ABC meeting – Napoli, 10-12 Aprile 2017

ASPETTI GENETICI E MOLECOLARI DELLA MALATTIA DI CUSHING

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Ospedale S. Orsola-Malpighi – Bologna

Agenda

La genetica nella sindrome di Cushing

- **Adenomi surrenalici sporadici**
- **Iperplasie surrenaliche macronodulari**

Background genetico della malattia di Cushing

- ***USP8* breakthrough**

Conclusioni e take home message

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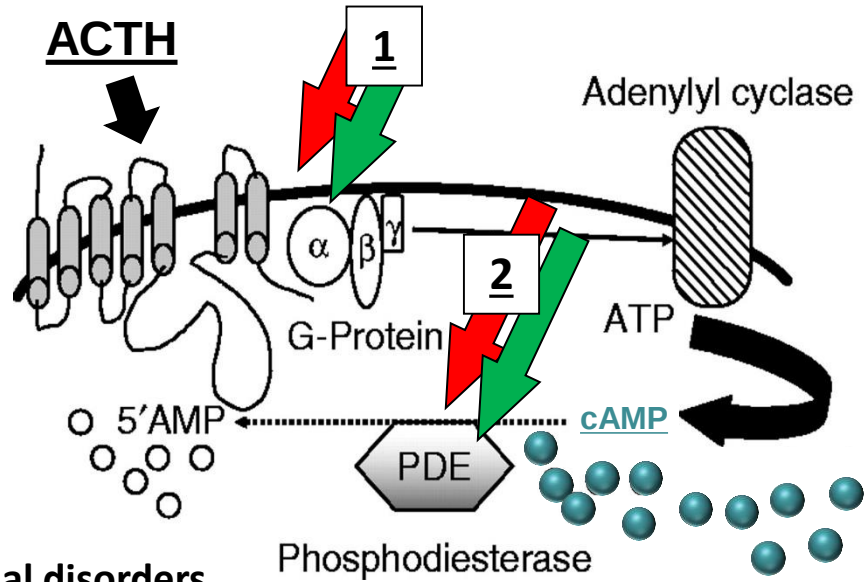
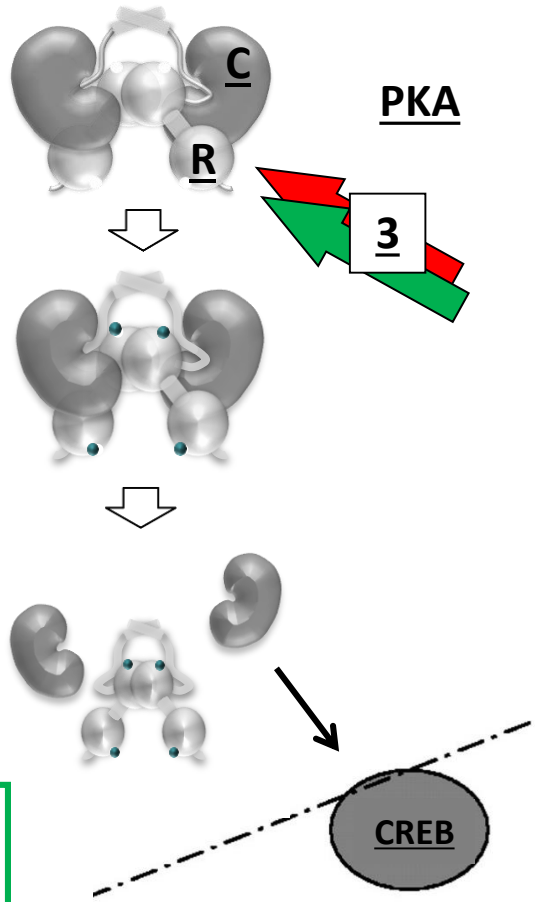
Conclusioni e take home message

cAMP-PKA pathway e sindrome di Cushing
Mutazioni germinali e somatiche





Familial disorders

1. McCune Albright syndrome
- *Weinstein LS, NEJM, 1991*
- *Brown MJ, JCEM, 2010*
- *Carney JA, Am J Surg Pathol, 2011*

2. Micronodular hyperplasia/PPNAD
- *Horvath A, Nat Genet, 2006*
- *Libè R, Clin Cancer Res, 2008*

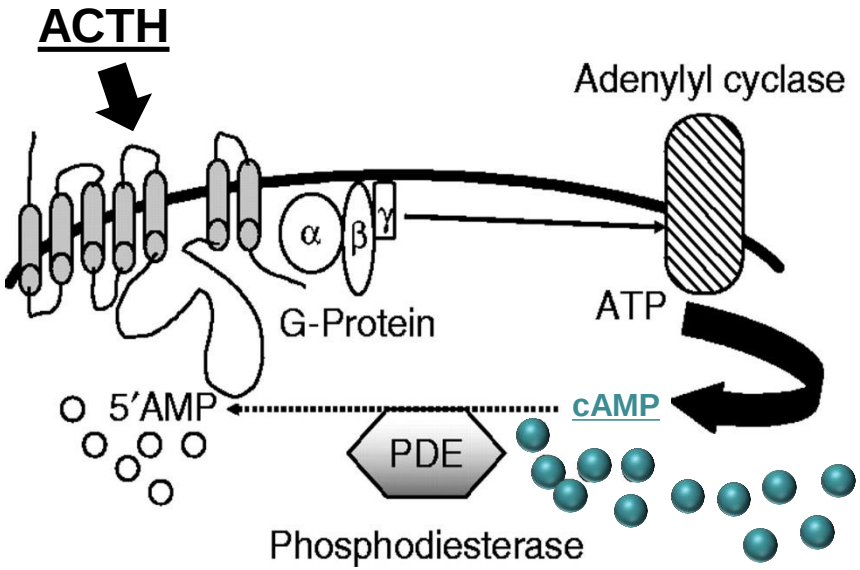
3. Carney complex (PPNAD)
- *Kirschner LS, Nat Genet, 2000*
- *Groussin L, Am J Hum Genet, 2002*
- *Horvath A, Nat Genet, 2006*

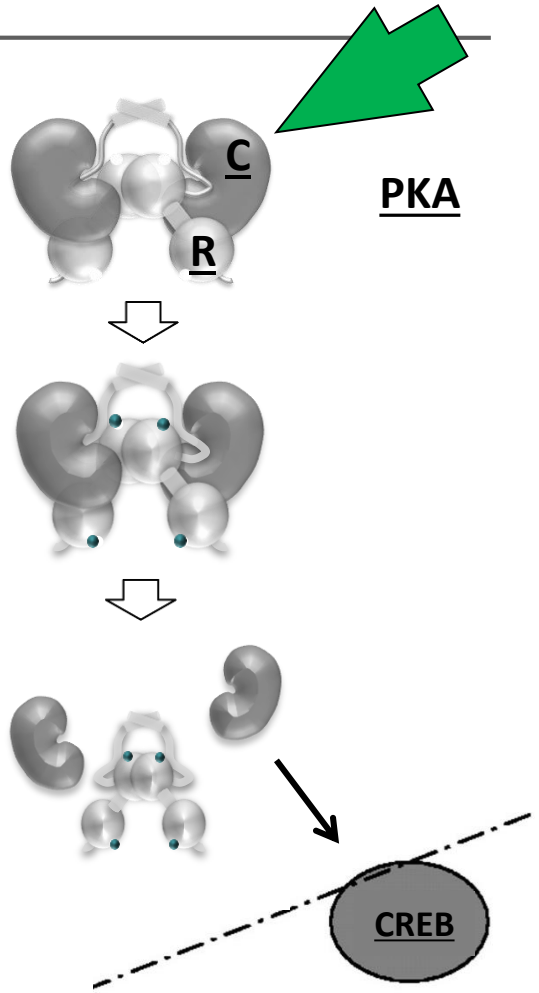
Sporadic adenomas

1. *Fragoso MC, JCEM, 2003*
2. *Rothenbuhler A, Clin Endocrinol, 2012*
3. *Bertherat J, Cancer Res, 2003*

Adapted from Vezzosi D, Eur J Endocrinol, 2011

cAMP-PKA pathway e sindrome di Cushing
Mutazioni somatiche di *PRKACA*



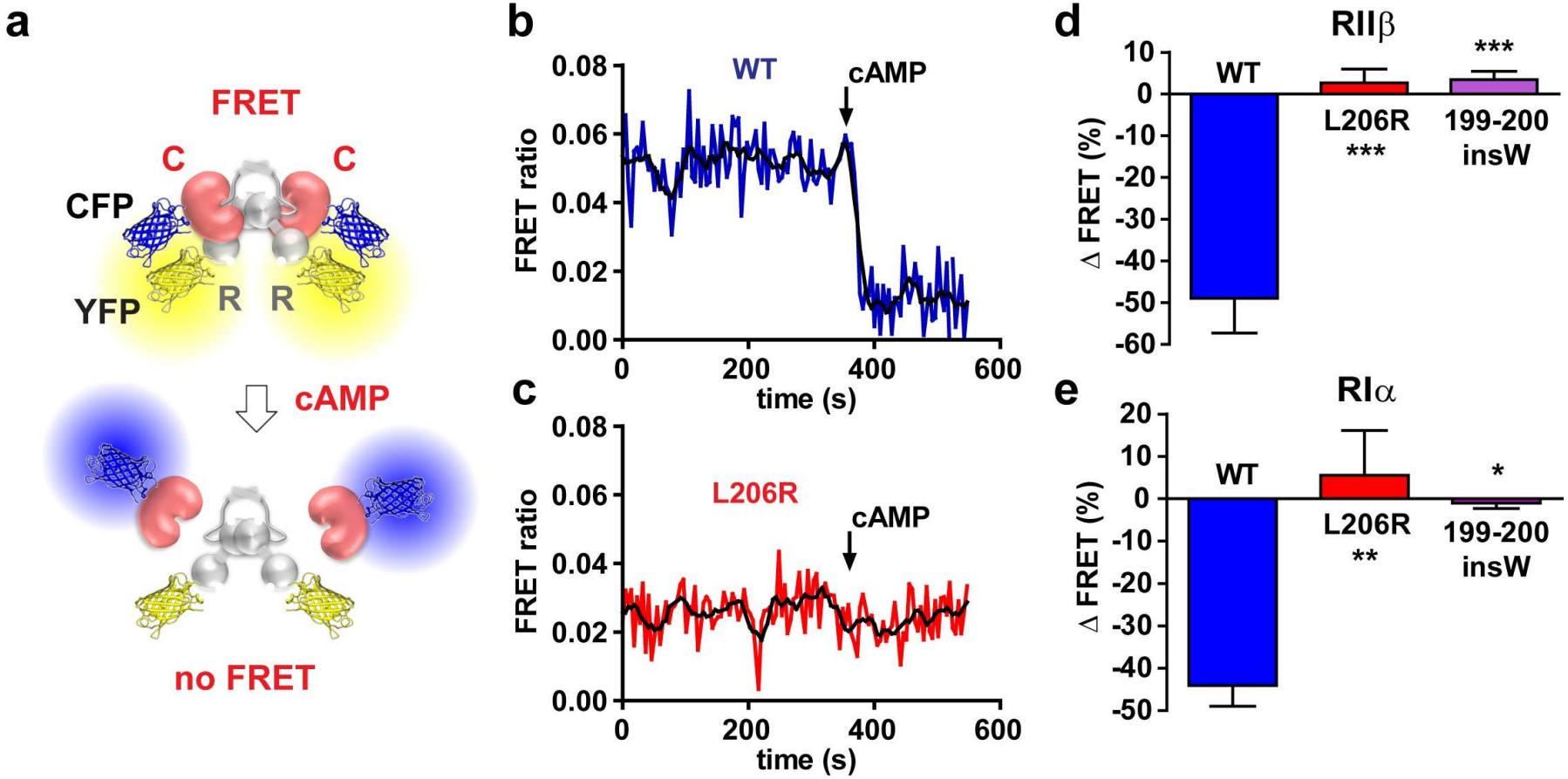


Author	*PRKACA* +
Beuschlein F, NEJM, 2014	37.3%
Goh G, Nat Genet, 2014	34.5%
Cao Y, Science, 2014	65.5%
Sato Y, Science, 2014	60.0%

