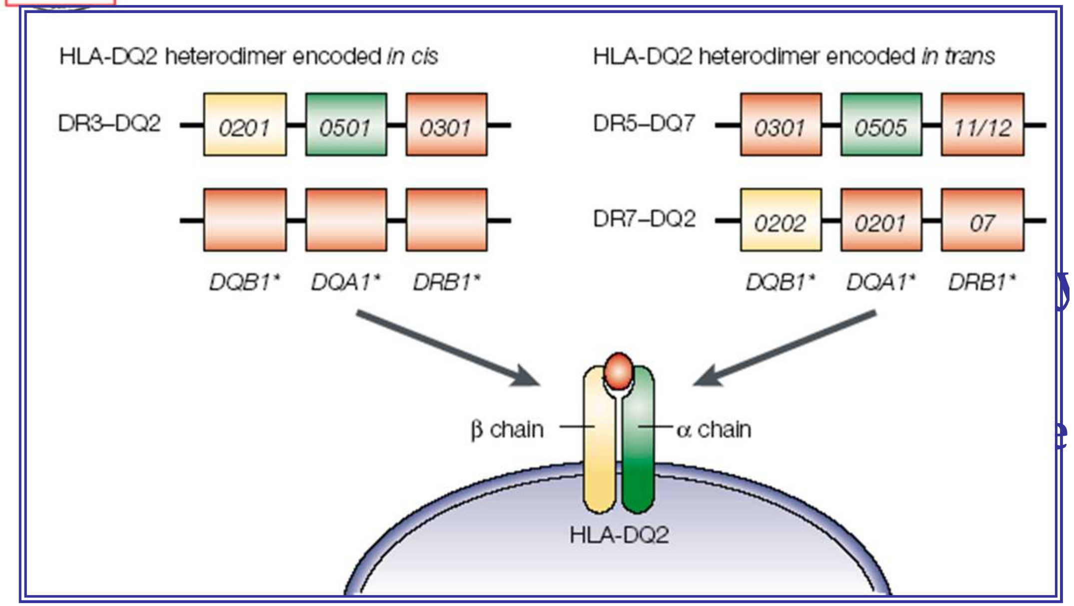
5 maggio 2012
La Celiachia nel terzo millennio: dalla diagnosi alla terapia

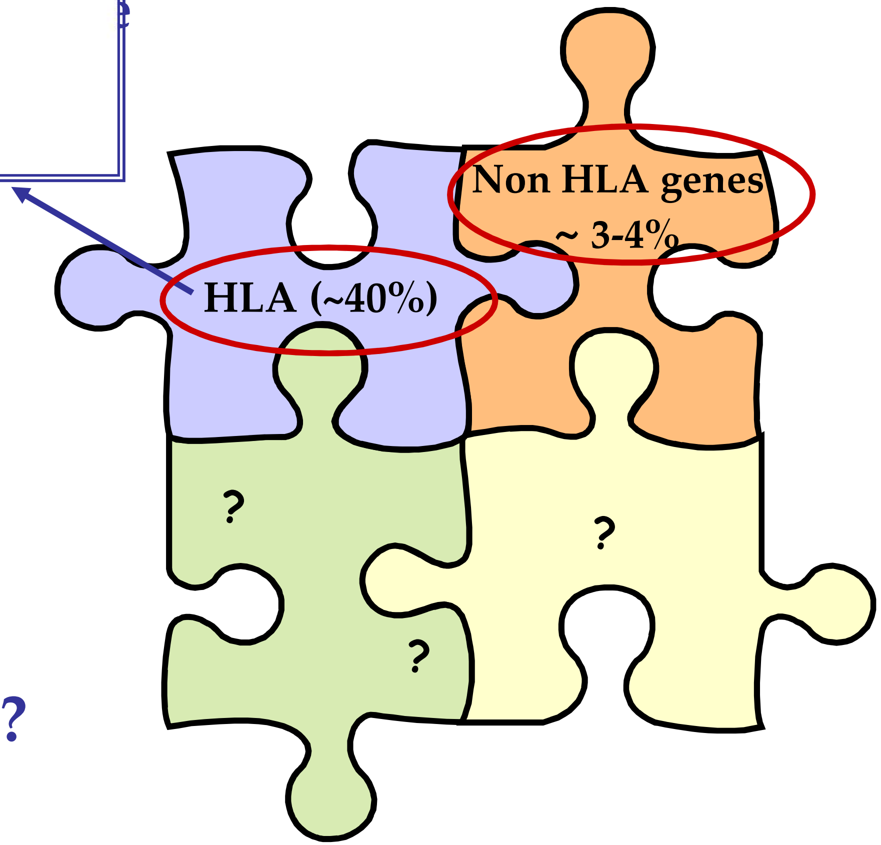
Contributo dell'epigenetica alla patogenesi della celiachia

Marina Capuano

BIOTECNOLOGIE AVANZATE s.c.a r.l.

Celiac Disease





Other ~56%?

? Other mechanisms?

Mechanism of gene regulation?

Micro-RNAs (miRNAs)

Sono sequenze di 20-24 nt che regolano l'espressione di geni bersaglio

let-7 ***in C. Elegans: I miRNA identificato*** **(*Reinhart et al.2000*)**

Sequenze altamente conservate (*Pasquinelli et al. 2000*)

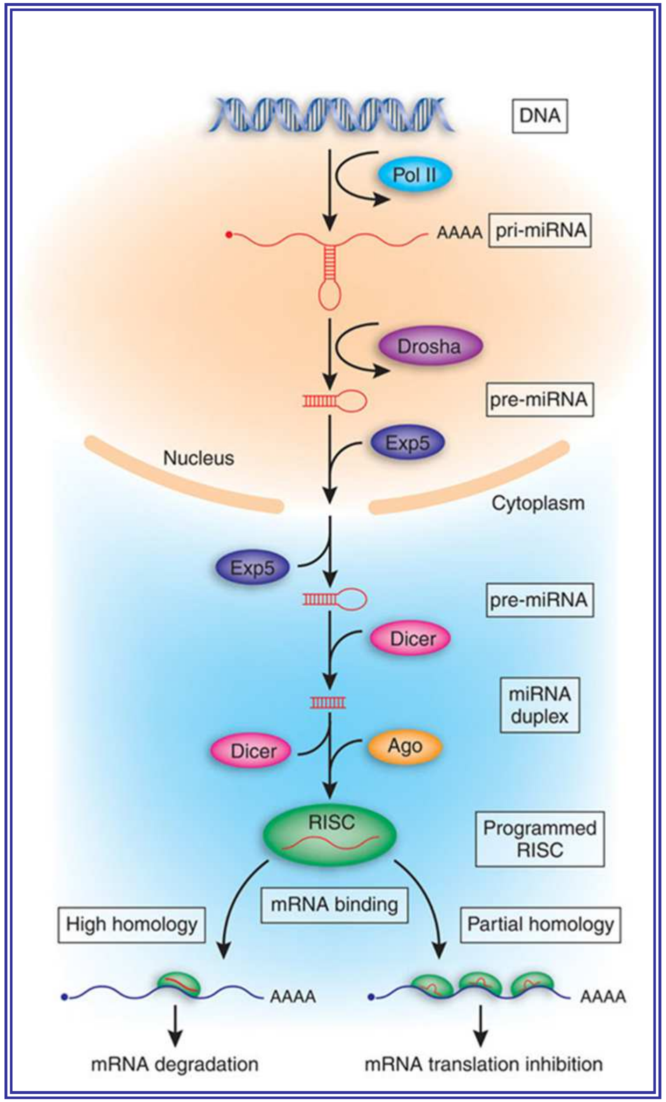
>4000 miRNA individuati (piante, virus, animali)

Circa 30% del genoma umano si pensa sia regolato con meccanismo miRNA dipendente

Meccanismo di regolazione genica risalente a oltre 425 milioni di anni fa) (*Zhang et al 2006*)

Si legano al 3'UTR (ma anche a regioni codificanti) del gene bersaglio (*Brodersen et al.2009*)

