site/Gly263-Arg

frameshift/?

Ala 465-Thr/ splice site

Arg486-Trp/Val335-Met

Arg486-Trp/Glu387-Gly

Arg486-Trp/Ala394-Val

Arg486-Trp/Ala394-Asp

Anemie diseritropoietiche congenite (CDAs)

Gruppo eterogeneo di disordini congeniti relativamente raro caratterizzato da :

1. Eritropoiesi inefficace
2. Alterazioni morfologiche degli eritroblasti
3. Emosiderosi secondaria

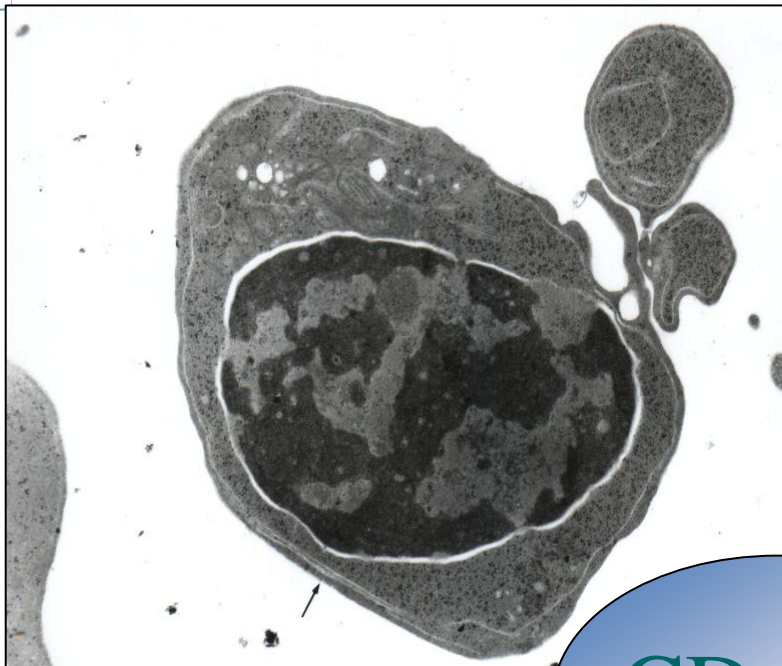
Congenital dyserythropoietic anemia type II (CDA II, HEMPAS)

La CDAII e' la piu' comune CDA (351 casi riportati in Europa)
Ed e' trasmessa come carattere autosomico recessivo

Presentazione clinica

- ✓ Mild to moderate anemia (mean Hb concentration 9.1-9.8 g/dL) with inadequate reticulocytosis
- ✓ Jaundice
- ✓ Variable splenomegaly in 50-60% of cases
- ✓ Iron overload due to both ineffective erythropoiesis and peripheral hemolysis

(Heimpel et al, Blood 2003)



BM electron microscopy:

double membranes close to the outer cell membrane due to residual endoplasmic reticulum as confirmed by detection of reticulum endoplasmic proteins on the cell surface (Alloisio et al., 1996).

CDA II

Band 3 hypoglycosylation:

Relevant hypoglycosylation of erythrocyte membrane proteins band 3 and 4.5 that carry altered N-glycans with truncated poly-lactosamine structures

Fukuda et al. 1984, Anselstetter V, 1977

CDAII CDAII Ctr