Adapted from Vezzosi D, Eur J Endocrinol, 2011

Mutazioni somatiche di *PRKACA*
Studi funzionali

p.Leu206Arg and Leu199_Cys200insTrp

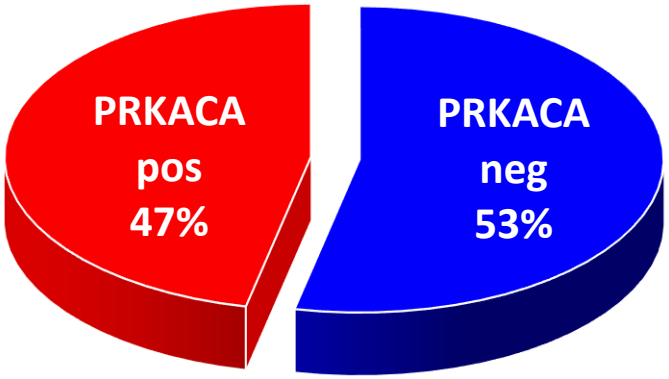


Calebiro D, Nat Commun, 2014

Mutazioni somatiche di *PRKACA* Prevalenza

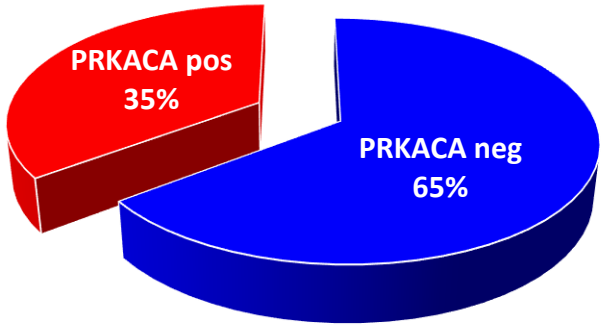
Overt Cushing's syndrome

Overall (n=379)

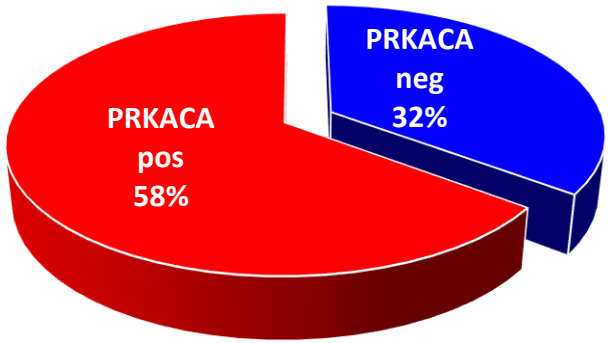


Author	*PRKACA* +
Beuschlein F, NEJM, 2014	37.3%
Di Dalmazi G, JCEM, 2014	34.4%
Goh G, Nat Genet, 2014	34.5%
Thiel A, EJE, 2015	34.4%
Cao Y, Science, 2014	65.5%
Sato Y, Science, 2014	60.0%
Nakajima Y, Endocr J, 2014	23.1%
Li X, Clin Endocrinol, 2016	52.5%
Mean pooled prevalence	**47.2%**

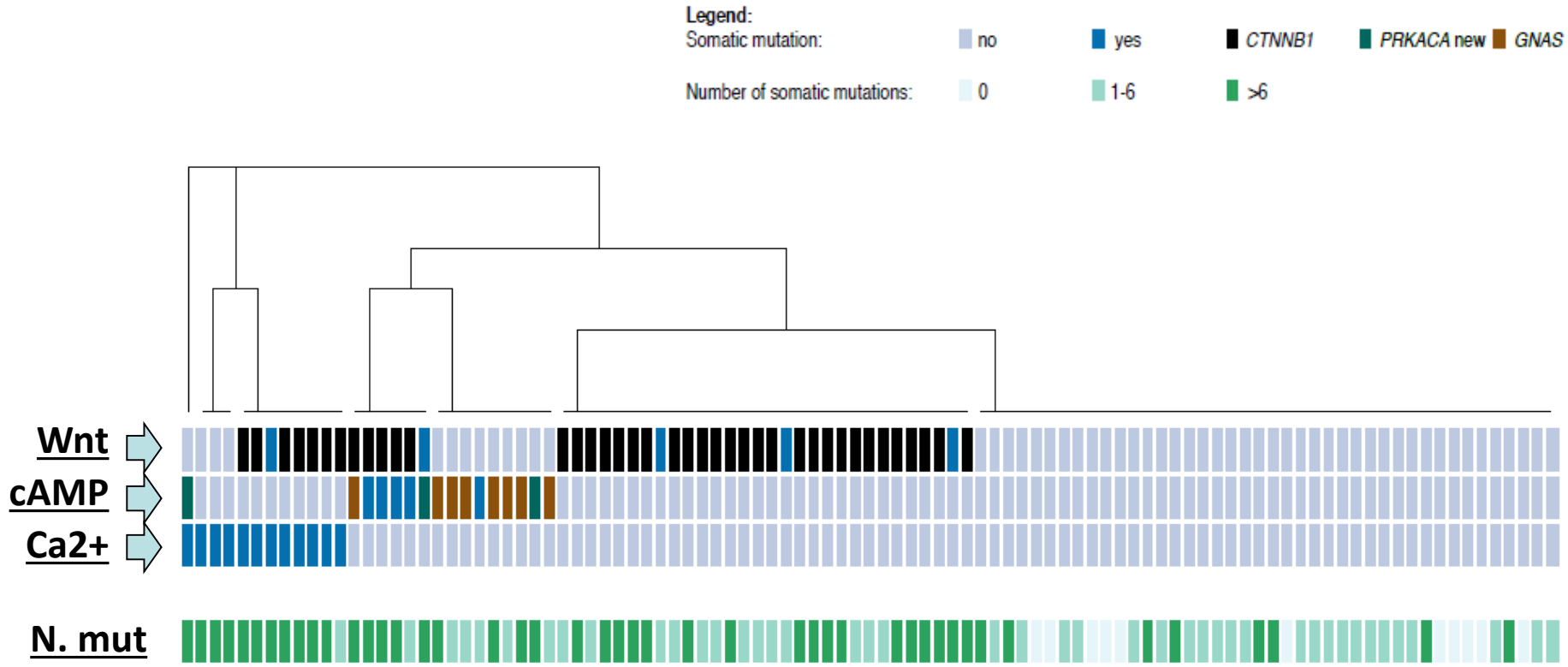
Europe/US (n=184)



Asian (n=195)