Ogni miRNA ha centinaia di geni target (altamente conservati e/o non conservati)



miRNA and disease

- ✓ MicroRNAs in Rheumatoid Arthritis: Potential Role in Diagnosis and Therapy.
BioDrugs. 2012
- ✓ microRNA involvement in human cancer.
Carcinogenesis. 2012
- ✓ microRNAs in metabolism and metabolic disorders.
Nat Rev Mol Cell Biol. 2012
- ✓ Epigenetics and microRNAs in Preeclampsia.
Clin Exp Hypertens. 2012
- ✓ MicroRNAs in diagnosis and prognosis in lung cancer].
Rev Invest Clin. 2011
- ✓ Role of microRNAs in Gynecological Pathology.
Curr Med Chem. 2012
- ✓ The Role of microRNAs in Cardiovascular Disease.
Curr Med Chem. 2012
- ✓ MicroRNAs in Human Diseases: From Lung, Liver and Kidney Diseases to Infectious Disease, Sickle Cell Disease and Endometrium Disease.
Immune Netw. 2011

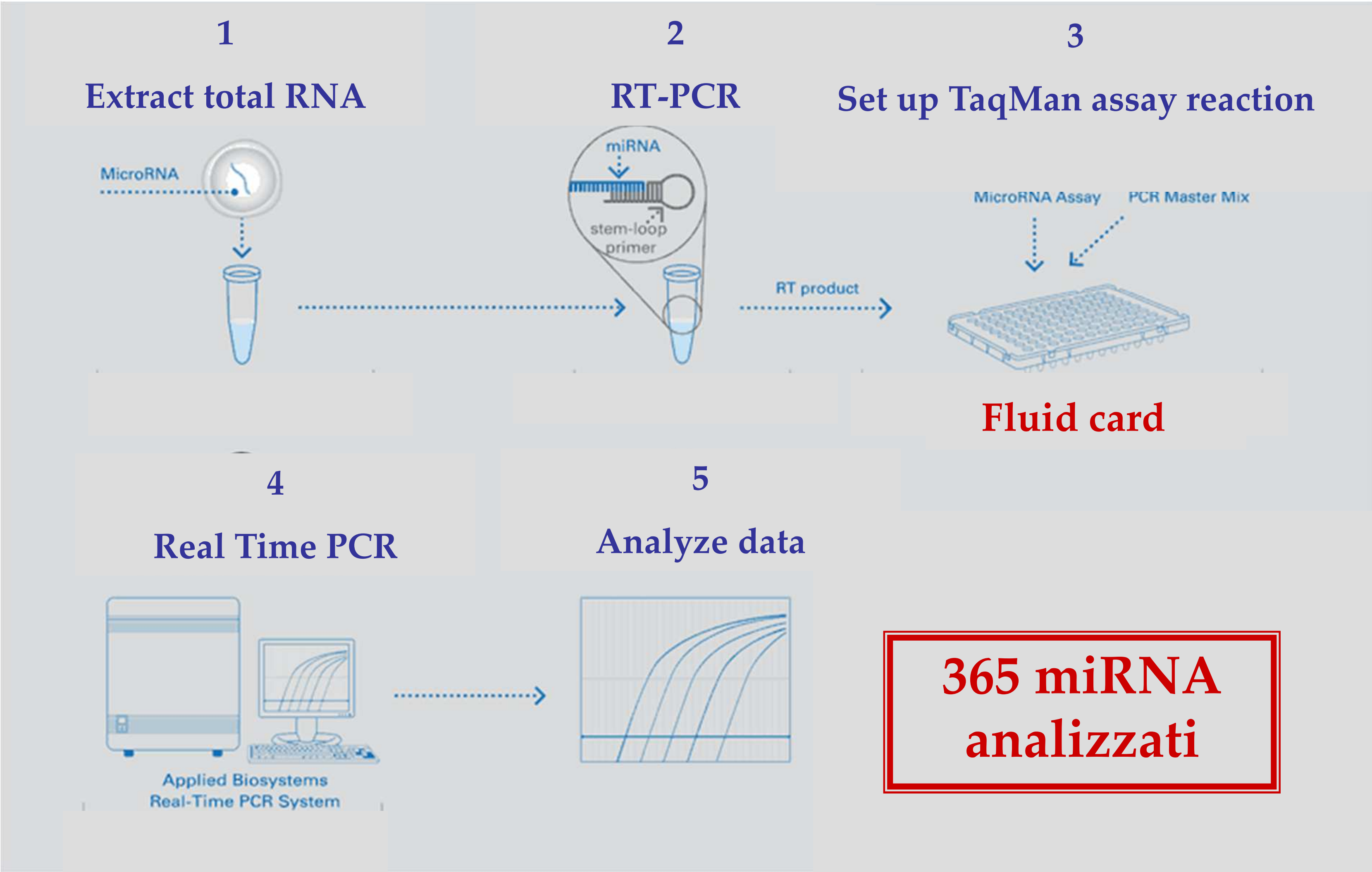
General characteristics of studied celiac patients and control children

Characteristics[a]	Subjects		
	Active CD (n=20)	GFD[b] (n=9)	CTRL (n=11)
Sex Female (%)	55	55	45
Age (years)	4.3 ±1.3	7.6 ± 2.5	6.1 ± 1.0
Clinical presentation:			
- Gastrointestinal symptoms (%)	80	22	82
- Villous atrophy % (Marsh stage)[c]	TIIIB 15 TIIIC 85	T0 56 TI 44	T0 64 TI 36
- Positive tTG or EMA (IgA)[d]	19	3	0
Familiarity for:			
- CD (%)	20	22	0
- Other autoimmune diseases (%)	5	11	0

a Data are expressed as percentage (%) or as mean ± standard error of the mean (SEM)
b At gluten free diet from at least 2 years
c According to Marsh classification
d Only 1 subject was negative for these antibodies but was positive for both AGA IgG/IgA antibodies

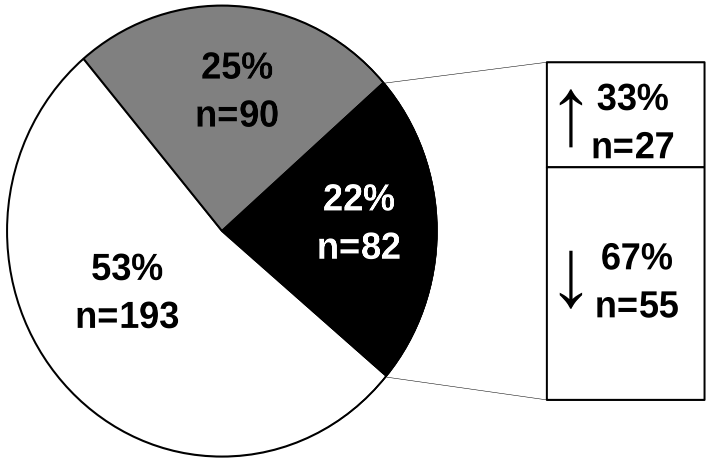
Capuano M, Sacchetti L et al., PLoS One, 2011

TaqMan microRNA assay workflow



MiRNA expression pattern in the small intestine of CD patients

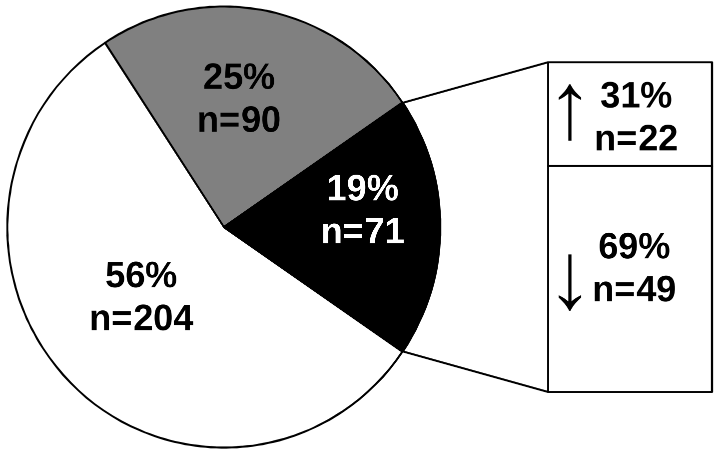
Active CD patients



upregulated ↑: RQ > 2

downregulated ↓: RQ < 0.5

GFD CD patients



Data are expressed as percentage of miRNAs tested (n=365)
White areas: miRNAs whose expression levels were similar in the two CD groups and controls
Gray areas: miRNAs not expressed
Black areas: miRNAs whose expression levels differed between CD patients and controls

