"A NEW GROUP OF AUTOPHAGIC VACUOLAR MYOPHATIES WHICH SHARE CLINICAL, PATHOLOGICAL AND BIOCHEMICAL FEATURES WITH ADULT LATE ONSET POMPE DISEASE (LOPD)"

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Consiglio Nazionale delle Ricerche

Glycogen Storage Disease

EMEA e FDA approvano l'uso nella forma infantile e in quelle tardive

1954 G.T. Cori classifies Pompe disease as glycogen storage disease type II

1961-1970 First reports of a late-onset form

1979 Gene for GAA traced to chromosome 17

2000 rhGAA therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial (n=3)

April 2006 rhGAA gains FDA approval

2009 Chaperone therapy trials

May 2010 LUMIZYME® gains FDA approval

1932 J.C. Pompe describes first case in an infant

1963 H. G. Hers links disease to absence of lysosomal enzyme GAA

1973 First use of enzyme replacement therapy with placental GAA in patients

1996 Isolation and characterization of a recombinant precursor form of lysosomal GAA

2006 rhGAA: major clinical benefits in infantile-onset Pompe disease (n=18)

2007 LOTS results released

2010 AANEM consensuses

2010 EMBASSY trial starts

Abbreviations: GAA, acid alpha-glucosidase; rhGAA, recombinant human acid alpha-glucosidase; FDA, Food and Drug Administration; LOTS, late-onset treatment study; AANEM, American Association of Neuromuscular & Electrodiagnostic Medicine; EMBASSY, Exploratory Muscle Biopsy, Biomarker, and Imaging Assessment Study

Van der Ploeg et al, Lancet 2008; 372: 1342-1353; Dasouki M et al, Neurol Clin 2014; 32: 751-76.

Malattia di Pompe: impellenza di una diagnosi precoce

Indirizzo diagnostico e correlazione Geno-Fenotipo

MUTAZIONI BIALLELICHE DEL GENE GLUCOSIDASI ALFA ACIDA (*GAA*)

Very severe/Very severe

↓

Attivita' GAA < 1%

↓

Forma Classica Infantile
Esordio> 0-1 anno di vita

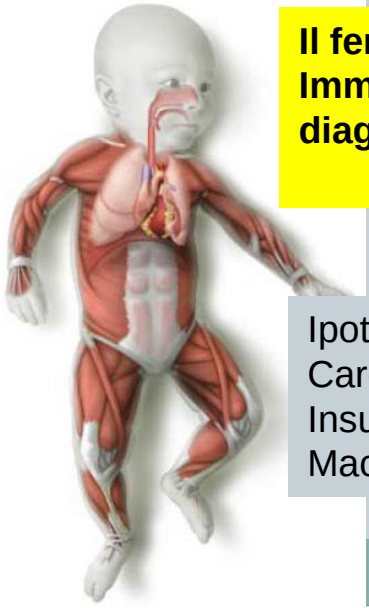
Very severe / Potentially less severe / Less severe / Potentially mild

↓

Attivita' GAA >1 <25%

↓

Forme "Tardive"
Esordio >2 anni



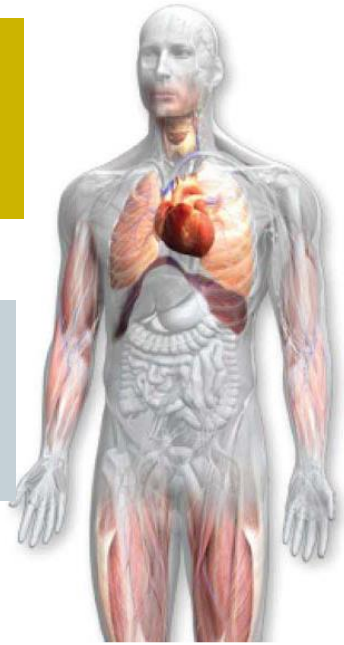
Il fenotipo indirizza Immediatamente la diagnosi

Ipotono
Cardiomegalia ipertrofica
Insuficienza ventilatoria
Macroglossia

Il fenotipo è aspecifico nelle maggioranza dei pazienti

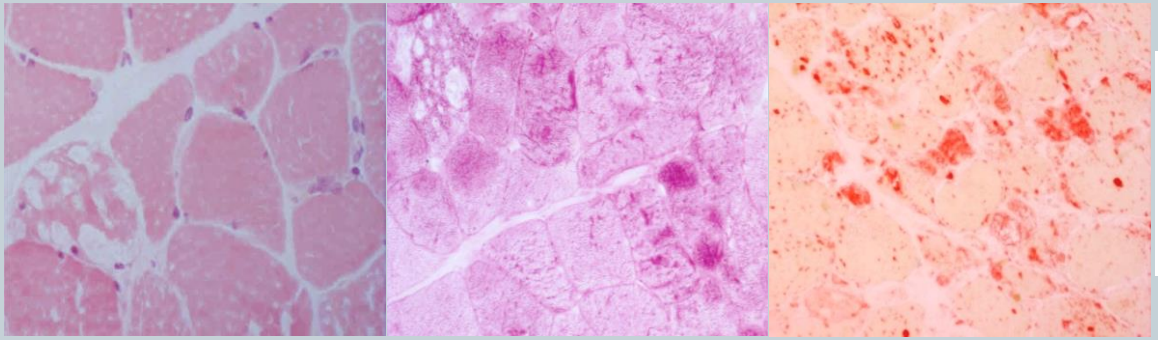
Diagnosi precoce: difficile

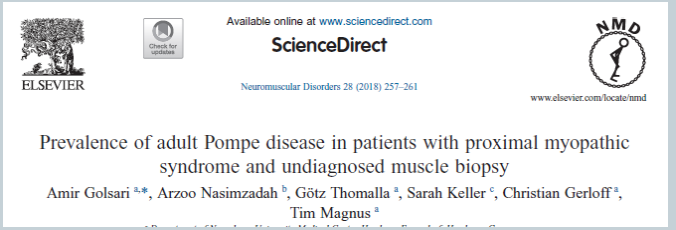
Debolezza a distribuzione cingolare
Insufficienza ventilatoria (30%)
Dolori muscolari e lombari
Scoliosi (20%)