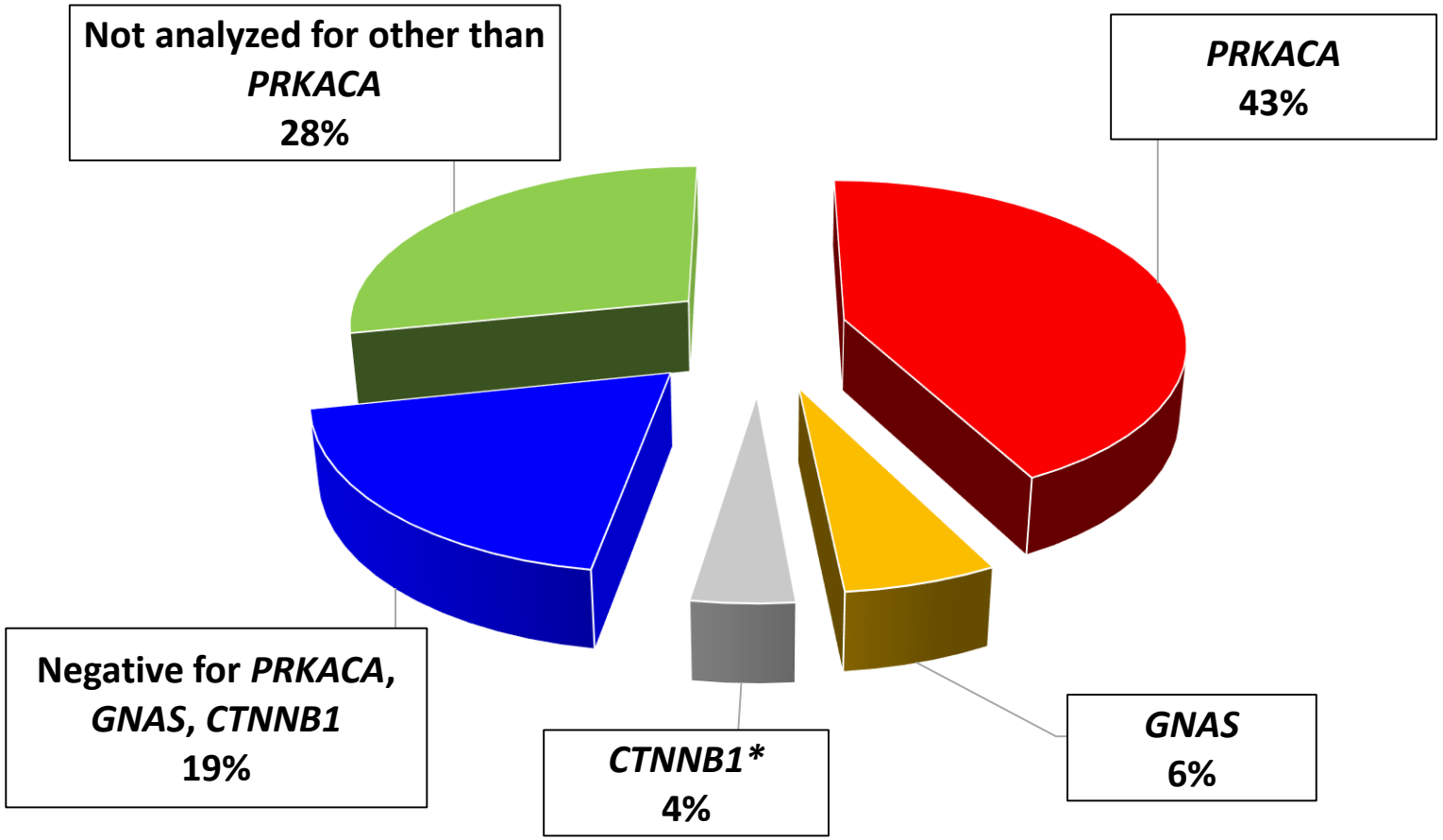


Mutazioni somatiche in tumori PRKACA negativi



Ronchi CL, Di Dalmazi G, Faillot S, JCEM, 2016

Prevalenza di mutazioni in tutte le serie pubblicate



*May be not correctly estimated

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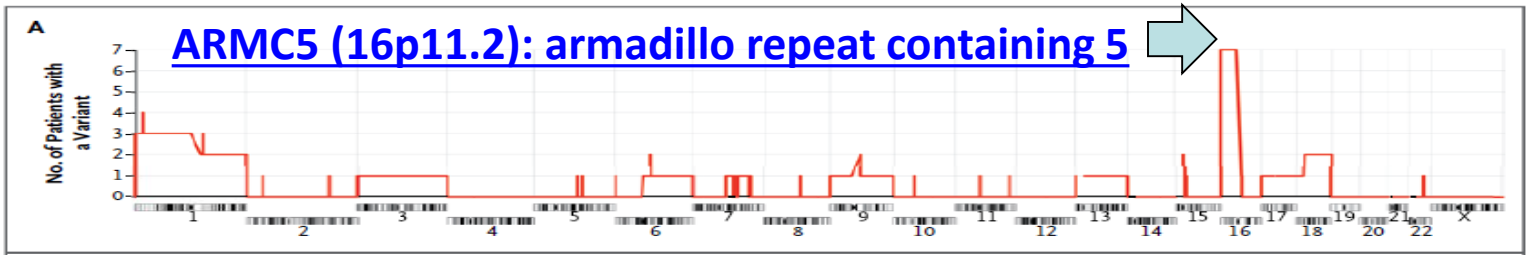
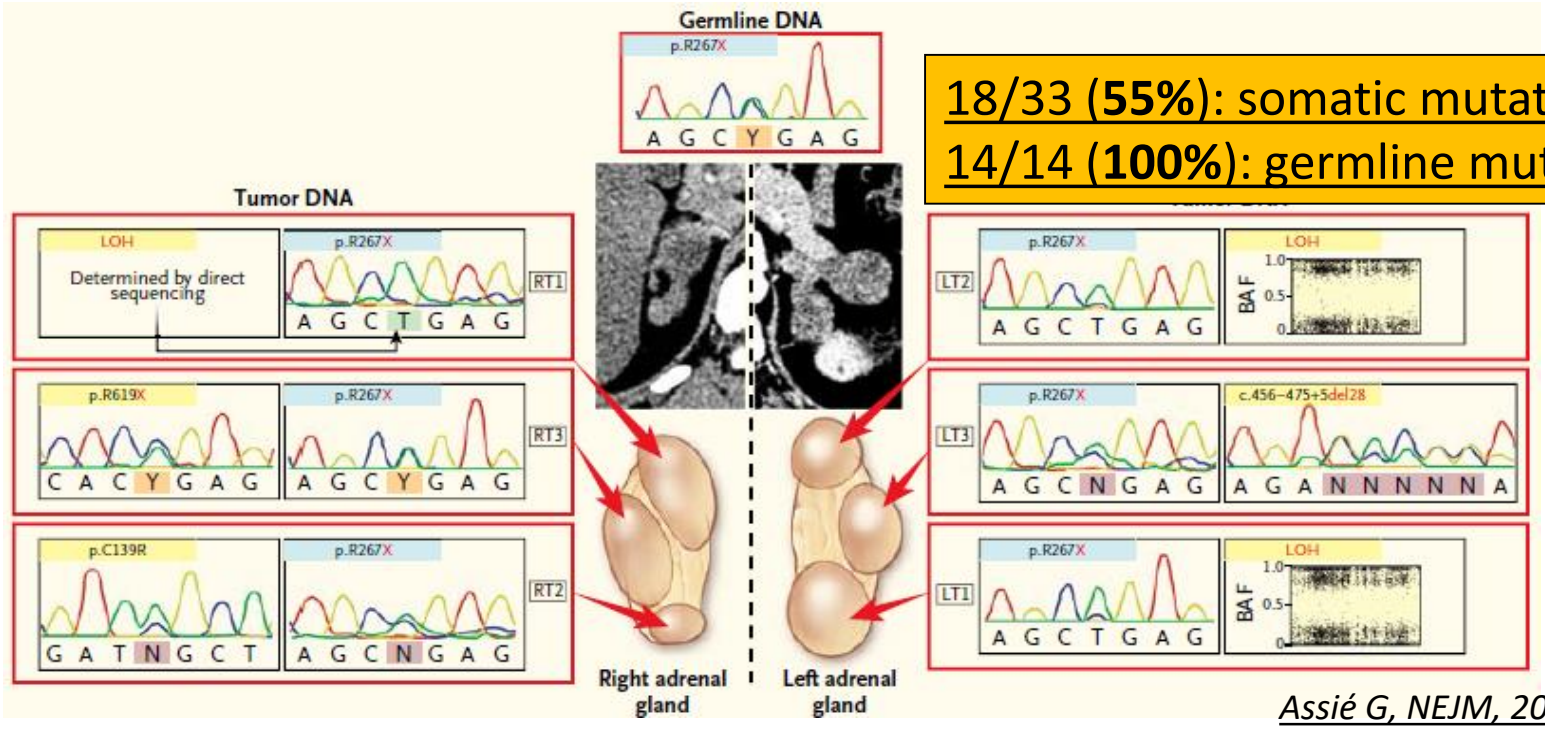
Conclusioni e take home message

ARMC5 e iperplasia macronodulare

GWAS and sequencing in 33 patients with macronodular hyperplasia





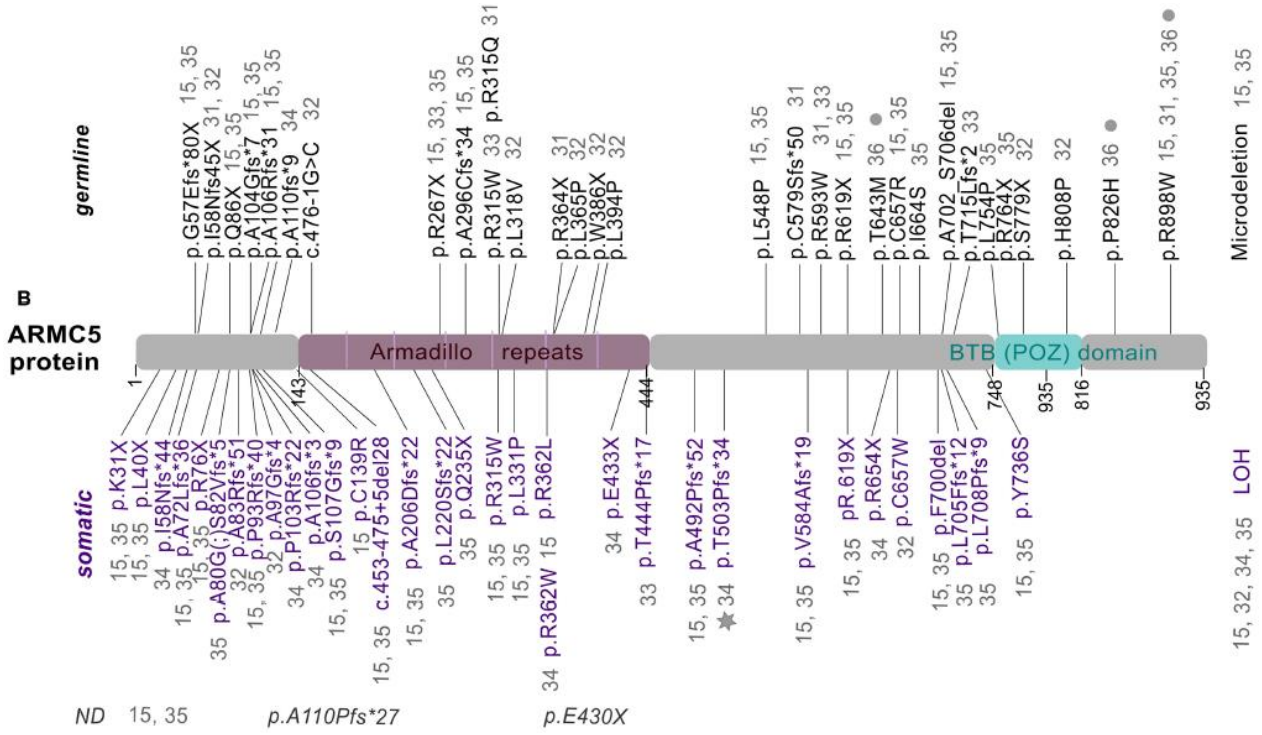
18/33 (**55%**): somatic mutations
14/14 (**100%**): germline mutations

Assié G, NEJM, 2013

ARMC5 e iperplasia macronodulare

Confirmation studies

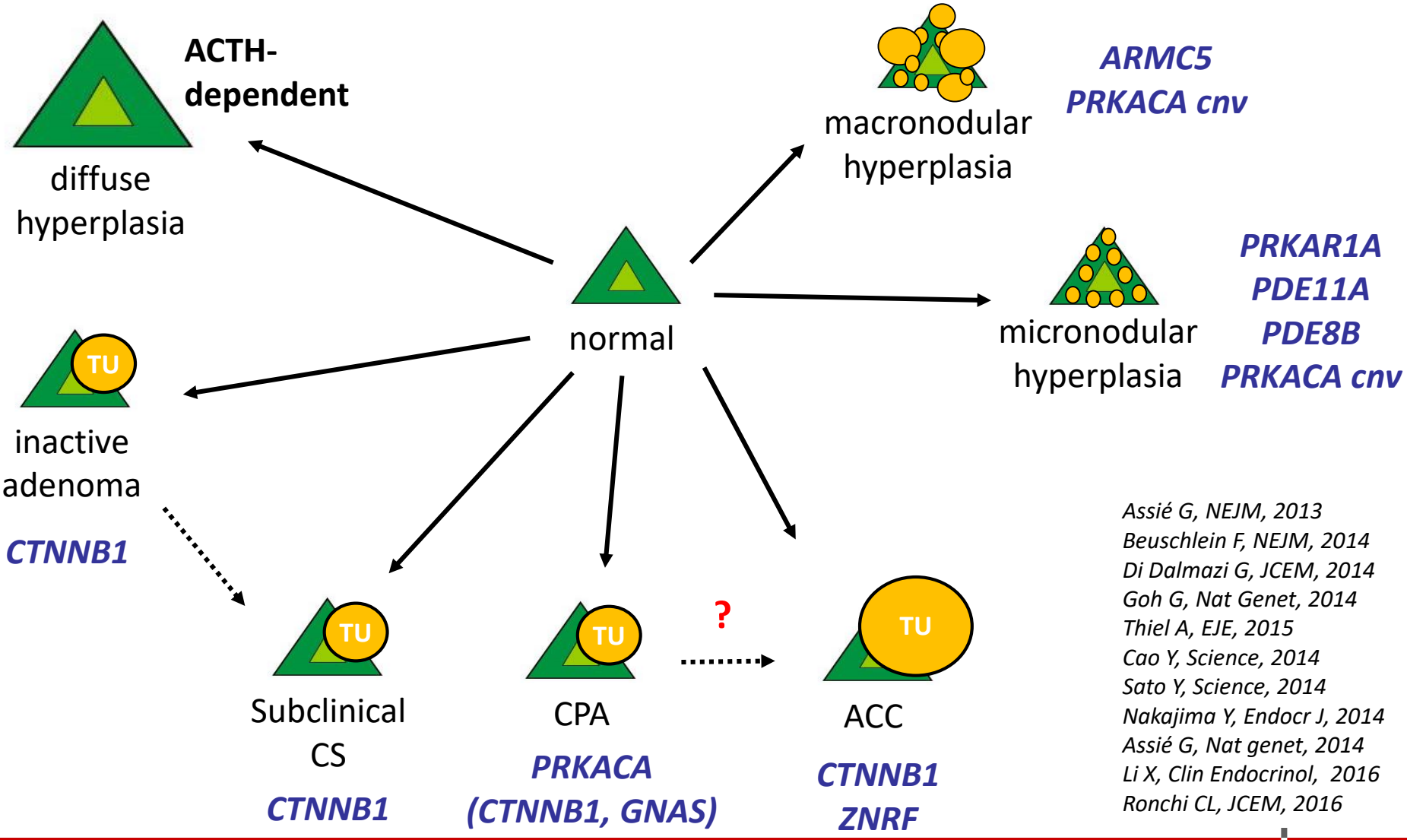
Mutations in 61/218 patients (28%) with PBMAH



Author	*ARMC5* +
Assié G, NEJM, 2013	18/33 (55%)
Faucz FR, JCEM, 2014	7/34 (21%)
Espiard S, JCEM, 2015	24/98 (26%)
Albiger NM, JCEM, 2016	12/53 (23%)
Pooled mean prevalence	**61/218 (28%)**

Drougat L, Front Endocrinol, 2015

Sindrome di Cushing: background genetico



Assié G, NEJM, 2013
Beuschlein F, NEJM, 2014
Di Dalmazi G, JCEM, 2014
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Li X, Clin Endocrinol, 2016
Ronchi CL, JCEM, 2016