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List of miRNAs differently expressed in active CD patients and controls

Up regulated miRNAs

miR-182
miR-196a
miR-330
miR-449a
miR-492
miR-500
miR-503
miR-504
miR-644
miR-18a
miR-187
miR-196b
miR-213
miR-223
miR-337
miR-383
miR-424
miR-425
miR-432
miR-554
miR-565
miR-575
miR-589
miR-597
miR-630
miR-639
miR-656

Down regulated miRNAs

miR-105
miR-124a
miR-135a
miR-189
miR-202
miR-219
miR-299-5p
miR-323
miR-379
miR-380-5p
miR-409-5p
miR-412
miR-512-3p
miR-566
miR-576
miR-600
miR-614
miR-616
miR-618
miR-631
miR-659
miR-30a-3p
miR-30b
miR-30c
miR-30e-3p
miR-31
miR-34b

miR-96
miR-133a
miR-135b
miR-139
miR-145
miR-185
miR-192
miR-194
miR-198
miR-199a
miR-204
miR-217
miR-224
miR-369-3p
miR-422b
miR-485-3p
miR-509
miR-515-3p
miR-520h
miR-542-5p
miR-548d
miR-556
miR-579
miR-606
miR-608
miR-624
miR-651
miR-653

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List of miRNAs differently expressed in GFD patients and controls

Up regulated miRNAs	*Down regulated miRNAs*	
miR-182	miR-105	miR-99a
miR-196a	miR-124a	miR-99b
miR-330	miR-135a	miR-125a
miR-449a	miR-189	miR-125b
miR-492	miR-202	miR-130a
miR-500	miR-219	miR-132
miR-503	miR-299-5p	miR-133b
miR-504	miR-323	miR-143
miR-644	miR-379	miR-148a
miR-184	miR-380-5p	miR-153
miR-190	miR-409-5p	miR-193a
miR-193b	miR-412	miR-203
miR-205	miR-512-3p	miR-376a
miR-338	miR-566	miR-383
miR-422a	miR-576	miR-410
miR-422b	miR-600	miR-411
miR-489	miR-614	miR-432
miR-490	miR-616	miR-433
miR-518d	miR-618	miR-518b
miR-524	miR-631	miR-589
miR-591	miR-659	miR-630
miR-627	miR-27b	miR-622
	miR-17-3p	miR-639
		miR-650

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List of miRNAs differently expressed in CD patients and controls but with similar expression levels both in active CD and GFD children

miRNA	Active CD	GFD
miR-449a	55.18 ± 16.45	15.43 ± 7.69
miR-492	48.88 ± 14.56	26.86 ± 9.00
miR-644	47.80 ± 8.80	37.53 ± 18.85
miR-503	19.84 ± 2.36	20.55 ± 8.07
miR-196a	11.06 ± 2.84	8.45 ± 1.01
miR-504	5.54 ± 0.83	8.02 ± 2.86
miR-500	5.49 ± 0.70	7.88 ± 1.56
miR-330	3.84 ± 0.45	2.48 ± 0.11
miR-182	2.95 ± 0.42	2.75 ± 0.13
miR-105	0.37 ± 0.03	0.25 ± 0.03
miR-124a	0.20 ± 0.02	0.21 ± 0.05
miR-135a	0.21 ± 0.05	0.38 ± 0.05
miR-189	0.15 ± 0.05	0.21 ± 0.06
miR-202	0.12 ± 0.06	0.17 ± 0.08
miR-219	0.10 ± 0.01	0.27 ± 0.08
miR-299-5p	0.11 ± 0.006	0.15 ± 0.05
miR-323	0.11 ± 0.01	0.23 ± 0.08
miR-379	0.30 ± 0.05	0.23 ± 0.1
miR-380-5p	0.25 ± 0.03	0.28 ± 0.04
miR-409-5p	0.35 ± 0.04	0.31 ± 0.05
miR-412	0.13 ± 0.03	0.18 ± 0.01
miR-512-3p	0.27 ± 0.03	0.26 ± 0.04
miR-566	0.29 ± 0.02	0.23 ± 0.03
miR-576	0.15 ± 0.04	0.4 ± 0.1
miR-600	0.19 ± 0.02	0.22 ± 0.06
miR-614	0.26 ± 0.02	0.21 ± 0.02
miR-616	0.17 ± 0.04	0.11 ± 0.03
miR-618	0.18 ± 0.03	0.32 ± 0.07
miR-631	0.34 ± 0.03	0.27 ± 0.04
miR-659	0.33 ± 0.03	0.30 ± 0.05

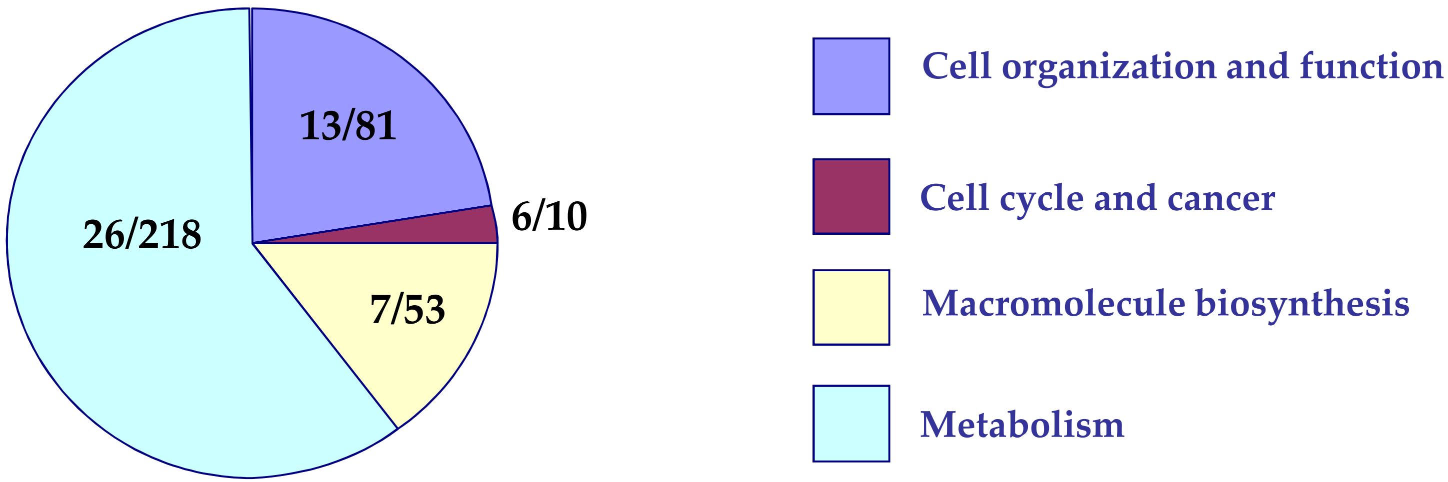
Data are reported as RQ levels (mean ± SEM); RQ of controls = 1

qRT-PCR for miR449a

ACTIVE CD (RQ±SEM)	2.8±0.9

Capuano M, Sacchetti L et al., PLoS One, 2011

Functional pathways predicted for miR-449a



miR-449a putative target genes with most favorable context score, selected by bioinformatics, were sorted into pathways using GOTM and then combined into functional groups. In each functional group are reported the genes belonging to NOTCH pathway/total gene number