Malattia di Pompe: protocollo classico per le forme tardive

MIOPATIA CON VACUOLI PAS E FOSFATASI ACIDA POSITIVI> ASPECIFICA NEL 9 – 35% DEI PAZIENTI





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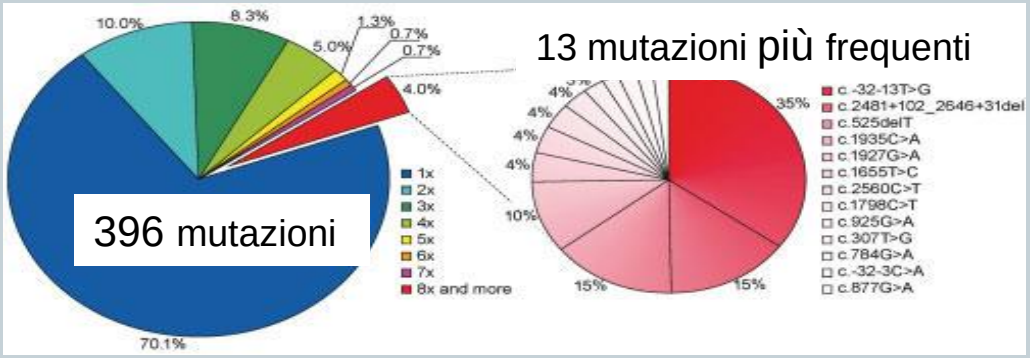
Neuromuscular Disorders 28 (2018) 257–261

www.elsevier.com/locate/nmd

Prevalence of adult Pompe disease in patients with proximal myopathic syndrome and undiagnosed muscle biopsy

Amir Golsari [a,*], Arzoo Nasimzadah [b], Götz Thomalla [a], Sarah Keller [c], Christian Gerloff [a], Tim Magnus [a]

ATTIVITA' DI GAA IN LEUCOCITI, FIBROBLASTI MUSCOLO > 10% DI FALSI NEGATIVI IN UNA POPOLAZIONE DI SOSPETTA MALATTIA DI POMPE TARDIVA



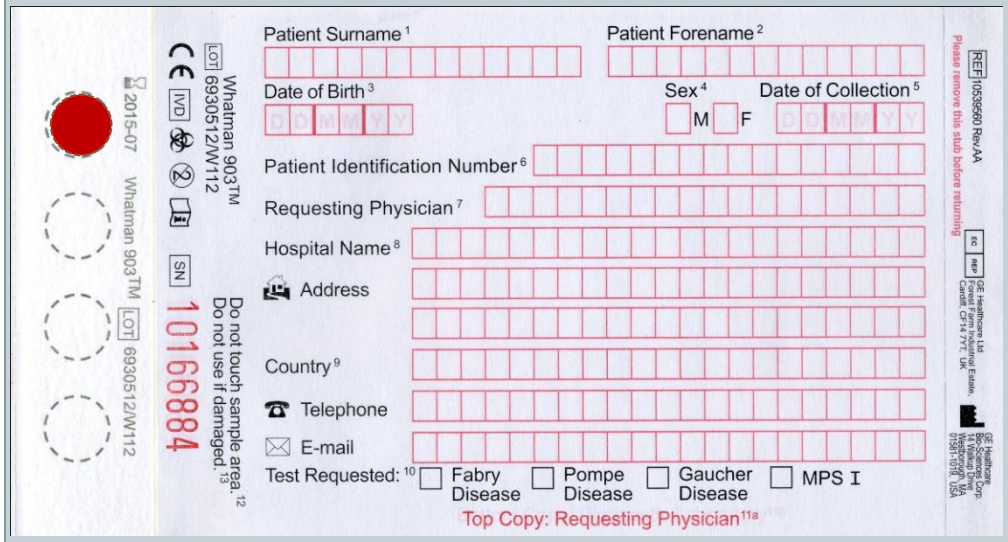
MUTAZIONI BIALLELICHE DI *GAA*> LA MAGGIORANZA SCONOSCIUTE, INTRONICHE, NUMEROSE IN ETEROZIGOSI SEMPLICIE

Malattia di Pompe>Screening di popolazioni a rischio >dosaggio della attività di GAA su goccia di sangue secco (DBS) conferma diagnostica >sequenziamento di *GAA*

SCREENING FOR POMPE DISEASE USING A RAPID DRIED BLOOD SPOT METHOD: EXPERIENCE OF A CLINICAL DIAGNOSTIC LABORATORY

JENNIFER L. GOLDSTEIN, PhD, MS, SARAH P. YOUNG, PhD, MOHITA CHANGELA, BS, GWEN H. DICKERSON, BS, HAOYUE ZHANG, PhD, JIAN DAI, BS, DENISE PETERSON, BS, DAVID S. MILLINGTON, PhD, PRIYA S. KISHNANI, MD, and DEEKSHA S. BALI, PhD