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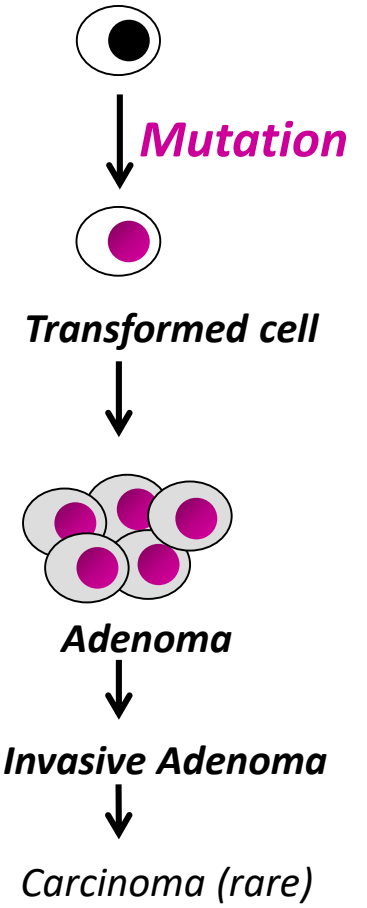
Malattia di Cushing: background genetico

Monoclonal



Gene	Ref.
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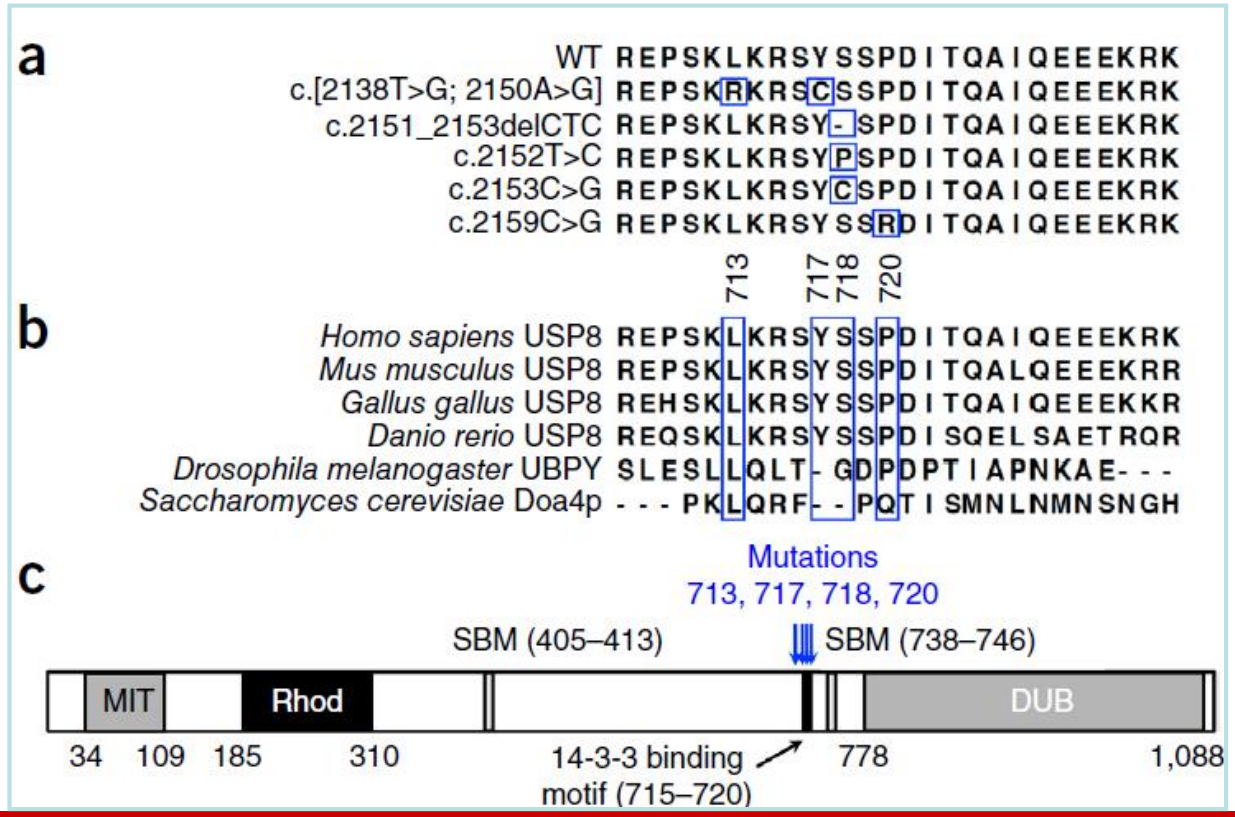
NR0B1	De Menis, 2005
DICER1	Sahakitrungruang, 2014
GNAS	Williamson, 1995 Riminucci, 2002
CDKN1B	Pellegata, 2006
TSC2	Stratakis, 2010

Mutazioni di *USP8* nella malattia di Cushing

Exome sequencing: n=10

Replication cohort n=7 corticotroph + 36 other tumors (NFA, GH, prolactinoma)

Somatic mutations in ubiquitin-specific protease 8 (*USP8*) in 6/17 CD (35%), 0/36 in other pituitary tumors



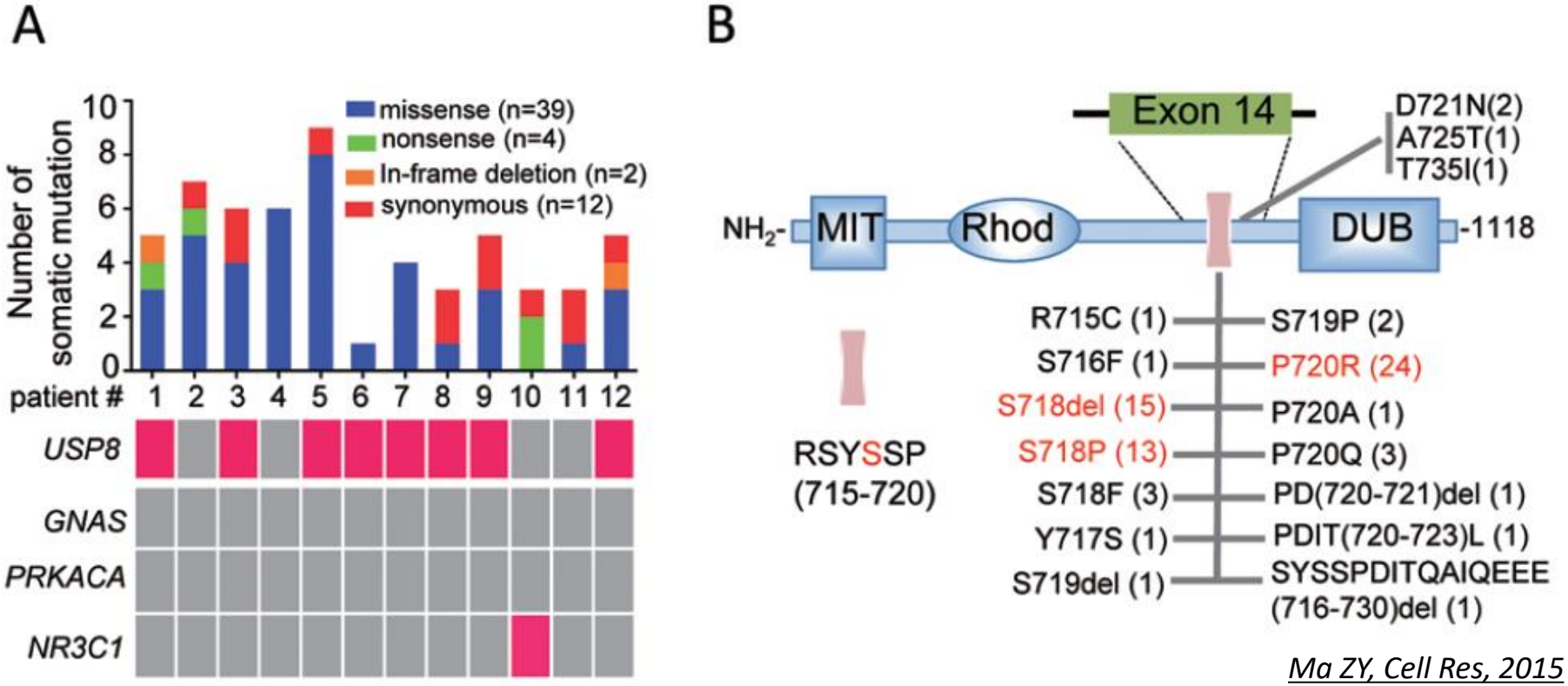
Reincke M. Nat Genet. 2015

Mutazioni di *USP8* nella malattia di Cushing

Exome sequencing: n=12

Replication cohort n=108 corticotroph + 36 other tumors (NFA, GH, nonfunctioning)

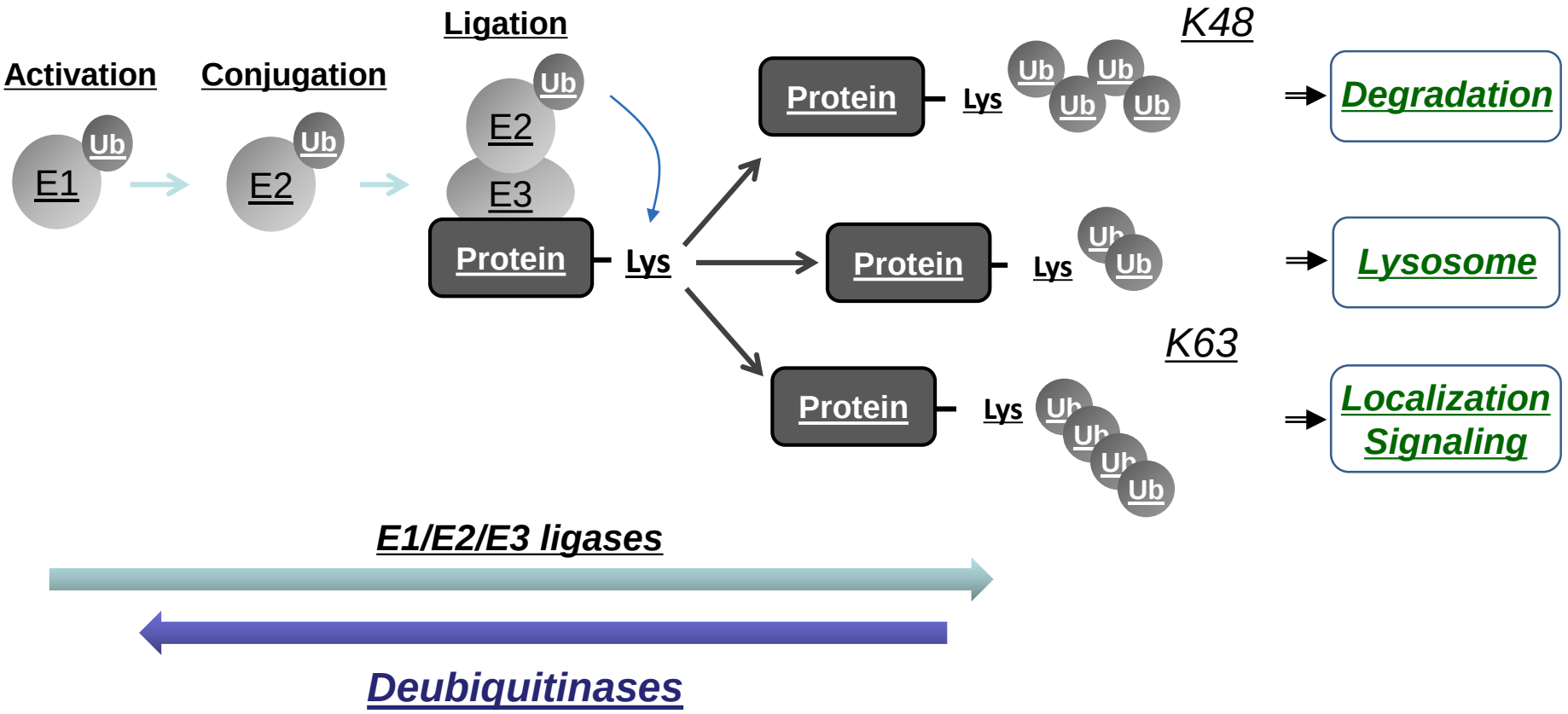
Somatic mutations in *USP8* in 62% of CD tumours