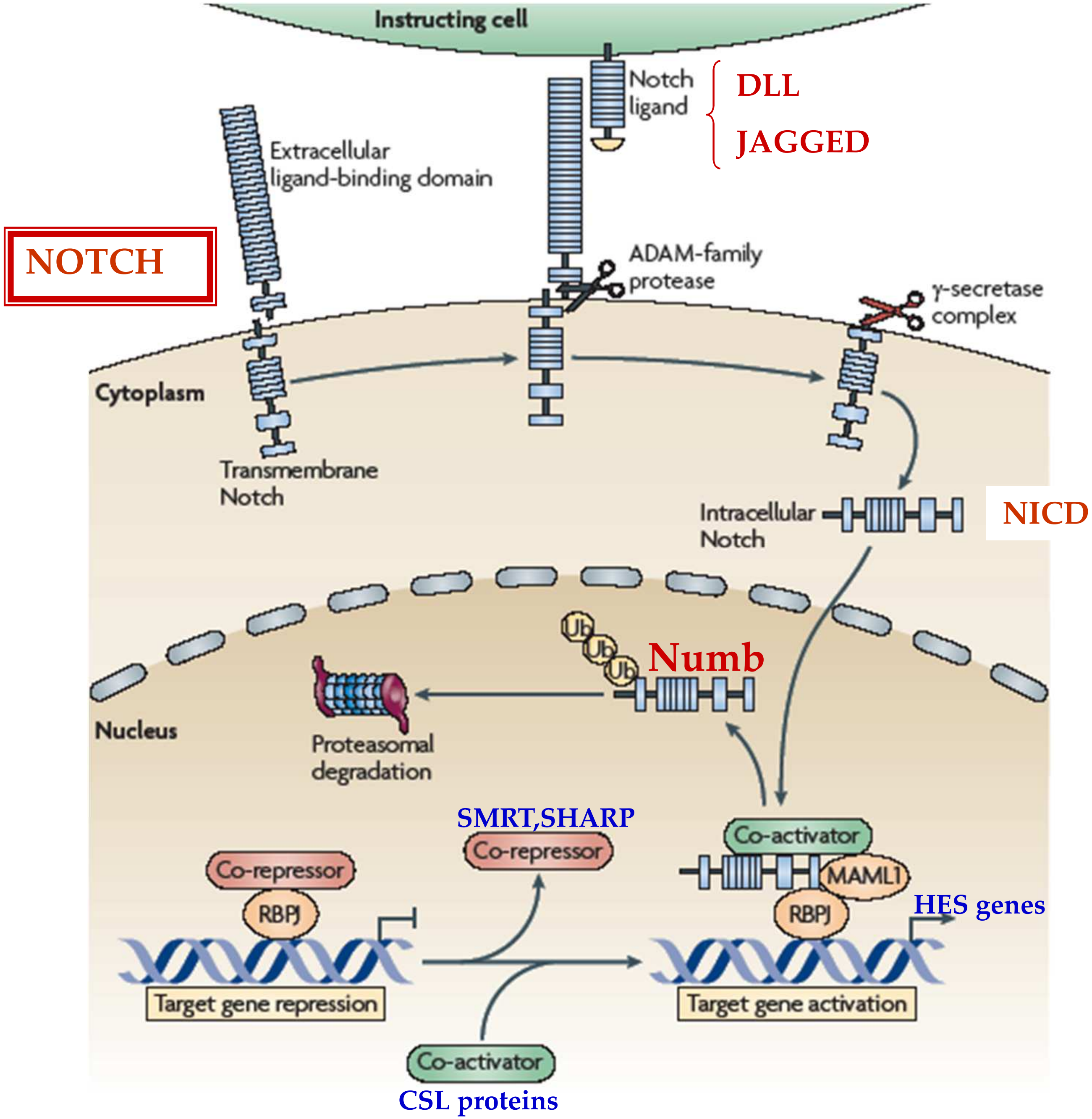
Geni target del miR449a

- NOTCH 1
- NUMBL
- DLL1
- KLF4

Signaling di Notch

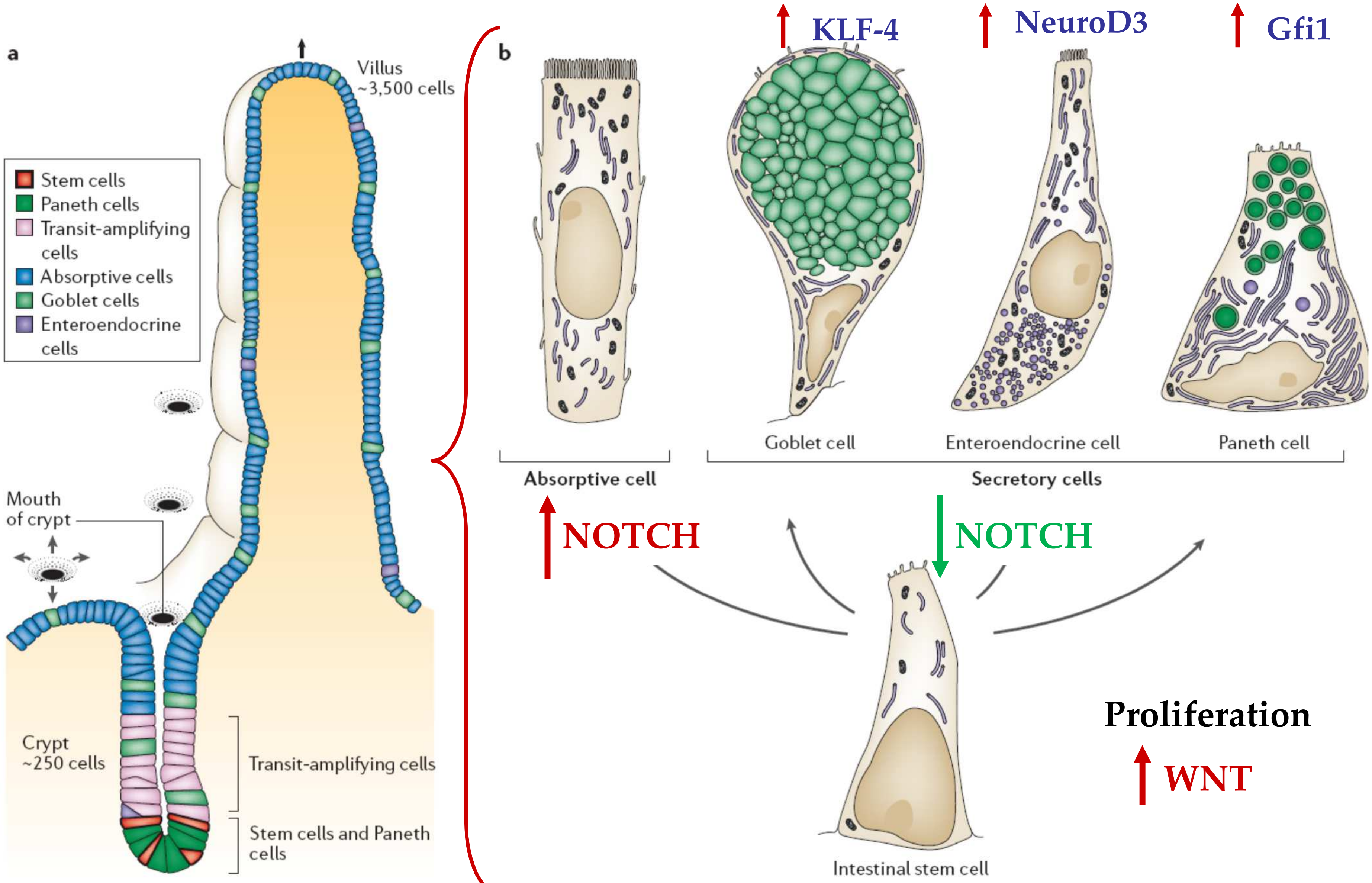
Capuano M, Sacchetti L et al., PLoS One, 2011