Muscle Nerve **40**: 32–36, 2009



Popolazione sintomatica con sospetta m. di Pompe

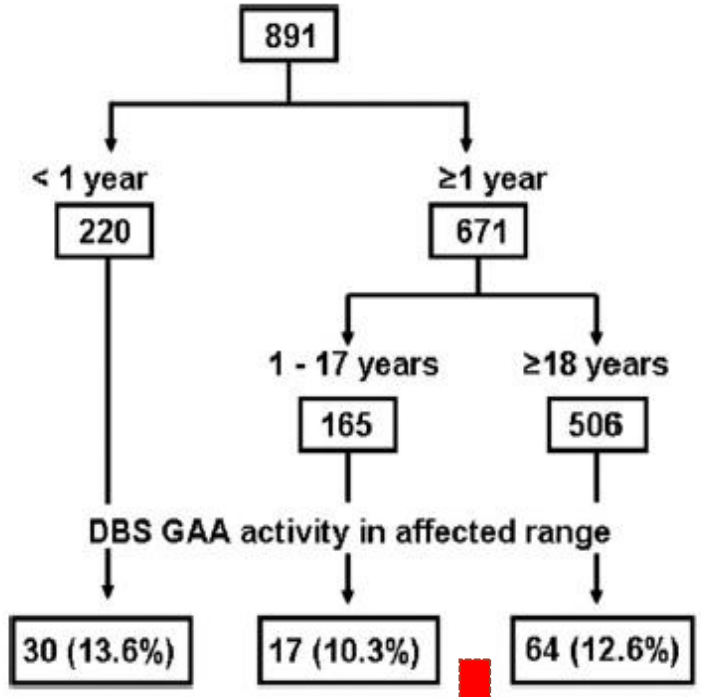


FIGURE 1. Flowchart showing the numb of patients in each age group referr results in the affec

DBS-GAA NELLE LOPD
SPECIFICITA'? (*PRESUNTA* 100%)
SENSIBILITA' ??

10 % FALSI POSITIVI (3%) E FALSI NEGATIVI (7%)

CONTROLLO DELLA DIAGNOSI?

Malattia di Pompe> Controllo della specificità del DBS-GAA

>conta di linfociti con vacuoli PAS positivi su striscio di sangue

Vacuolated PAS-Positive Lymphocytes on Blood Smear: An Easy Screening Tool and a Possible Biomarker for Monitoring Therapeutic Responses in Late Onset Pompe Disease (LOPD)

Daniela Parisi[1†], Olimpia Musumeci[1†], Stefania Mondello[2], Teresa Brizzi[1,3], Rosaria Oteri[1], Alba Migliorato[2], Annamaria Ciranni[1], Tiziana E. Mongini[4], Carmelo Rodolico[1], Giuseppe Vita[1] and Antonio Toscano[1]*

Studio di specificità e sensibilità
contro controlli sani (??)
e altre glicogenosi selezionate (??)

of 1.00 (95%CI 1.00–1.00; $p < 0.0001$). PAS-positive lymphocyte cutoff level of >10 yielded sensitivity of 100% (95%CI 78–100%), specificity of 100% (95%CI 96–100%), and positive predictive value of 100%. Patients studied before and after ERT showed

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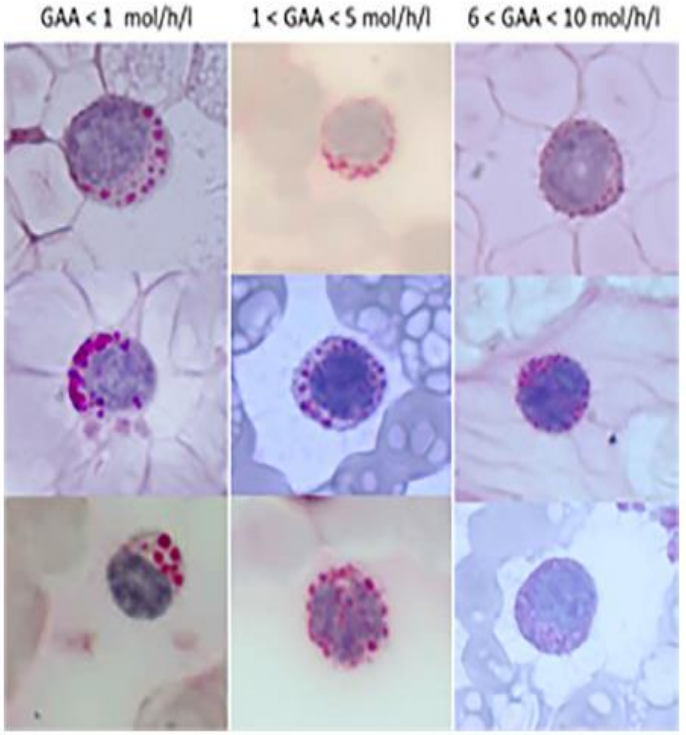
ORIGINAL RESEARCH ARTICLE