Ma ZY, Cell Res, 2015

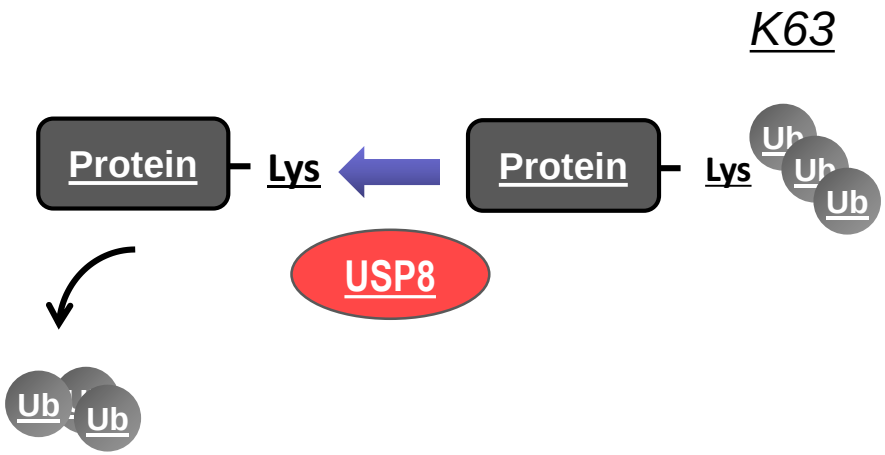
Mutazioni somatiche di *USP8*
Analisi funzionale

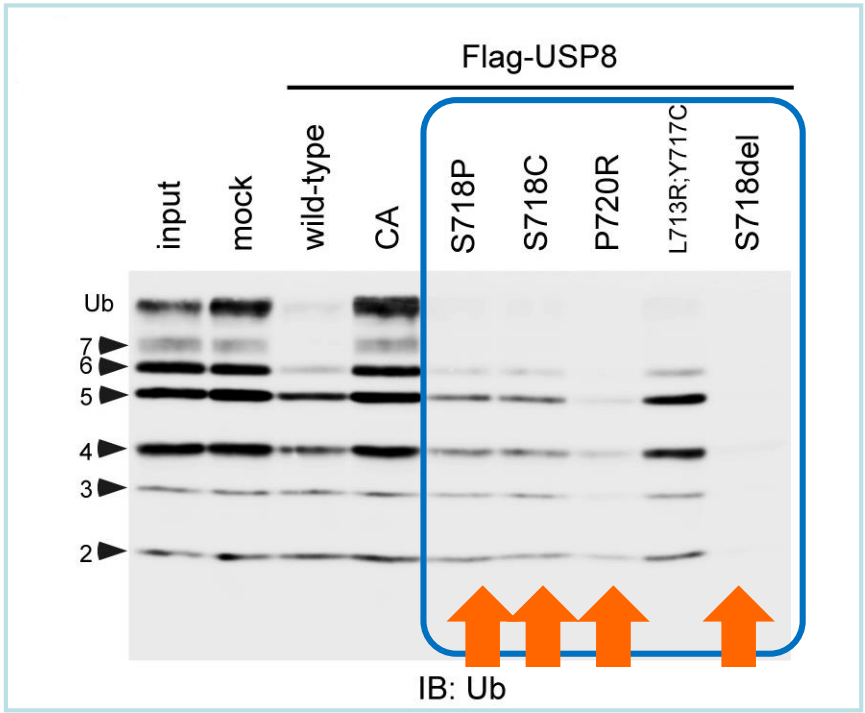
Ubiquitinazione



Mutazioni somatiche di *USP8*
Analisi funzionale

Mutazioni di USP8 aumentano la deubiquitinazione





Lys63-linked Ub oligomers

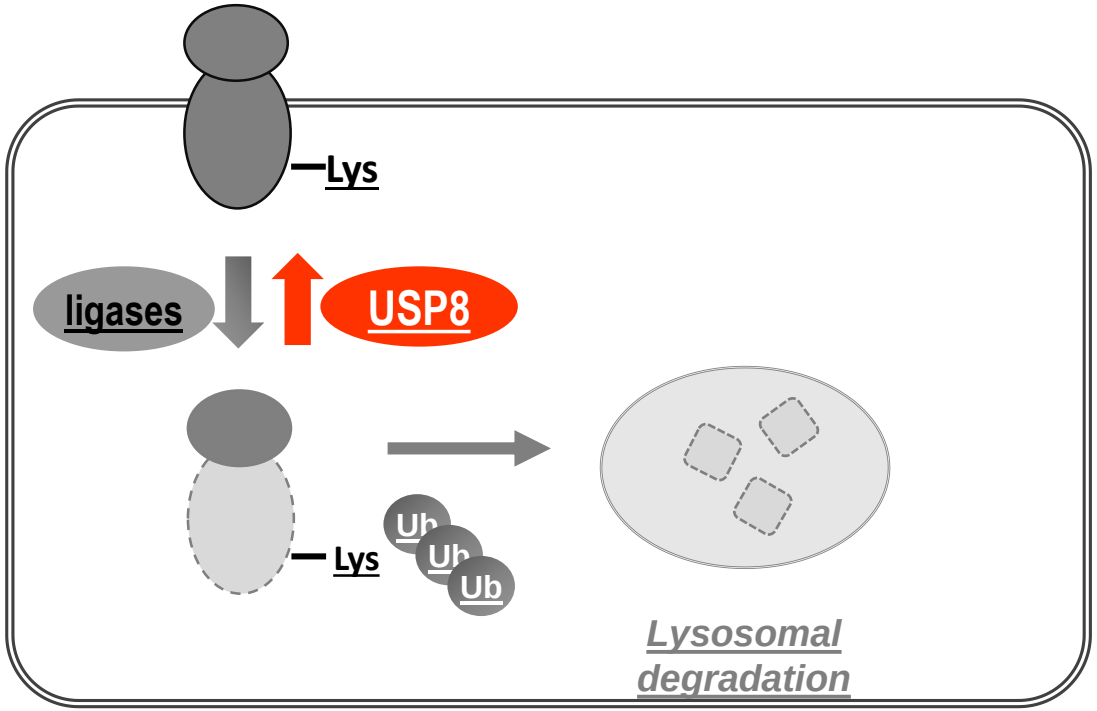
Reincke M. Nat Genet. 2015

Mutazioni somatiche di *USP8*
Analisi funzionale

Implicato nel traffico lisosomiale

Salva i recettori attivati dalla degradazione lisosomiale

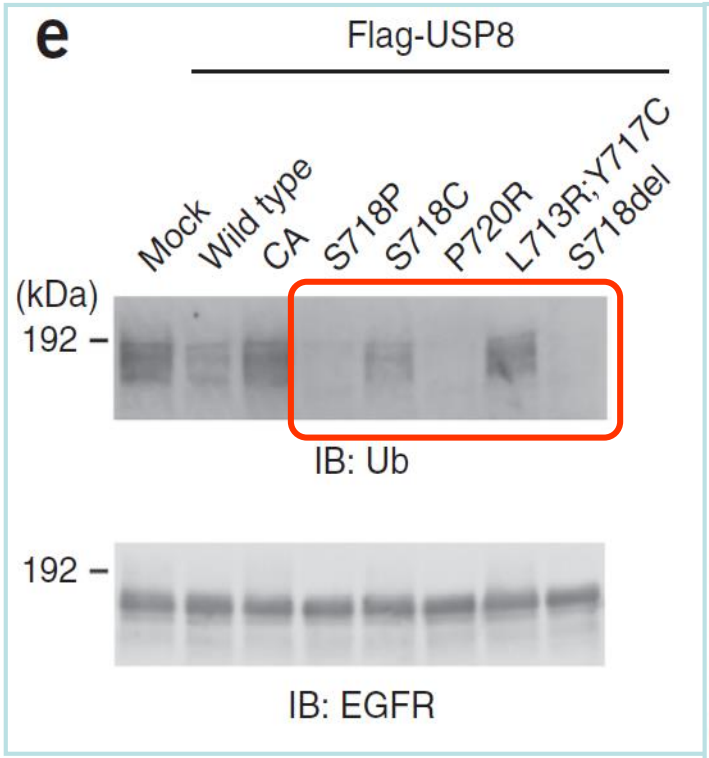
- **Effetti noti su epidermal growth factor receptor (EGFR)**



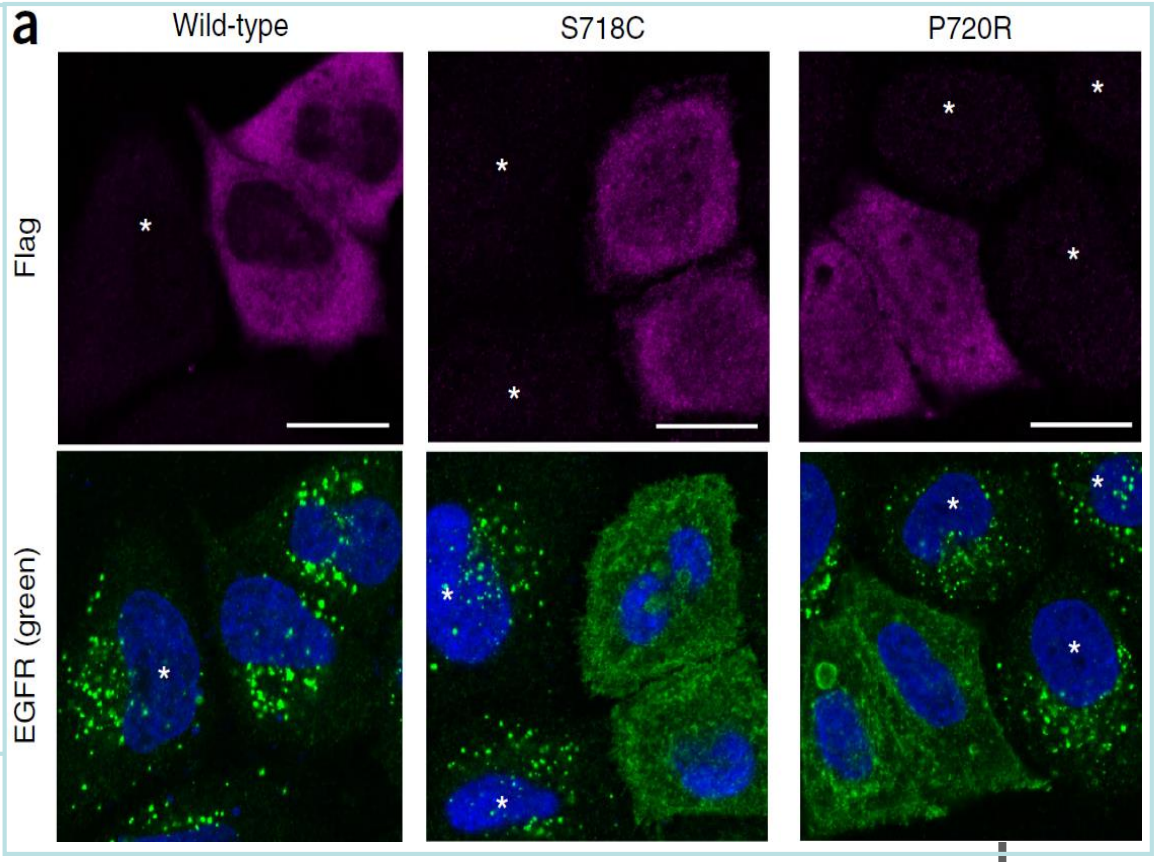
Komada M, Current Drug Discovery Technologies. 2008