The canonical Notch signalling pathway



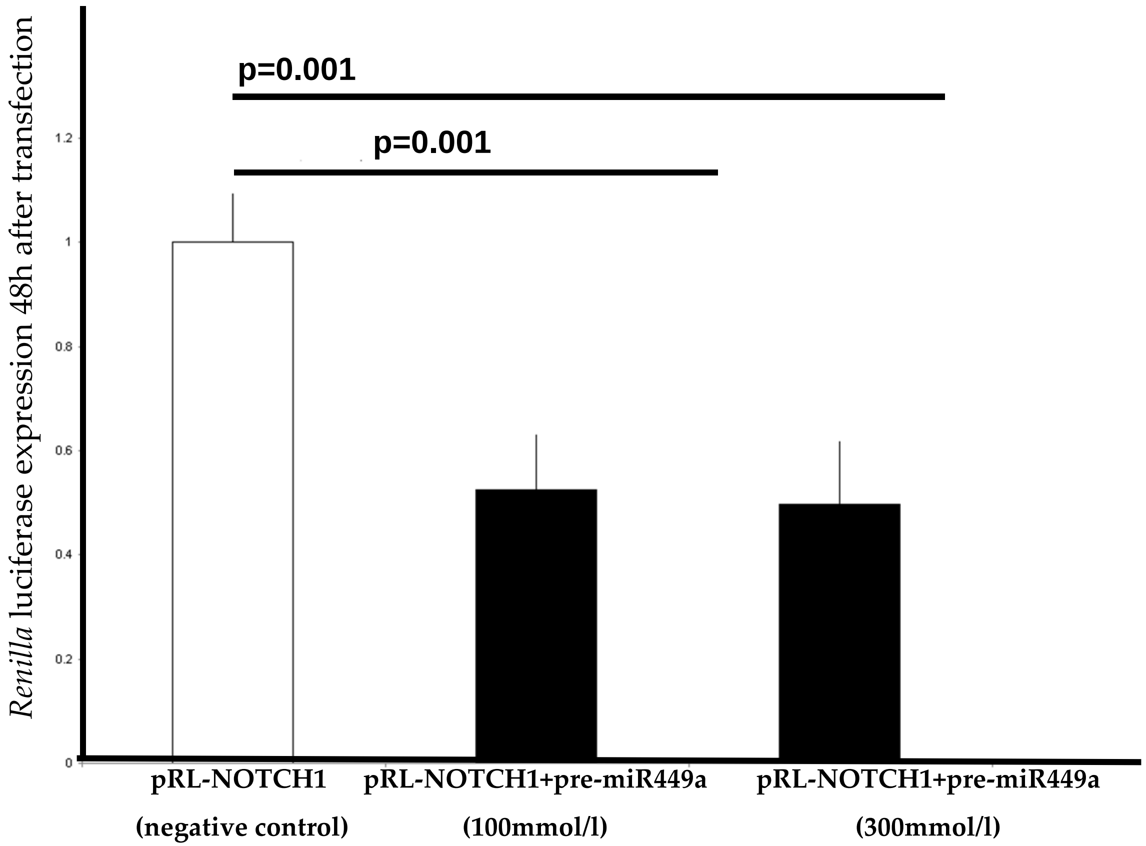
Nature, 2009

Distribution of epithelial cell types in the mammalian small intestine



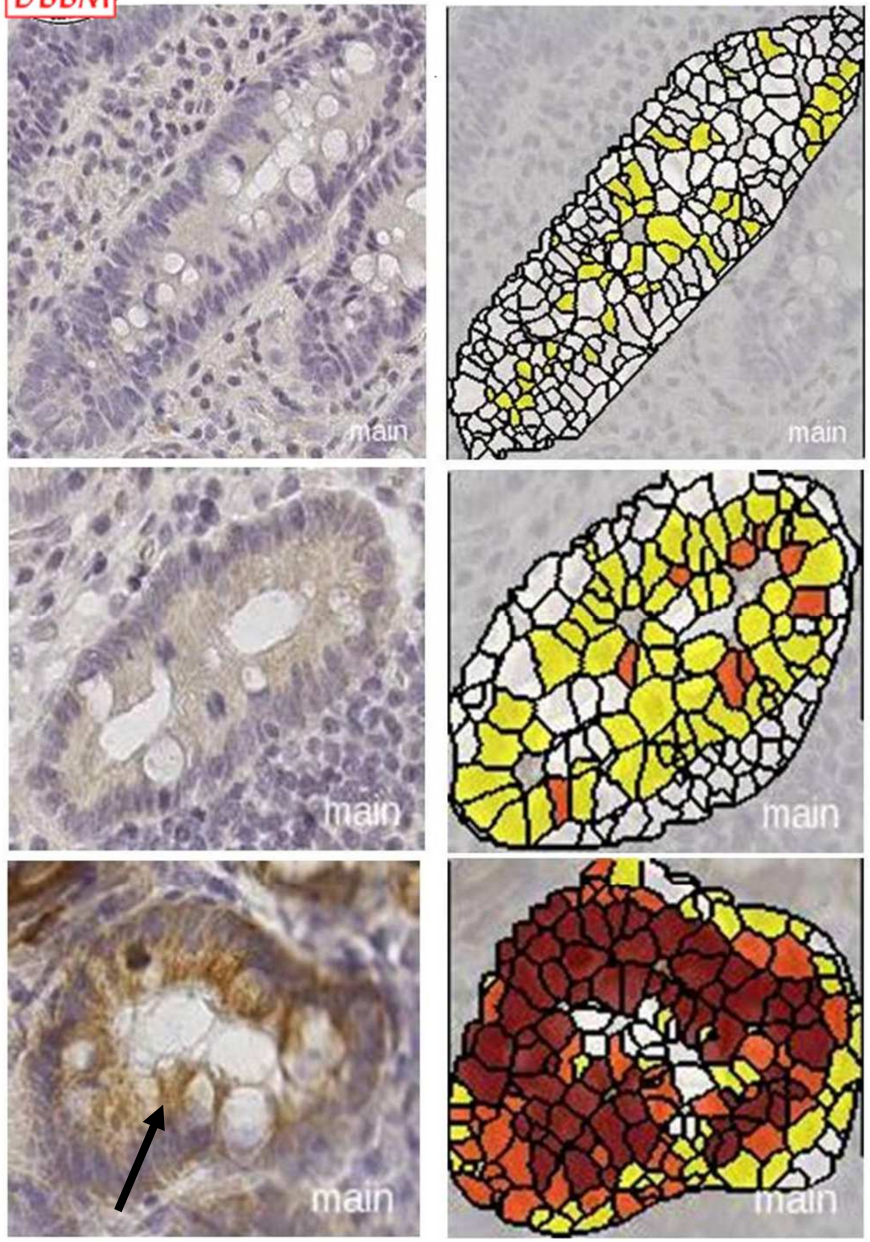
Nature Review Genetics, 2006

Validation of miR-449a and 3′-UTR NOTCH1 mRNA interaction



Capuano M, Sacchetti L et al., PLoS One, 2011

Immunohistochemistry of NOTCH1 in small intestine



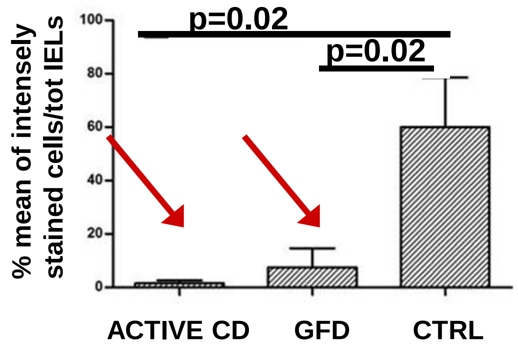
ACTIVE CD

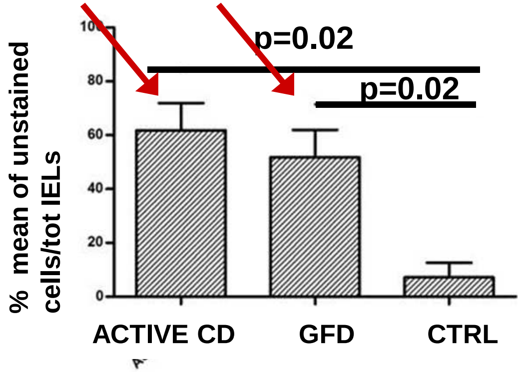
GFD

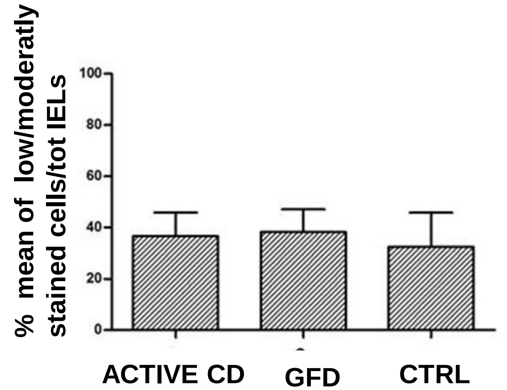
CTRL

NOTCH1 staining