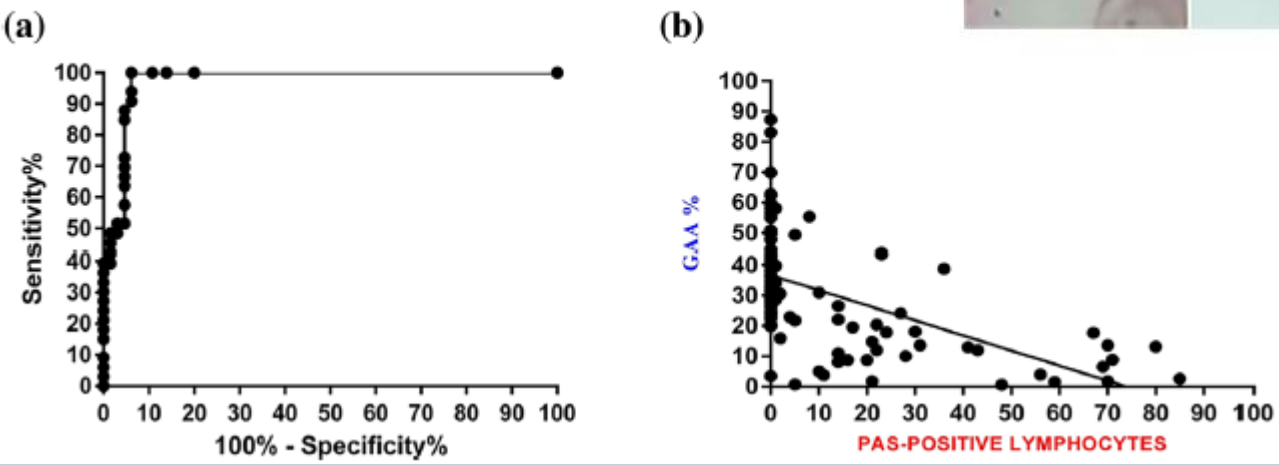
WILEY Journal of Cellular Physiology

Vacuolated PAS-positive lymphocytes as an hallmark of Pompe disease and other myopathies related to impaired autophagy

Angelo Pascarella[1,2] | Chiara Terracciano[3] | Olimpia Farina[1] | Luca Lombardi[1] | Teresa Esposito[4,5] | Filomena Napolitano[1] | Giuseppina Franzese[1] | Giovanni Panella[1] | Francesco Tuccillo[1] | Giancarlo la Marca[6] | Sergio Bernardini[3] | Silvia Boffo[7] | Antonio Giordano[7,8] | Giuseppe Di Iorio[1] | Mariarosa A.B. Melone[1,7] | Simone Sampaolo[1]



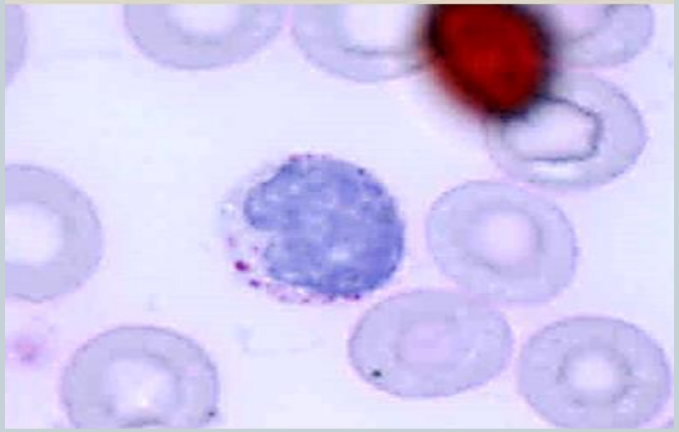
Striscio di sangue
4 o più linfociti PAS+ a x40
100 sensibilità e 94% specificità



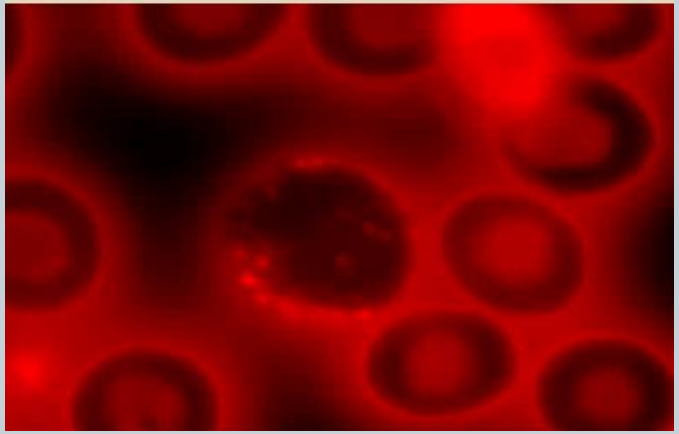
4 o più linfociti PAS positivi sono presenti anche in miopatie diverse dalla Pompe

Malattia di Pompe
vacuoli PAS positivi su striscio di sangue e vacuoli PAS negativi su biopsie di muscolo hanno natura autofagica

PAS EMATOSSILINA



ANTICORPO ANTI-LC3



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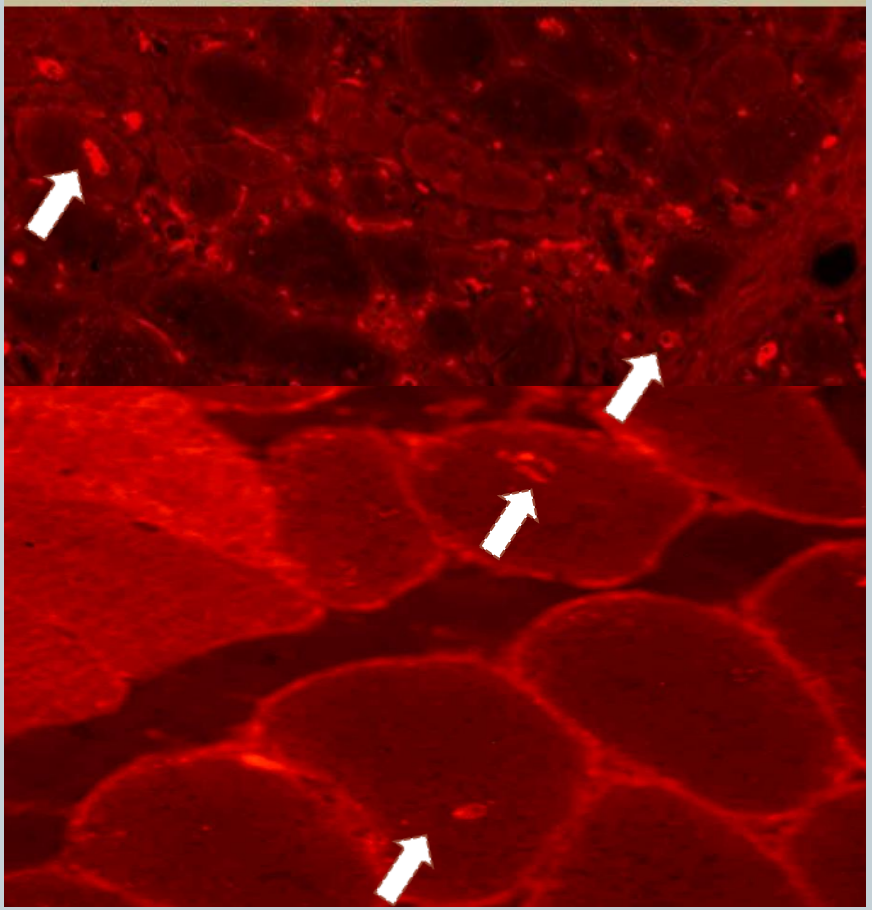