Mutazioni somatiche di *USP8*
Analisi funzionale

USP8 deubiquitina EGFR

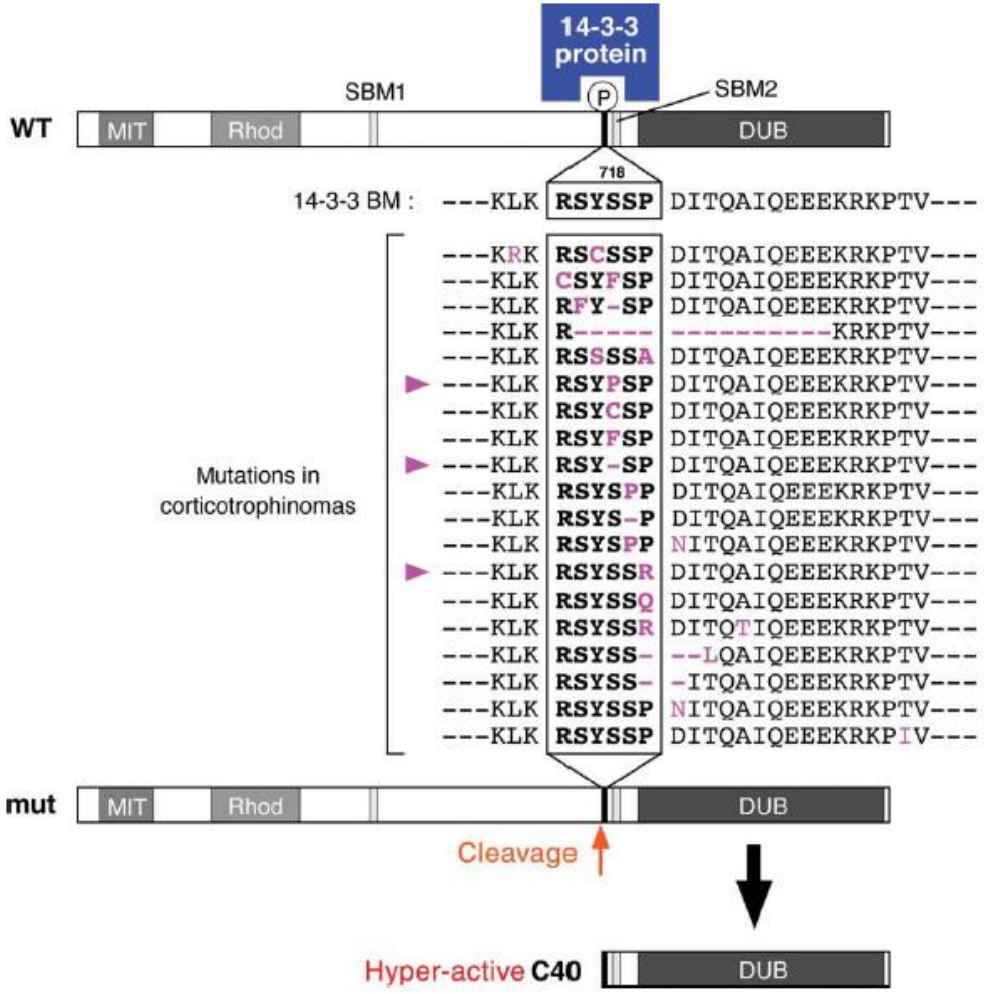


USP8 impedisce internalizzazione e downregulation di EGFR

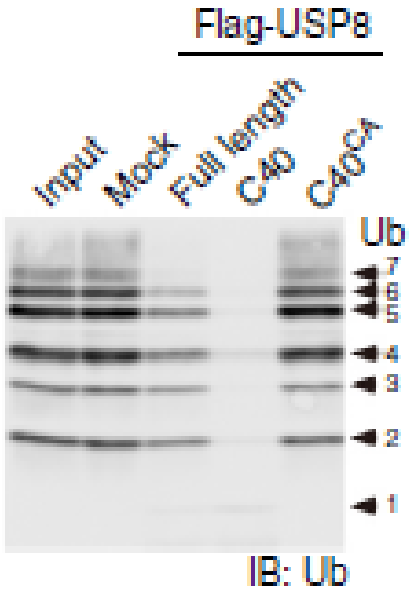


Reincke M. Nat Genet. 2015

Mutazioni somatiche di *USP8*
Analisi funzionale



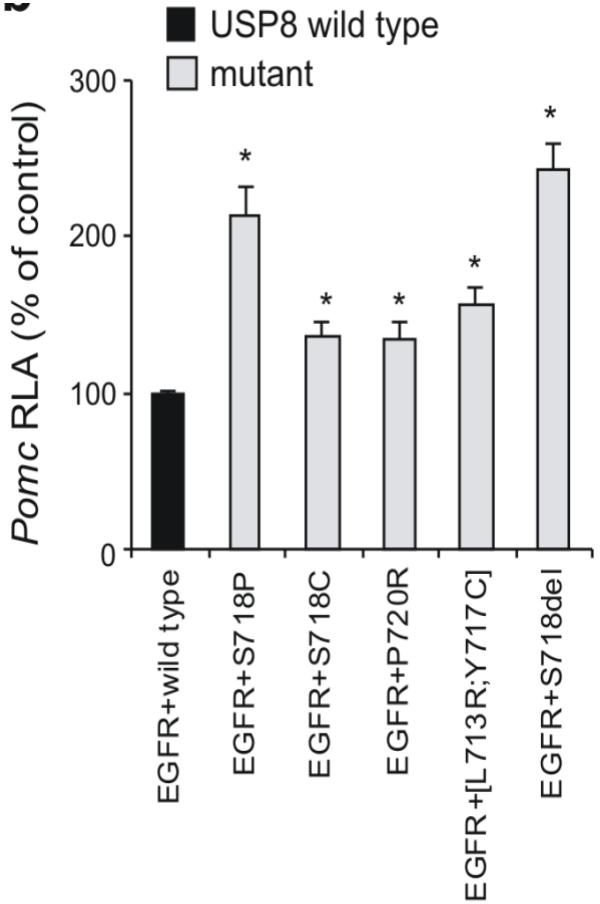
Theodoropoulou M. Eur J Endocrinol, 2015