Original magnification 40X

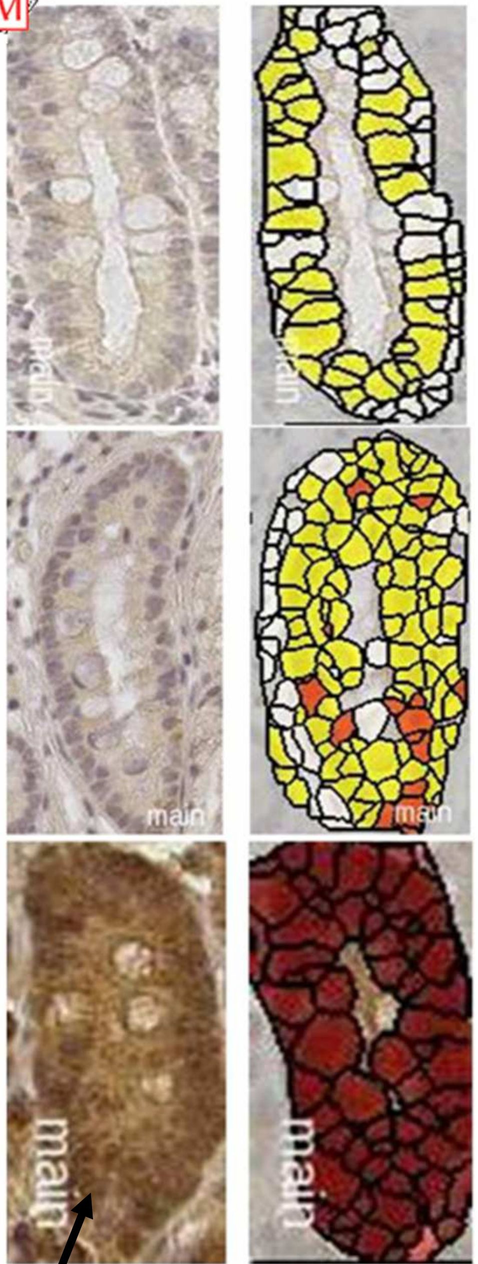






Capuano M, Sacchetti L et al., PLoS One, 2011

Immunohistochemistry of HES1 in small intestine



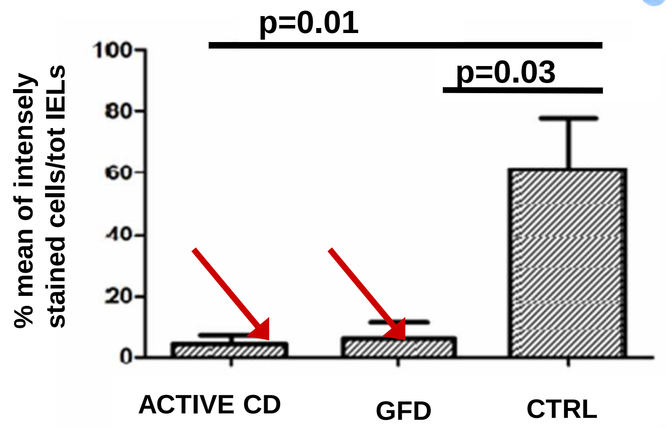
ACTIVE CD

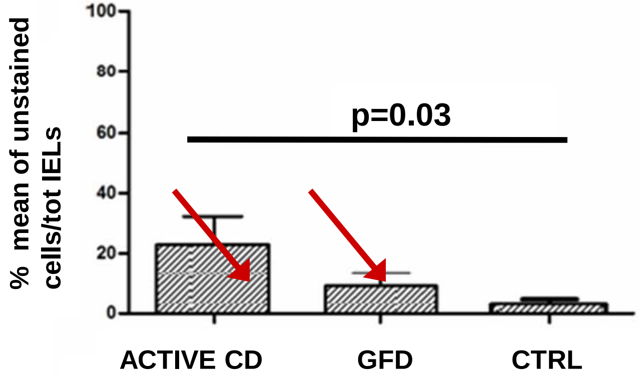
GFD

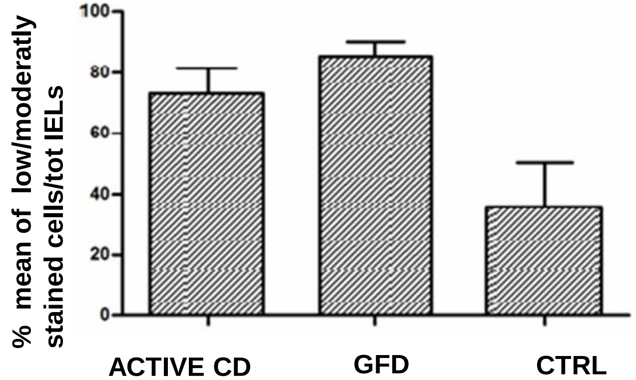
CTRL

HES1 staining

Original magnification 40X







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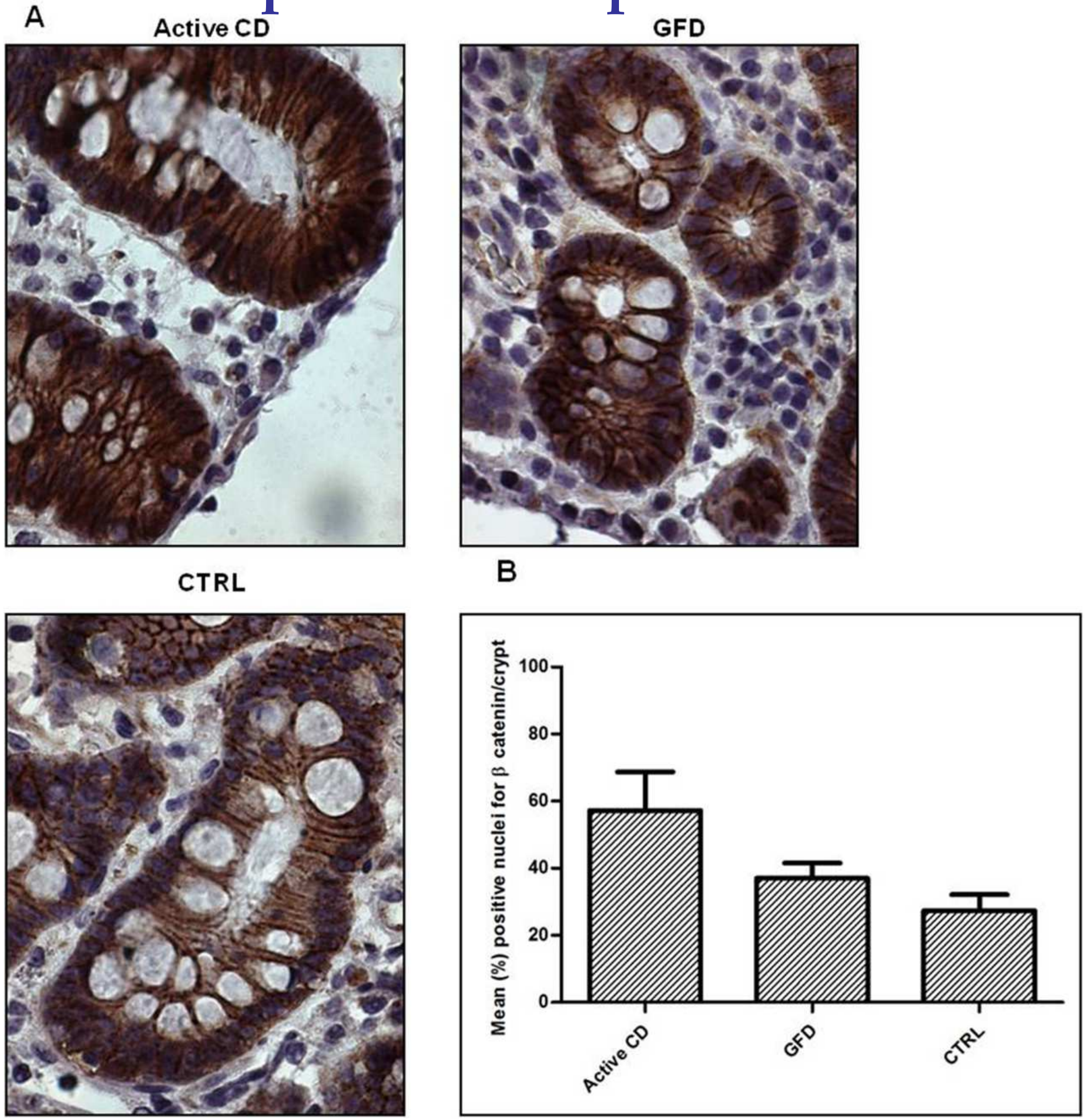
Increased expression of β-catenin in small intestine from CD patients compared with controls