400 PAZIENTI CONSECUTIVI CON CPK ↑ E MIOPATIA PROSSIMALE > <u>Protocollo diagnostico per LOPD</u> > linfociti su striscio+DBS-GAA>biopsia di muscolo>sequenziamento di GAA

60 PRESENTAVANO

Fenotipo clinico compatibile con LOPD

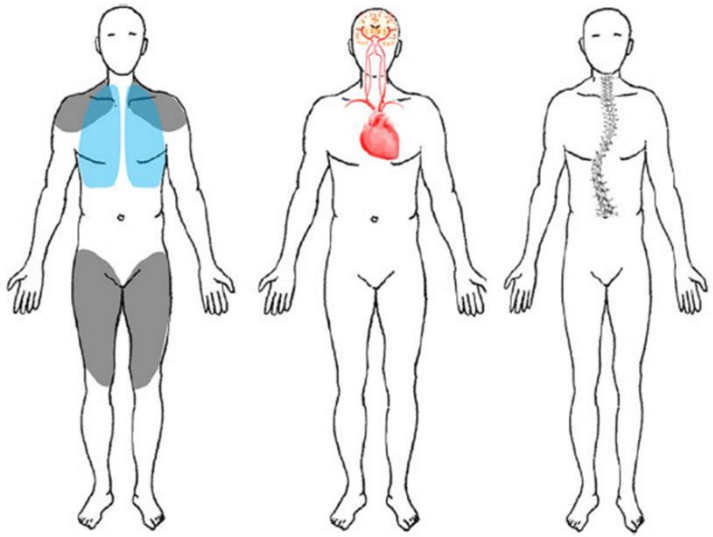
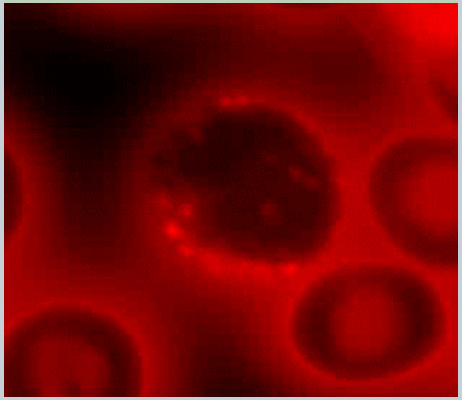
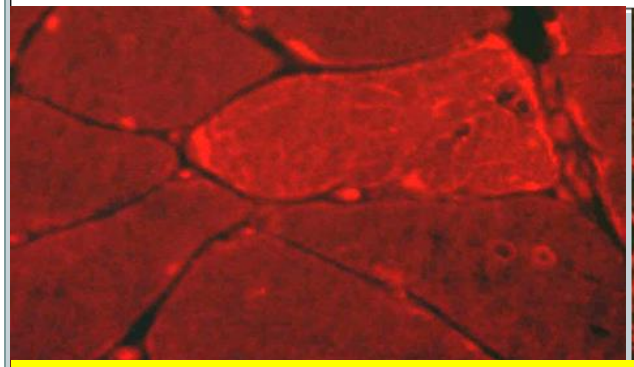
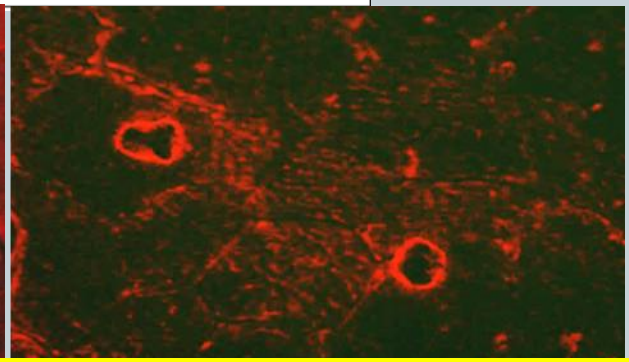


Figure 1. Clinical Manifestation Patterns by organ System Subtypes of Late-Onset-Pompe disease. Left: The Limb girdle and diaphragmatic muscle weakness manifestations. Middle: The Cardio-Cerebrovascular Pattern. Right: The Rigid Spine Syndrome, Scoliosis, and Low Body Weight Pattern. Please note that in all patterns of LOPD a muscular involvement could be found.