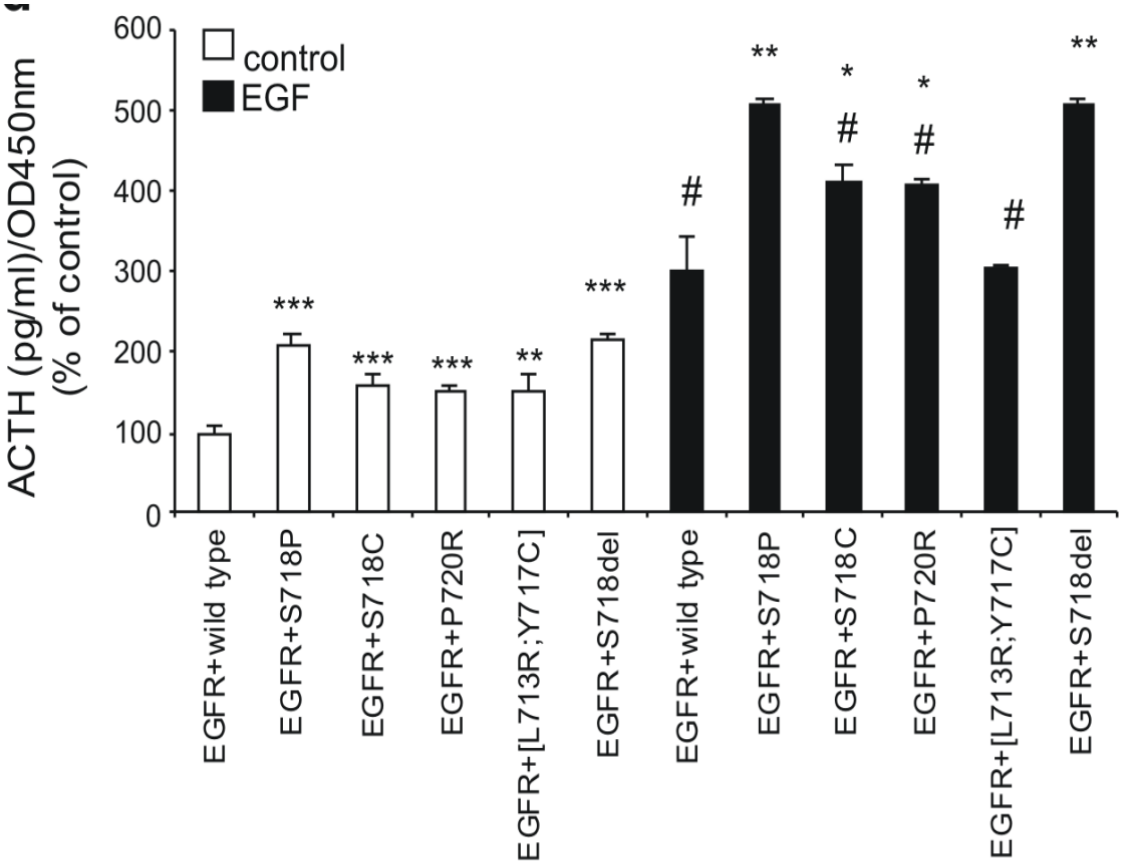
Reincke M, Nat Genet, 2015

Mutazioni somatiche di *USP8*
Analisi funzionale

POMC

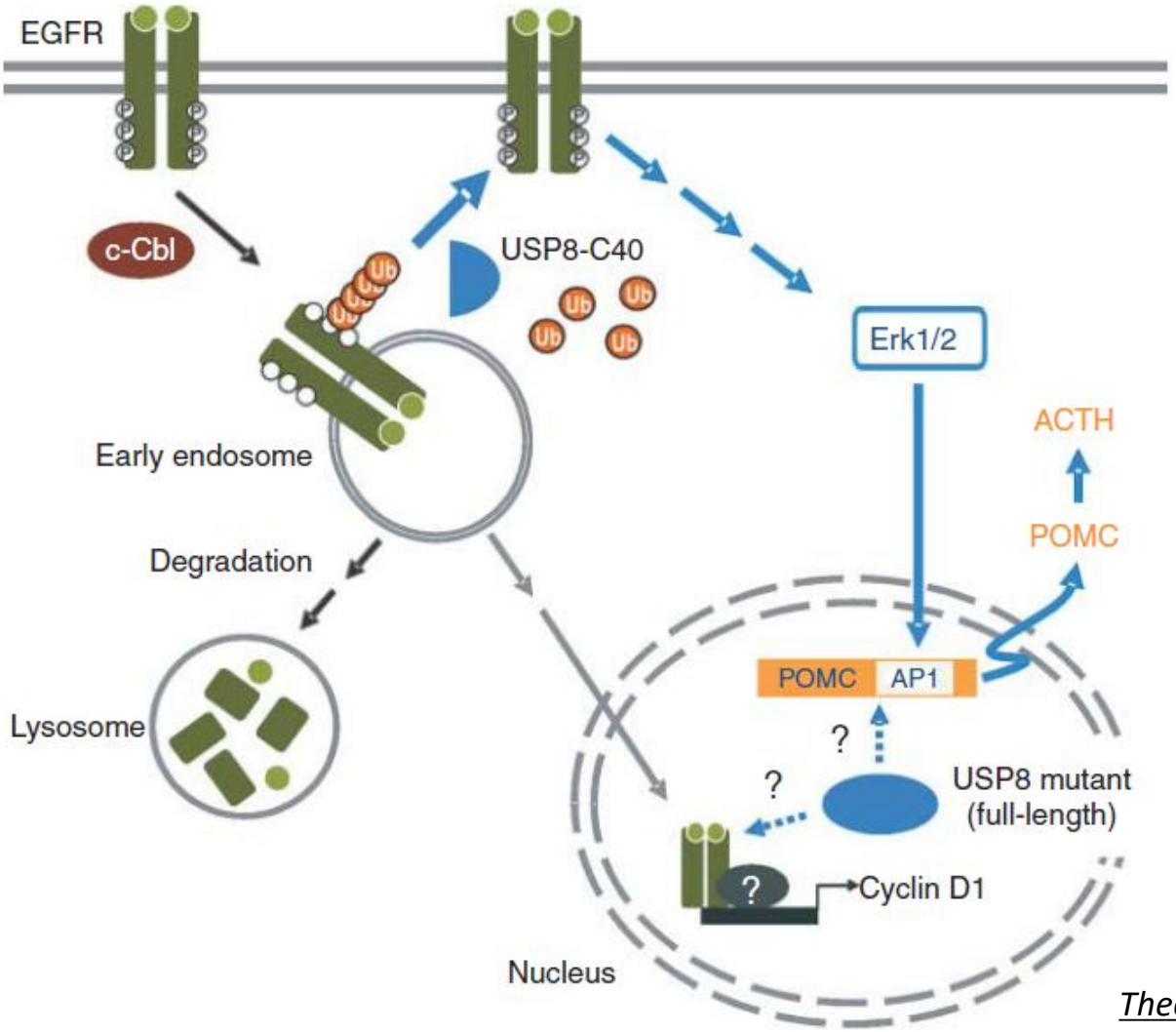


ACTH



Reincke M. Nat Genet. 2015

Mutazioni somatiche di *USP8*
Ipotesi del meccanismo di azione



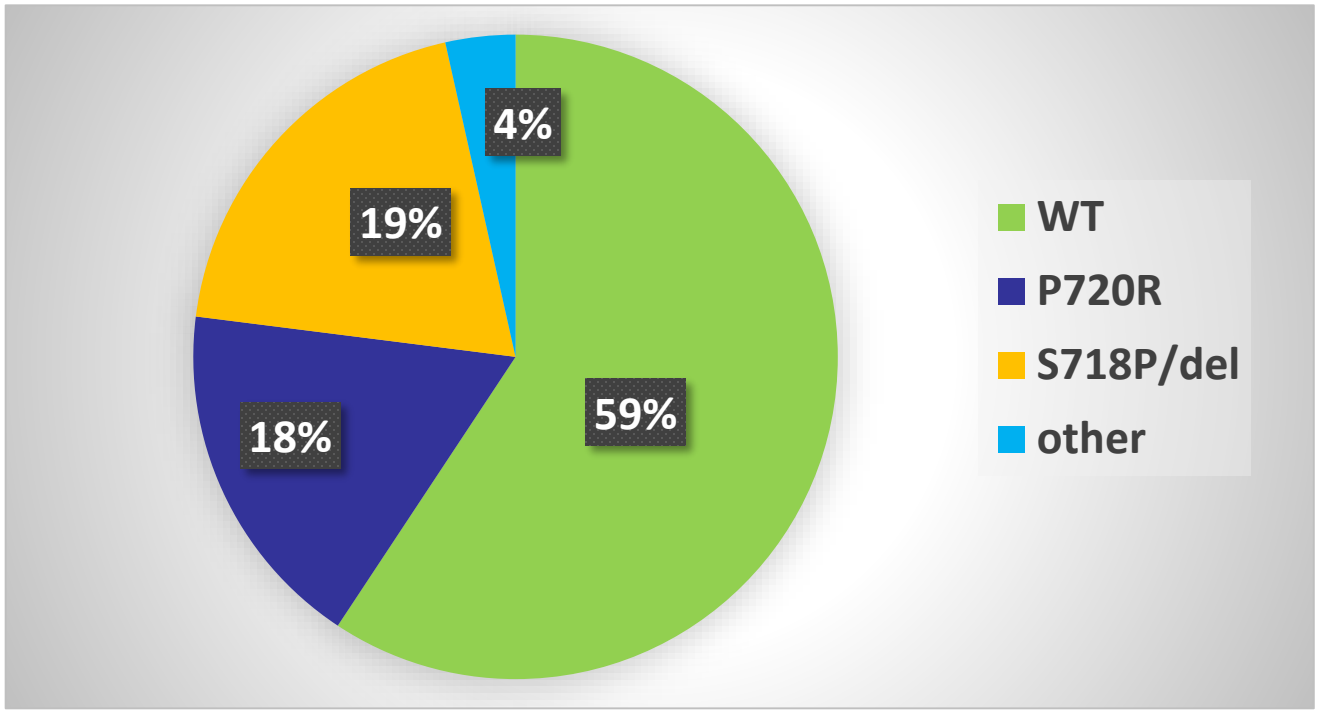
Theodoropoulou M, EJE, 2015

Mutazioni somatiche di *USP8*
Coorte di replicazione

Studio retrospettivo, multicentrico

145 adenomi ACTH-secernenti

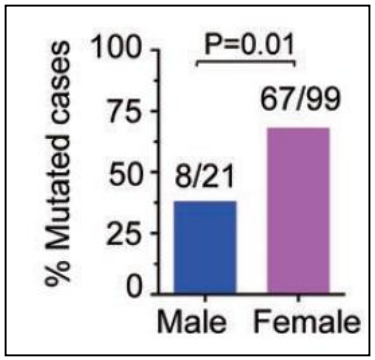
Mutazioni somatiche di *USP8* in 36% dei tumori



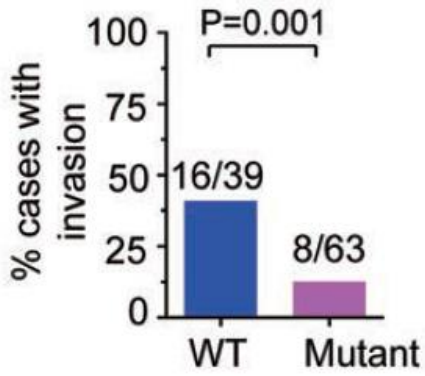
Perez-Rivas LG, JCEM, 2015

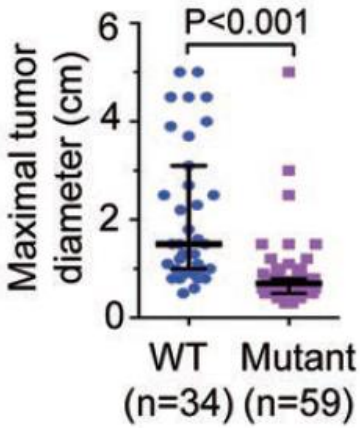
Mutazioni somatiche di *USP8*
Genotipo/fenotipo

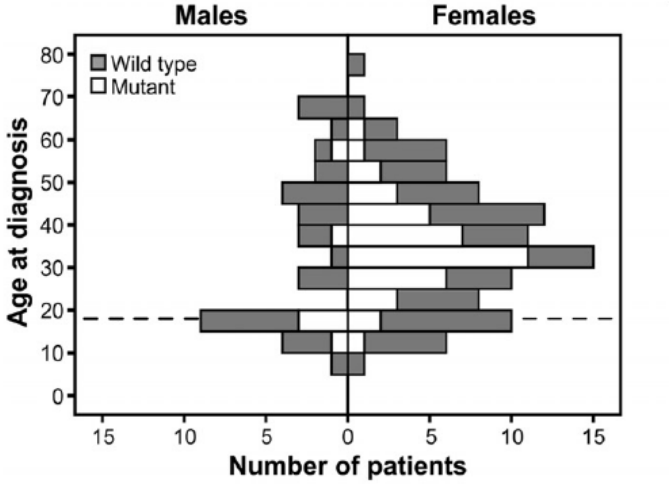
Prevalenza fra sessi

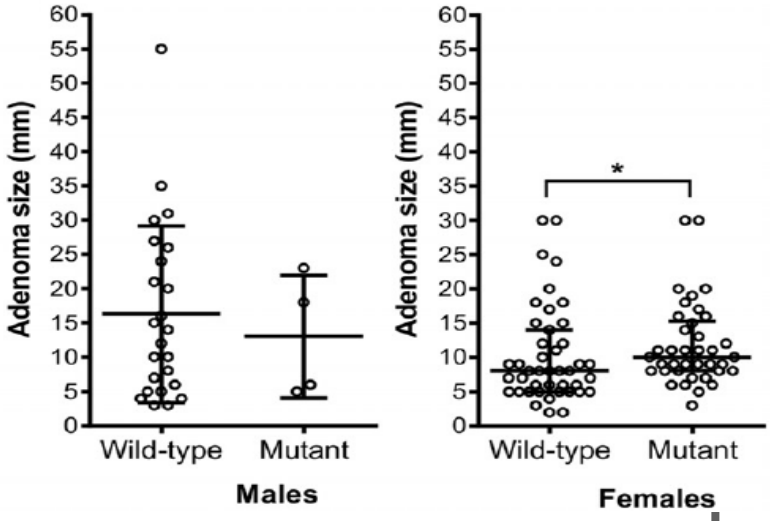


Dimensione del tumore









Perez-Rivas LG, JCEM, 2015

Ma ZY, Cell Res, 2015

Mutazioni somatiche di *USP8* Genotipo/fenotipo

Profilo ormonale

Ma, Cell Res, 2015	**WT**	***USP8* mut**	**P value**
Age (yrs)	40 (30-46)	36 (26-42)	0.08
ACTH (pg/ml)	94 (61-163)	69 (51-106)	0.05
ACTH/size [(pg/ml)/cm]	**51 (32-105)**	**103 (76-202)**	**<0.001**
Midnight F (mcg/dl)	22 (16-31)	23 (18-29)	0.8
UFC (mcg/day)	650 (412-1168)	552 (262-977)	0.4
Post-op remission	73%	84%	0.2

Reincke, Nat Genet, 2015	**WT**	***USP8* mut**	**P value**
ACTH (pg/ml)	90 ± 48	50 ± 46	NS
F after DST (mcg/dl)	**21.1 ± 12.5**	**7.8 ± 9.7**	**<0.05**

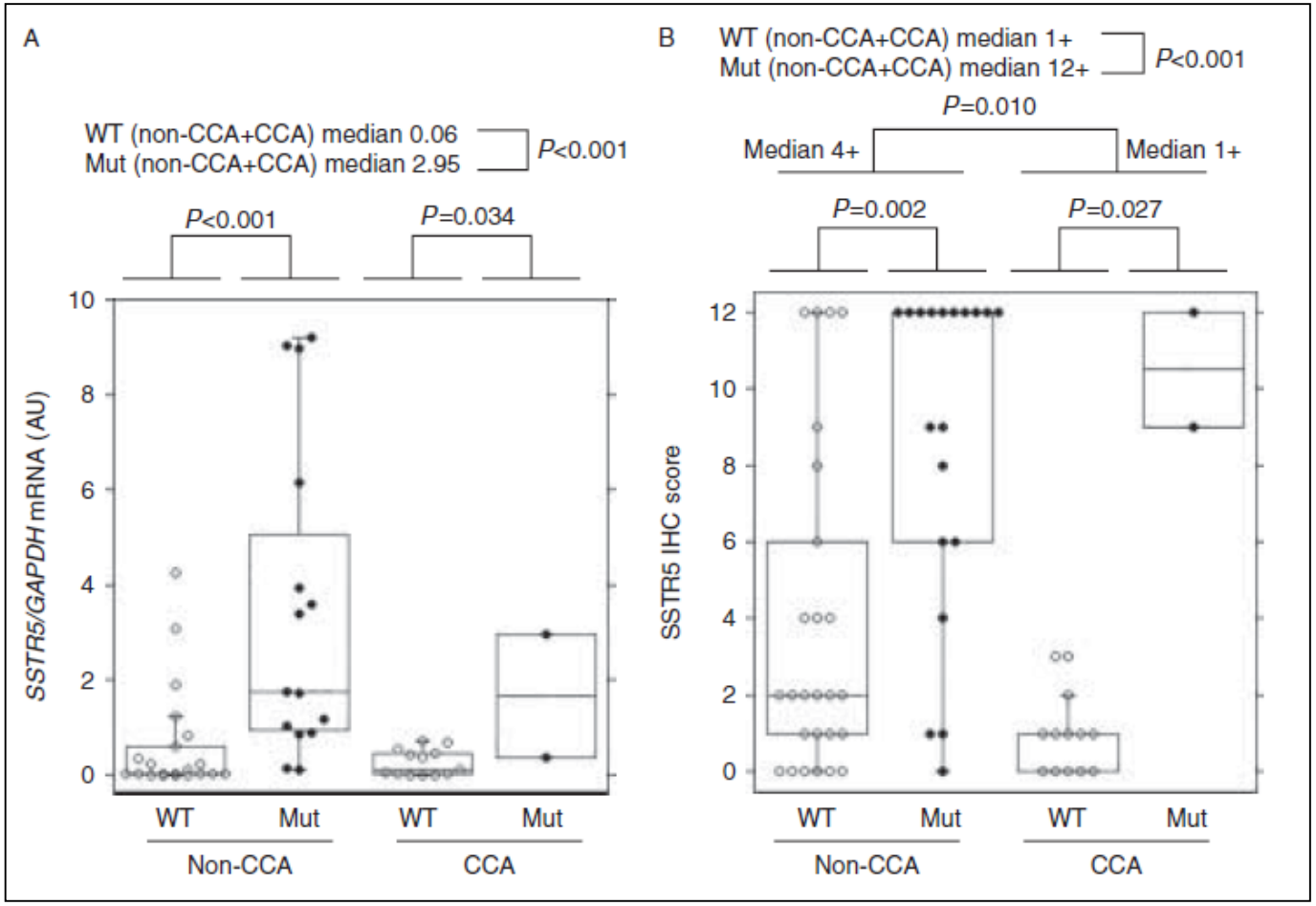
Mutazioni somatiche di *USP8*
Genotipo/fenotipo