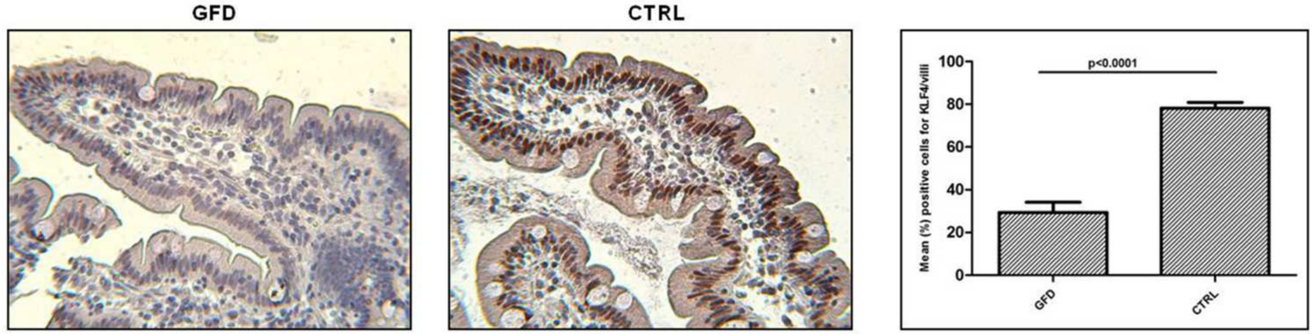
Immunostaining with beta-catenin in small intestinal crypts from active CD, GFD and controls. We counted the beta-catenin labeled nuclei. Similar counts of beta-catenin labelled nuclei were detected in the crypts of the small intestine in all groups. However, higher even if not statistical significant mean percentage counts (beta-catenin positive nuclei/crypt) were obtained in active CD and GFD than in controls, respectively 57.0±11.5 and 37.0±4.6 vs 27.0±4.6 (Original magnification 63×). (CTRL: controls; GFD: gluten free diet; CD: celiac disease).

Capuano M, Sacchetti L et al., PLoS One, 2011

Decreased KLF4 and increased Ki67 expression in small intestine from CD patients compared with controls

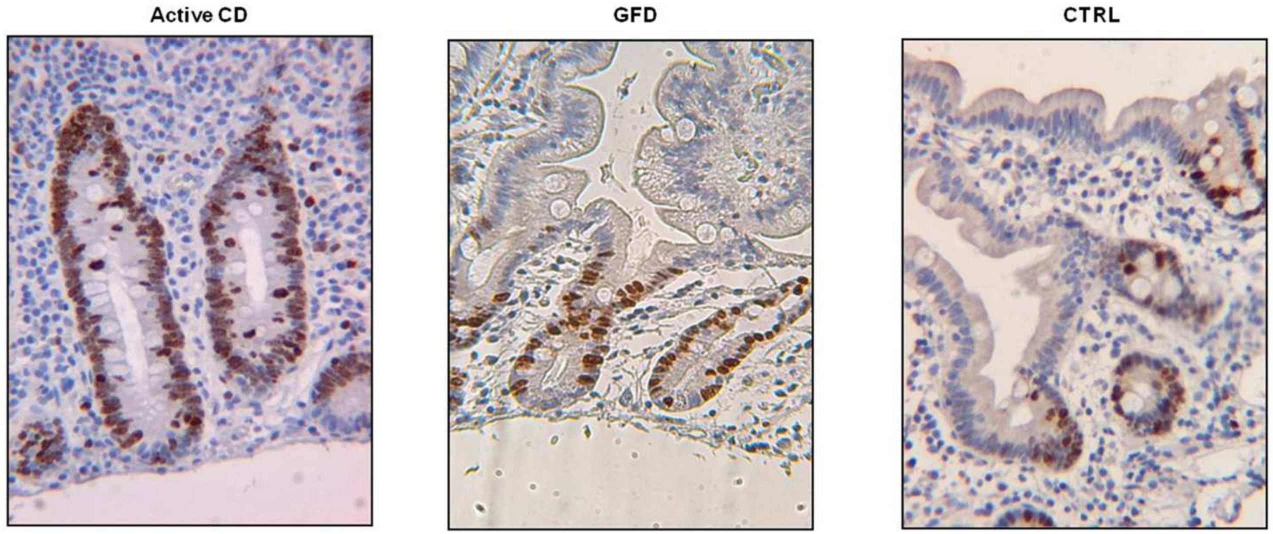
KLF4 staining of small intestinal villi in GFD patients and Controls

A



Ki67 staining in small intestinal crypts of active CD, GFD patients than in controls

B

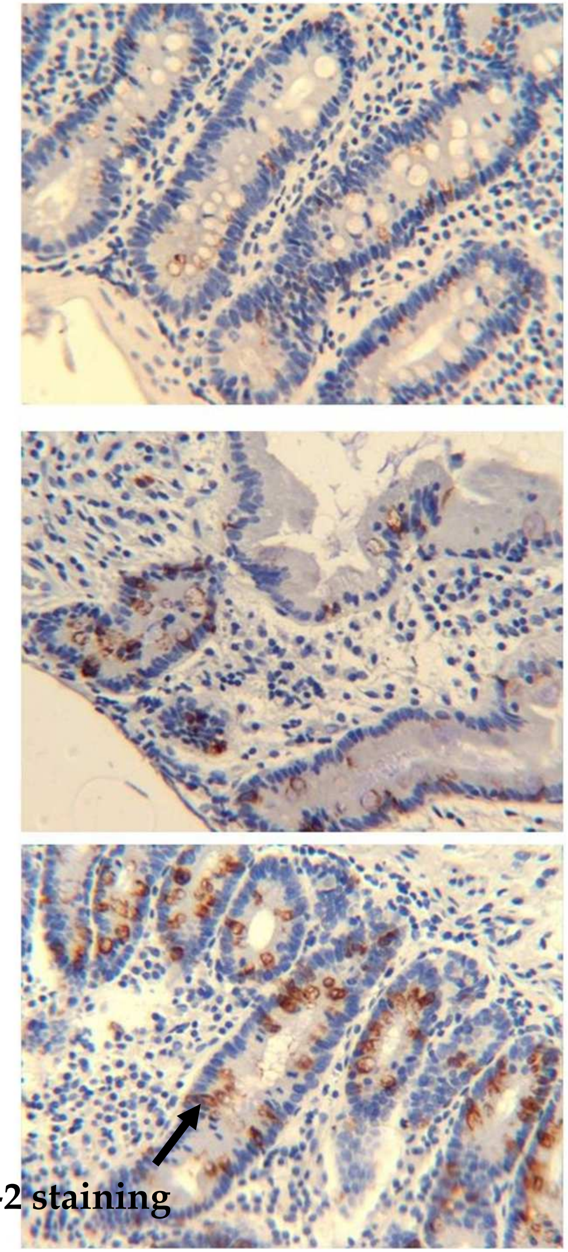


A. KLF4 staining of small intestinal villi in GFD patients and Controls (Original magnification 20×). A statistically significant reduced KLF4-positive cells/villi were counted in GFD patients than in controls, respectively 29.0±5.0 vs 79.0±3.0 (mean±SEM) ($p<0.0001$). **B.** Increased Ki67 signal is present in small intestinal crypts of active CD, GFD patients than in controls (Original magnification 20×). (CTRL: controls; GFD: gluten free diet; CD: celiac disease).