Linfociti PAS/LC3 positivi







Miopatia Vacuolare con vacuoli PAS + o PAS - LC3/P62 positivi (autofagici)

RISULTATI DEL DBS-GAA nei 60 pazienti

Parameter	Result	Reference range	
Diagnostics of Pompe Disease from Dried Blood			
alpha-glucosidase at pH 3.8	0.12	– 1,5 - 10	nmol/spot*21h
alpha-glucosidase at pH 7.0	0	– 1,8 - 17,1	nmol/spot*21h
alpha-glucosidase with inhibition	0.14	– 0,9 - 7,2	nmol/spot*21h

Evaluation
Dear colleague,
the enzyme activities above are generally below their respective reference ranges. Especially, the activity at pH 7.0 (reference enzymes) is diminished. This may indicate a pre-analytical problem. We therefore, recommend to measure the activity of alpha-glucosidase in another dried blood specimen.
If you have any questions feel free to contact us anytime.

Possibile errore preanalitico

Parameter	Result	Reference range	
Diagnostics of Pompe Disease from Dried Blood			
alpha-glucosidase at pH 3.8	0.78	– 1,5 - 10	nmol/spot*21h
alpha-glucosidase at pH 7.0	3.81	1,8 - 17,1	nmol/spot*21h
alpha-glucosidase with inhibition	0.18	– 0,9 - 7,2	nmol/spot*21h
Lysosomal Enzymes from Dried Blood			
beta-galactosidase	1.22	0,5 - 3,2	nmol/spot*21h

16 eterozigoti semplici in GAA; **7 altri geni**

Pompe geneticamente confermato!

Evaluation
Dear colleague,
the activities of alpha-glucosidase at pH 3.8, with and without specific inhibition, are below their respective reference ranges. This is in agreement with Pompe disease. We recommend to verify the diagnosis in another dried blood specimen (if possible also in lymphocytes/fibroblasts). In addition, especially if enzyme replacement therapy is under consideration, a molecular genetic work-up should out.

22 mutati in GAA
in eterozigosi composta

Diagnostics of Pompe Disease from Dried Blood			
alpha-glucosidase at pH 3.8	0.36	– 1,5 - 10	nmol/spot*21h
alpha-glucosidase at pH 7.0	4.02	1,8 - 17,1	nmol/spot*21h
alpha-glucosidase with inhibition	0.05	– 0,9 - 7,2	nmol/spot*21h

Nessuna mutazione di GAA ???

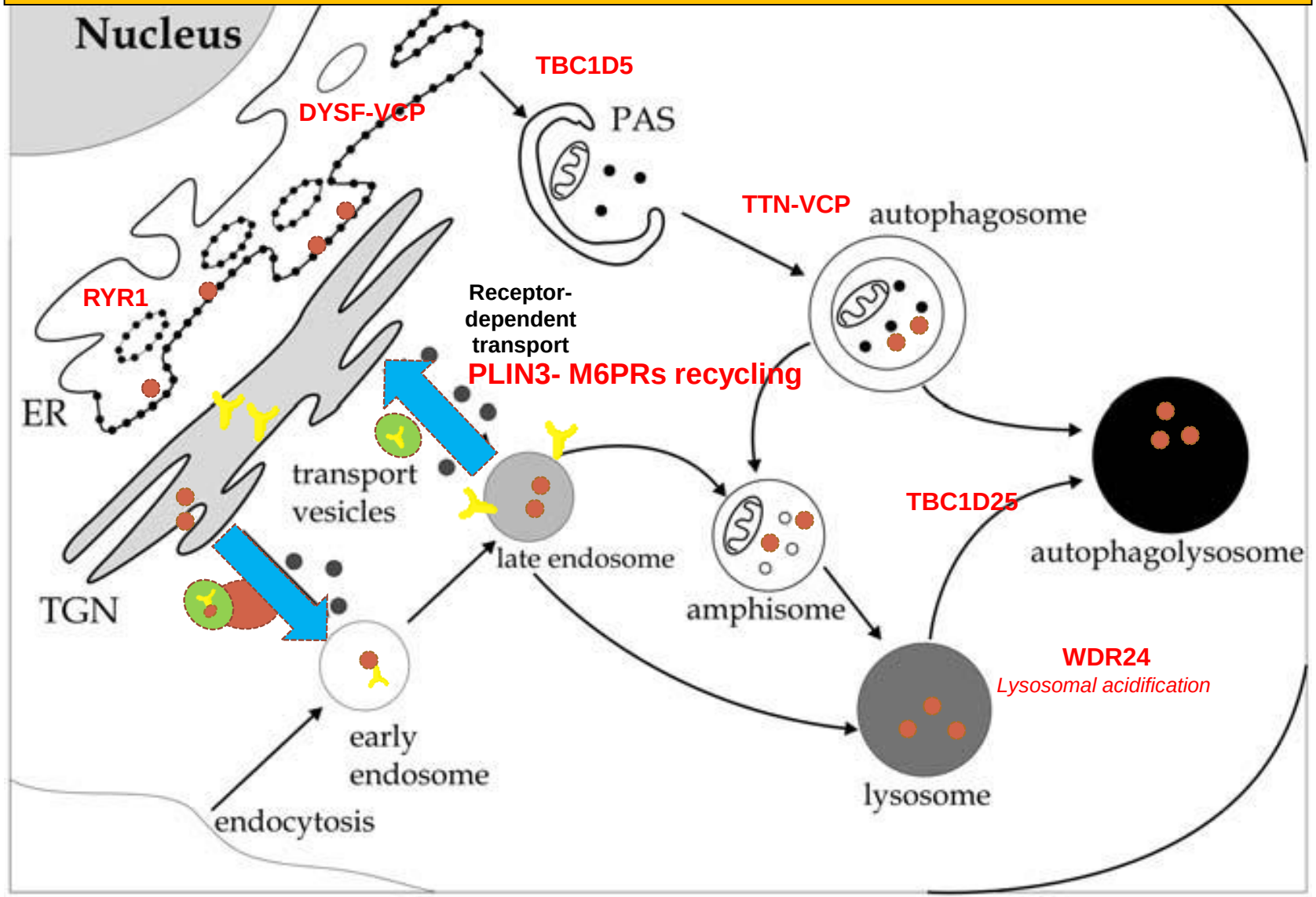
Evaluation
Dear colleague,
The activities of alpha-glucosidase at pH 3.8, with and without specific inhibition, are below their respective reference ranges. This is in agreement with Pompe disease. We recommend to verify the diagnosis in another dried blood specimen (if possible also in lymphocytes/fibroblasts). In addition, especially if enzyme replacement therapy is under consideration, a molecular genetic work-up should be carried out.