Profilo ormonale

Perez-Rivas, JCEM, 2015	**WT**	***USP8* mut**	**P value**
ACTH (pg/ml)	74 (IQR 68)	67 (IQR 57)	0.8
UFC (mcg/day)	380 (IQR 415)	370 (IQR 490)	0.6
F post-8mg DST (mcg/dl)	**5.2 (IQR 6.8)**	**2.5 (IQR 2.5)**	**0.01**
Post-op UFC	**2.5 (IQR 6.8)**	**22.5 (241.3)**	**0.007**
No post-op adr. insuff.	**29%**	**51%**	**0.03**

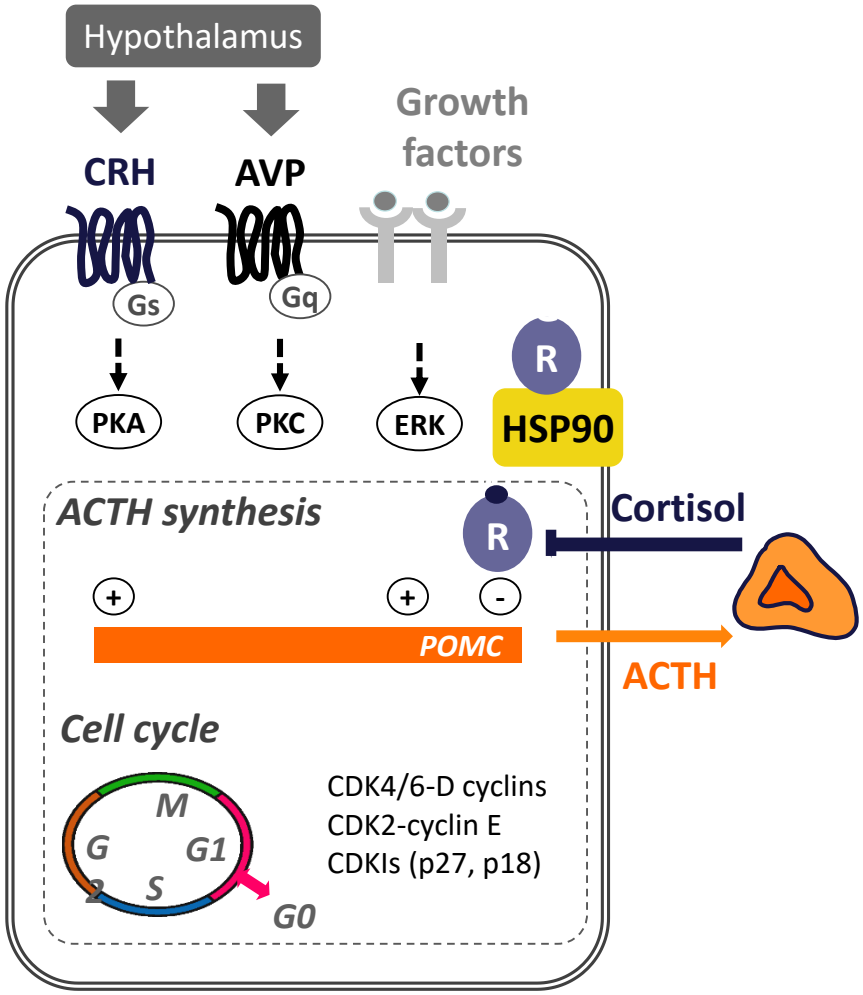
Mutazioni somatiche di *USP8*
Genotipo/fenotipo

Le mutazioni di *USP8* sono più frequenti in tumori che esprimono SSTR5



Hayaschi K, Eur J Endocrinol, 2016

Malattia di Cushing: background genetico Update 2017



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GNAS	Williamson, 1995 Riminucci, 2002
CDKN1B	Pellegata, 2006
TSC2	Stratakis, 2010
USP8	Reincke, 2015 Ma, 2015 Perez-Rivas, 2015 Hayaschi, 2016

35-60%

Dworakowska D, Eur J Clin Invest. 2012
Sbiera S, Trends Endocrinol Metab. 2015
Theodoropoulou M, Eur J Endocrinol. 2015

Conclusioni e take home message

- **Le tecniche di NGS hanno portato un sensibile avanzamento nello studio della patogenesi della sindrome di Cushing**

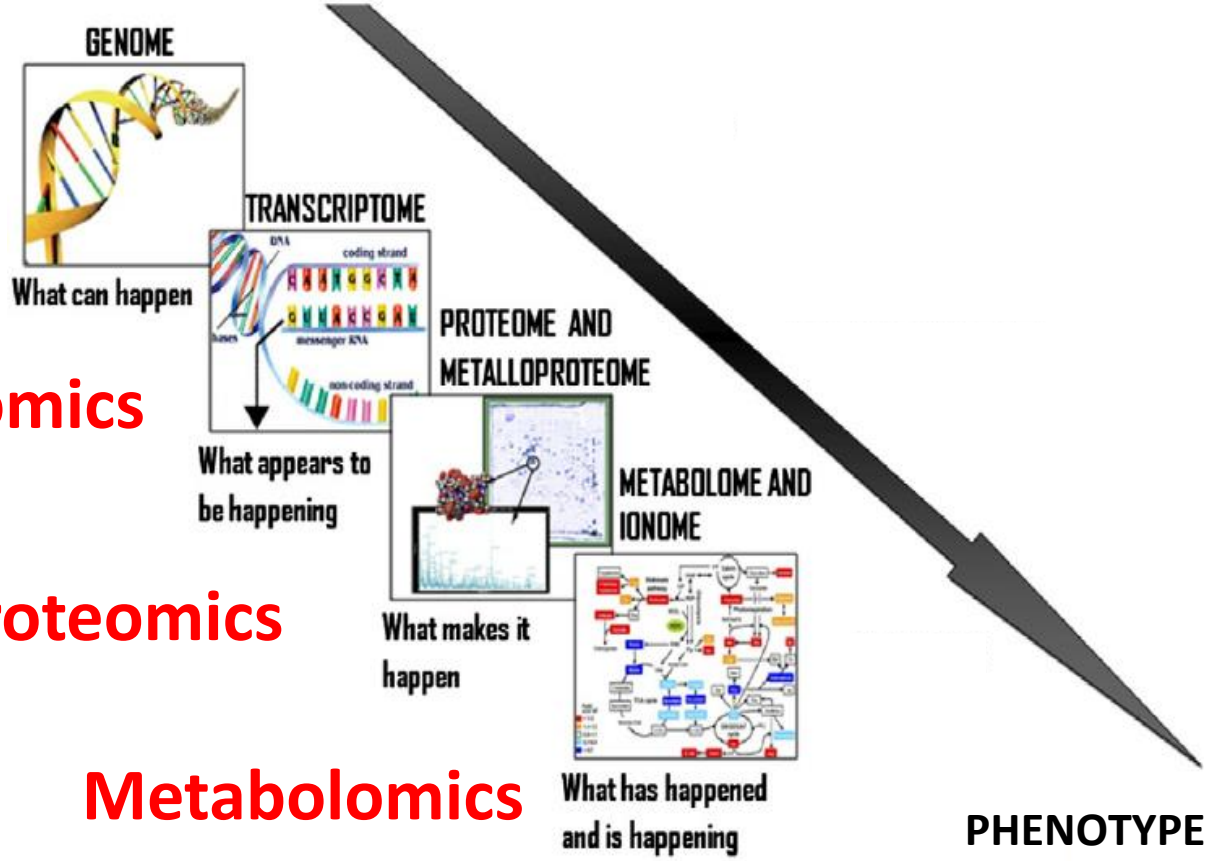
- **Le mutazioni di *PRKACA* sono l'evento predominante negli adenomi sporadici (30-60% dei casi)**

- **Le mutazioni di *ARMC5* (germinali + somatiche) sono un evento comune nelle iperplasie macronodulari (30% dei casi)**

- **Le mutazioni somatiche di *USP8* sono un evento frequente e specifico negli adenomi ipofisari ACTH-secernenti e hanno un ruolo patogenetico che coinvolge il signaling di EGFR**

- **Le mutazioni somatiche di *USP8* si associano ad un fenotipo clinico da definire e potrebbero avere nuove potenziali implicazioni farmacologiche**

The “omics” cascade



Genomic

Transcriptomics

Proteomics

Metabolomics

Modified from Sevillano MAG, J Proteomics, 2014

U.O. di Endocrinologia – Bologna

Università di Bologna - Ospedale S. Orsola Malpighi

Patologie surrenaliche

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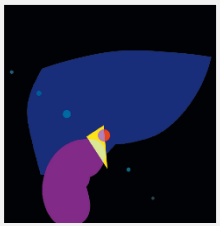

Percorso di Diagnosi, Terapia e Assistenza delle patologie surrenaliche - Bologna

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  - Cristina Mosconi
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  - Cristina Nanni
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  - Eugenio Cosentino
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USP8 mutational status & clinical presentation

Table 2. Hormonal status in patients with wild type versus USP8-mutated adenomas.

	Wild-type		Mutated		*P* value
Preoperative variables					
Basal plasma ACTH, pg/ml (median, IQR)	74.0	67.65	67.0	57.0	0.76
Basal serum cortisol, µg/dl, (median, IQR)	24.1	14.8	21.6	9.2	0.41
Urinary free cortisol, µg/24h, (median, IQR)	379.6	415.0	370.0	490.1	0.62
Serum cortisol after 1/2 mg DMX, µg/dl, (median, IQR)	14.7	14.1	17.2	16.1	0.60
Serum cortisol after 8 mg DMX, µg/dl, (median, IQR)	5.2	6.75	2.5	2.5	0.01
Postoperative variables					
Basal levels of plasma ACTH after OP, pg/ml, (median, IQR)	8.3	12.3	14.0	30	0.12
Minimum serum cortisol after OP, µg/dl, (median, IQR)	2.5	7	3.3	7.9	0.72
Urinary free cortisol after OP, µg/24h, (median, IQR)	2.5	6.0	22.5	241.3	0.007
Adrenal insufficiency, n (%)					
No	19	(29.2)	21	(51.2)	0.03
Yes	46	(70.8)	20	(48.8)	

Perez-Rivas et al., J Clin Endocrinol & Metabol 2015