Capuano M, Sacchetti L et al., PLoS One, 2011

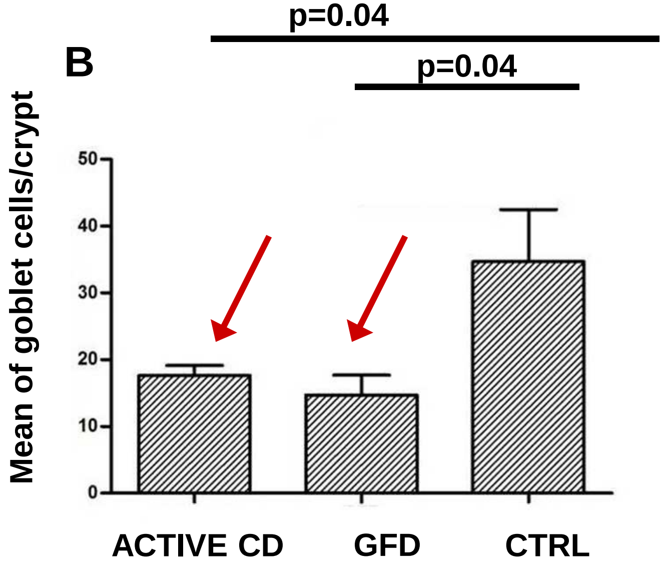
Immunohistochemistry of MUC-2 in small intestine

A



Original magnification 20X

B



	Mean number of cells± SD (in the crypts)
Active CD	18±1.6
GFD CD	15±3
Controls	35.0±7.7

	Mean number of cells± SD (in the villi)
GFD CD	7.0±1.8
Controls	20.0±4.9

Capuano M, Sacchetti L et al., PLoS One, 2011

Conclusions

- ✓ This study identified for the first time, both miRNAs expression pattern in human small intestine and miRNAs differently expressed in the jejunal biopsies of CD children vs controls
- ✓ Among the large set of expressed microRNAs, the 22% (n=82) and 19% (n=71) of miRNAs were differently expressed respectively in active CD and in GFD patients vs controls
- ✓ Among these miRNAs upregulated, we identified miR-449a as microRNA probably involved in CD pathogenesis in association with a reduced NOTCH1 pathway and with decrease of differentiation of intestinal cells towards the secretory goblet cell lineage

This finding could represent an epigenetic mechanism of gene regulation in the pathogenesis of CD

Prospettive future…

- ✓ Valutare miR449a su potenziali celiaci per confermarne l'alterazione genetica in assenza di malattia

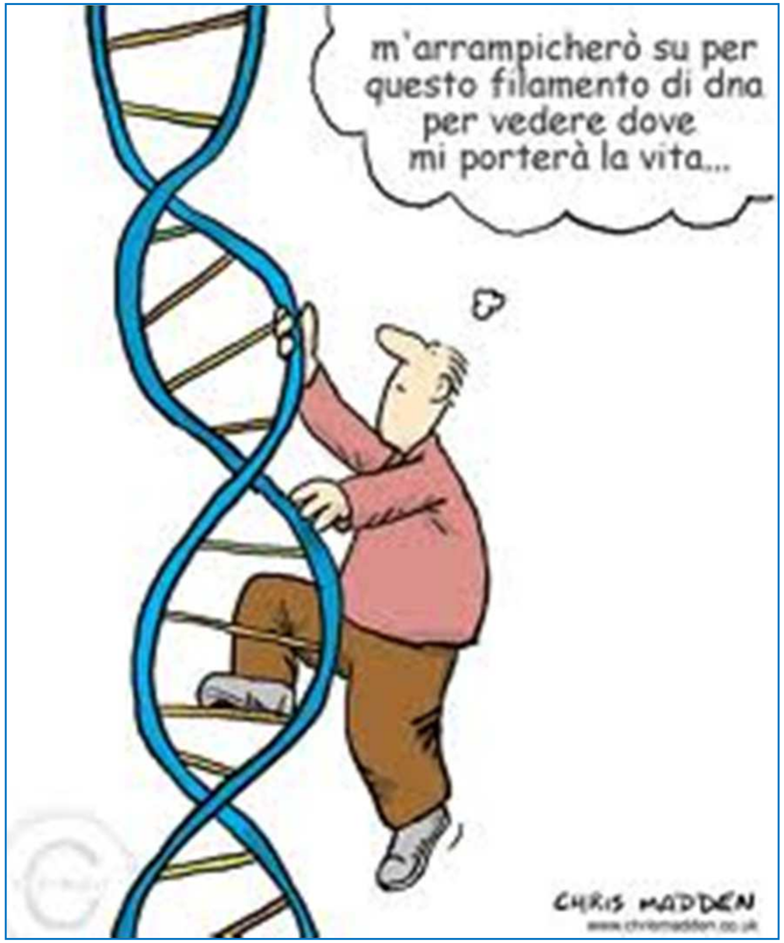
- ✓ Studio di altri miRNA differentemente espressi nei CA e GFD per ampliare lo studio ad altri pathways

- ✓ miR449a marcatore di diagnosi?

Studio dell'espressione del miR449a nel siero dei CA e GFD per eventuale uso in campo diagnostico

RESEARCH GROUP (CEINGE/DBBM)

- ✓ **Prof.ssa L. Sacchetti**
- ✓ **Prof.ssa N. Tinto**
- ✓ **Dr.ssa L. Iaffaldano**
- ✓ **Dr.ssa V. Capobianco**



In collaboration with Department of Pediatrics (Univ. degli Studi di Napoli Federico II)

- Prof. L. Greco

- Dr.ssa V. Izzo

- Dr.ssa F. Tucci

The luciferase assay confirms that miR-449a inhibits the expression of NOTCH1 and KLF4

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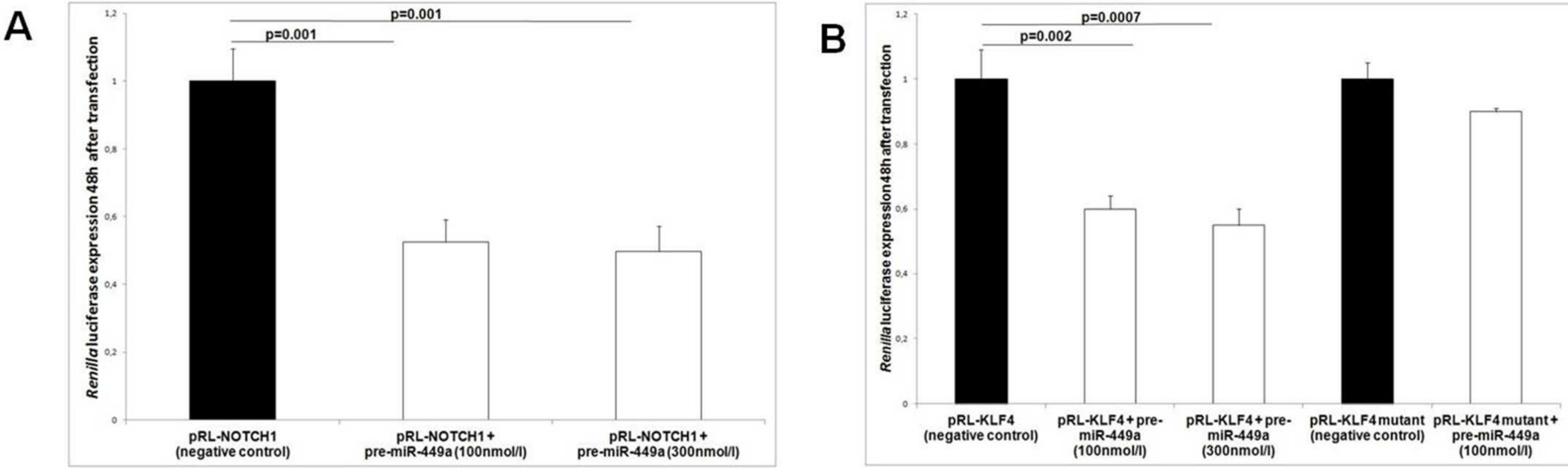

HEK293 cells co-transfected or with pRL-NOTCH1 vector (panel A) or with pRL-KLF4 vector (panel B), a pre-miR-449a concentration of 100 nmol/L was sufficient to significantly reduce (respectively, p=0.001 and p=0.002) *Renilla* luciferase activity versus control values.

***PLoS One*, 2011**

Epigenetica

Le modificazioni epigenetiche alterano l'espressione dei geni senza cambiarne la sequenza

MECCANISMO GENETICO	→	**Caratteri ereditari che risultano da cambi nella sequenza del DNA**
MECCANISMO EPIGENETICO	→	**Caratteri ereditari che non dipendono dalla sequenza del DNA**