7 eterozigoti semplici in GAA; **8 altri geni**

Malattia di Pompe
Risultati della WES in 7 di 15 pazienti LOPD-like

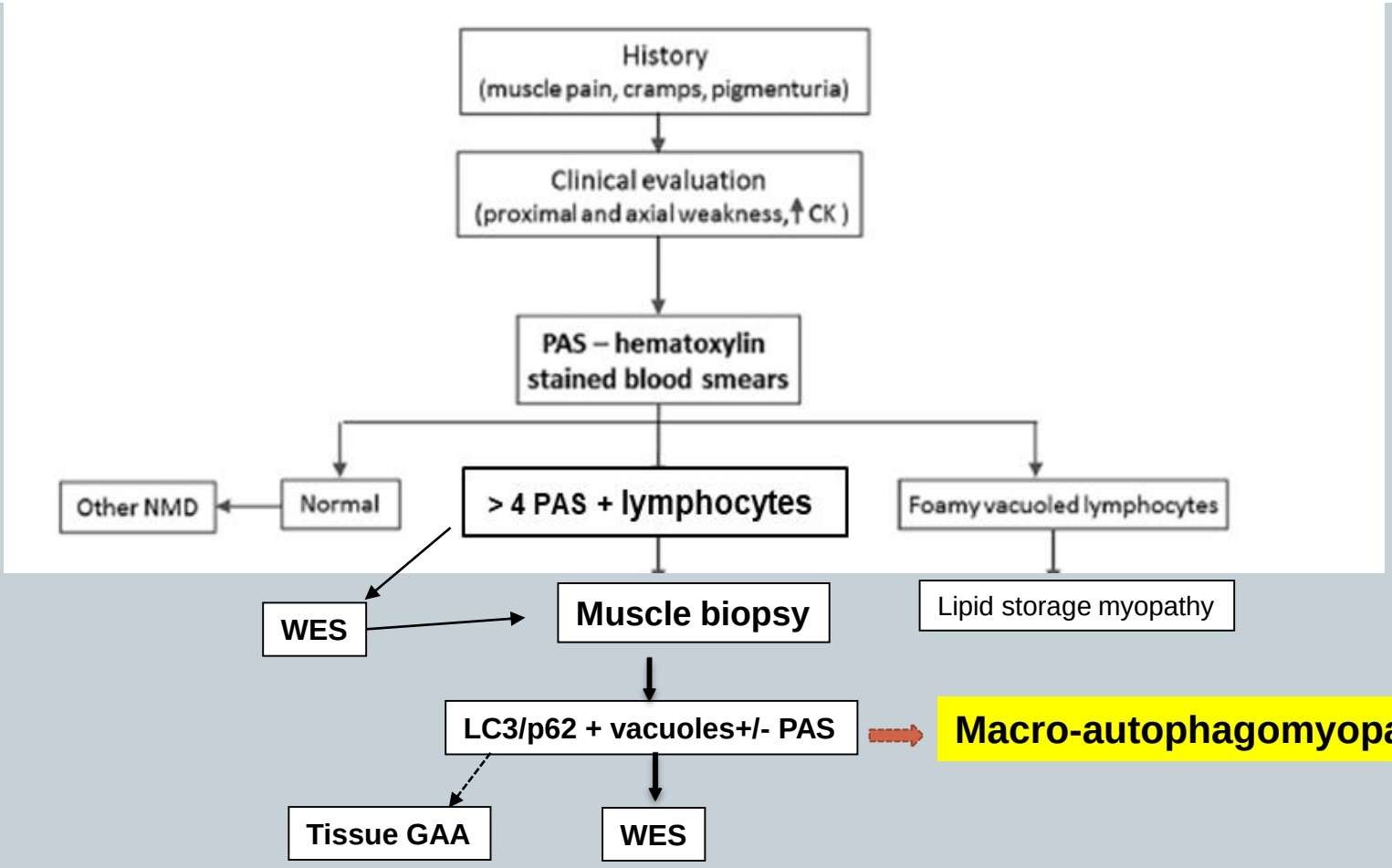
	ID1 gm	ID3 dcmr	ID 4 gl	ID5 on	ID6 vm	ID7 cg	ID8 cp
Age of onset [years]	49	56	13	45	19	50	42
Sex [F/M]	M	F	M	M	M	M	M
Serum CK [U/L]	350	334	1646	482	2946	390	370
PAS positive lymphocytes on PBS [%]	21	22,5	43	32	22,7	38	37
DBS [nmol/spot*21h]	**0,13**	**0,26**	**0,85**	**1,09**	**0,49**	**1,31**	**0,85**
GAA muscle activity (nmol/h/mg of proteins)	**0,237**	**0,63**	**0,79**	**0,47**	**0,37**	**0,47**	**1,18**
WES data	***GAA polym RYR1 DYSF***	***GAA het? MYOT WDR24***	***GAA het TTN TBC1D5***	***TTN ATL3 PLIN3***	***TTN MTMR9 TBC1D5***	***TTN PLEC AGL***	***TTN TRPA1 MAN2B1 TBC1D25***

Geni le cui mutazioni causano un fenotipo LOPD-like



Modified by Oxidative Stress and Autophagy in the Regulation of Lysosome-Dependent Neuron Death. October 2008 Antioxidants & Redox Signaling 11(3):481-96

Malattia di Pompe e altre macro-autofagomiopatie proposta di algoritmo diagnostico



Malattia di Pompe
Dogmi sfatati – Nuove verità

La riduzione della attività enzimatica di GAA nei tessuti tra 1 e 30% della norma

non è specifica di LOPD

trovare 4 o più linfociti PAS positivi a x40 su striscio di sangue

non è specifico di LOPD

la riduzione/blocco della sintesi di GAA

non è biunivocamente legata a mutazioni di GAA

ma è espressione di una down-regulation indotta dal blocco di uno qualsiasi degli stadi della via autofagica- lisosomiale

Cell Death and Disease (2017) 8, e2565; doi:10.1038/cddis.2016.475

Autophagy dysregulation in Danon disease

Anna Chiara Nascimbeni et al. ,

vacuoli autofagici sono dimostrabili con Ab LC3/P62 anche in biopsie apparentemente indenni in istochimica

Malattia di Pompe
Dogmi sfatati – Nuove verità

Una miopatia vacuolare con accumulo di glicogeno di variabile entità e vacuoli fosfatasi acida/LC3/P62 positivi, con attività tissutale di GAA tra 1 e 25% della norma, caratterizza tutte le miopatie con blocco della macroautofagia riconducibile a mutazioni dei geni regolatori della via autofago-lisosomiale.

Esiste verosimilmente una molecola segnale comune a tutte le miopatie autofagiche che reprime la sinstesi di GAA

La ERT-rhGAA può essere utile in molteplici condizioni di blocco autofagico oltre la malattia di Pompe

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Tecnici di Laboratorio
Giuseppina Franzese
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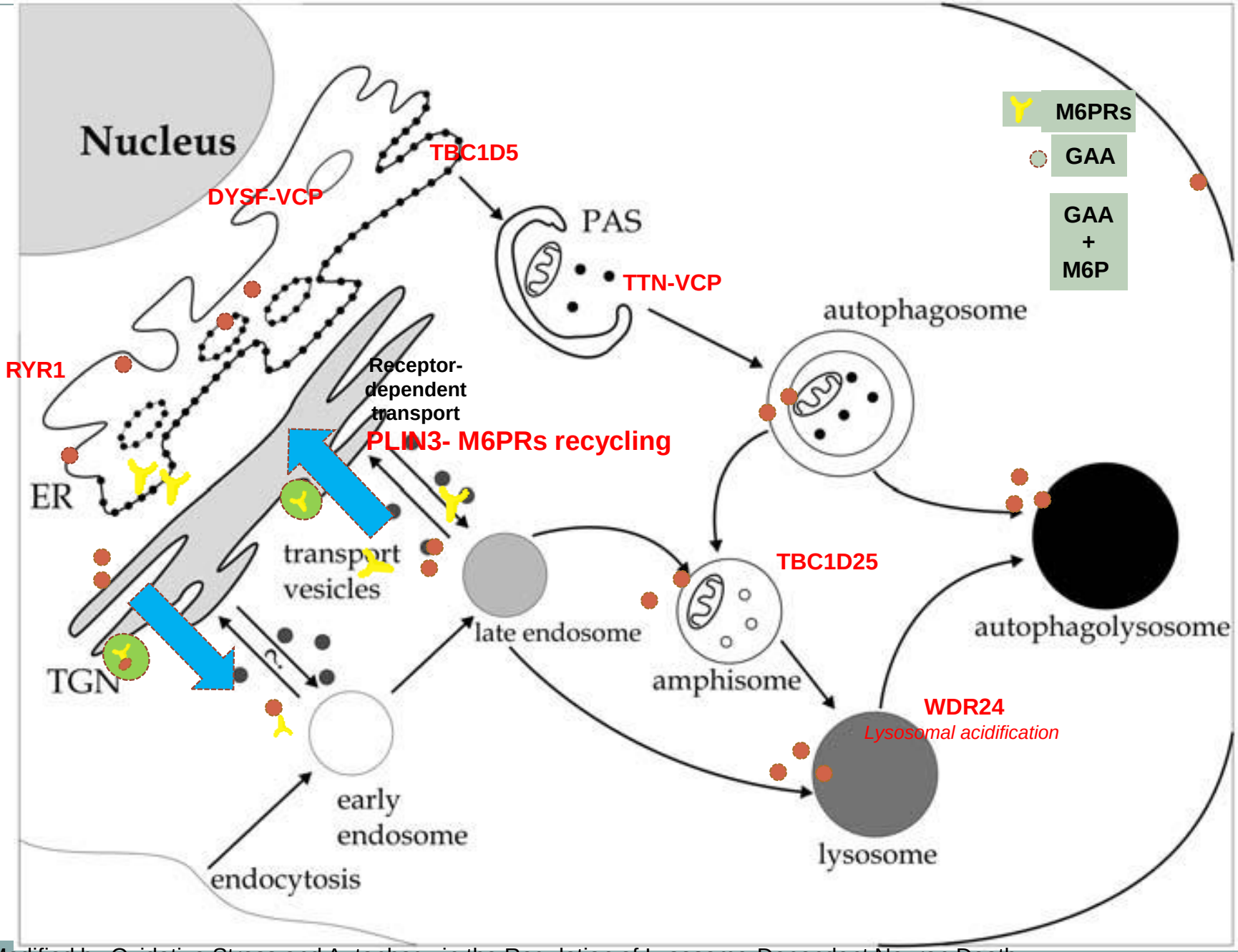

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