

CB2 e la Malattia Celiaca: nuovo biomarker di malattia e futuro target terapeutico?



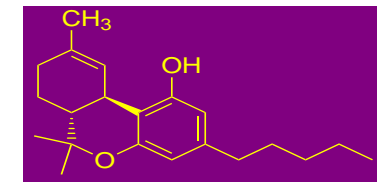
Francesca Rossi





Cannabis in Medicina

- **3000 a.c.:** Il più antico testo cinese sulle piante medicinali, il Pen T'sao ching, la raccomanda per disturbi femminili, reumatismo, gotta, malaria, stipsi e debolezza mentale
- **fin dal 2000 a.c.:** in India è usata per la cura della dissenteria, per facilitare la digestione e stimolare l'appetito e per rendere corpo e mente più pronti e vivaci
- **Il secolo d.c.:** Galeno la raccomanda per la flatulenza, il mal d'orecchio e tutti i tipi di dolore
- **1840 - 1900:** vengono pubblicati in Europa e negli USA più di 100 articoli sugli usi medici della cannabis come potente narcotico, analgesico, sonnifero, per il trattamento delle convulsioni, la corea, l'isteria, la depressione mentale, la pazzia, la dismenorrea e la menorragia (H.C.J Wood , William Osler, J.R Reynold).
- **1937 :** la cannabis viene inserita, negli USA, nella lista degli stupefacenti e il suo uso, anche a scopo terapeutico, diventa illegale. Di conseguenza, l'utilizzo della cannabis come farmaco crolla notevolmente anche in Europa, e le pubblicazioni scientifiche dedicate all'argomento scompaiono quasi del tutto nei decenni successivi.
- **1964:** scoperta del componente psicoattivo della cannabis , il Δ -9 tetraidrocannabinol (THC)



I Cannabinoidi naturali o Fitocannabinoidi

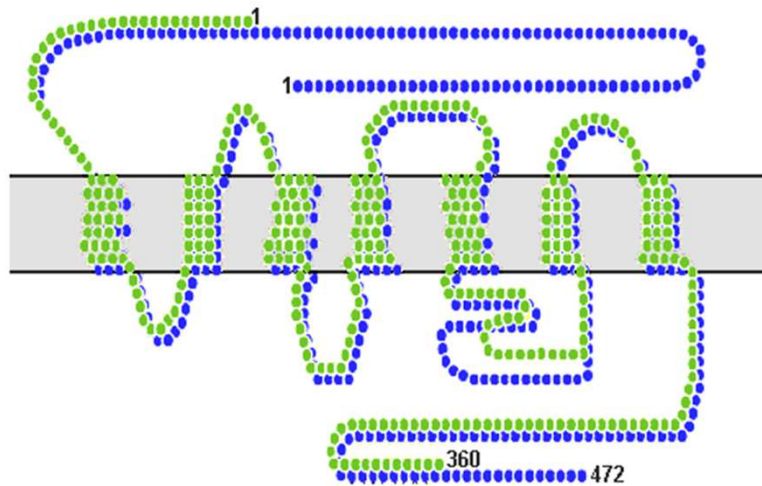


Cannabinoids Identified in Marijuana

Cannabinoid Group	Common Abbreviation	No. of Known Variants in Each Group
Δ^9 -Tetrahydrocannabinol	Δ^9 -THC	9
Δ^8 -Tetrahydrocannabinol	Δ^8 -THC	2
Cannabichromene	CBC	5
Cannabicyclol	CBL	3
Cannabidiol	CBD	7
Cannabielsoin	CBE	5
Cannabigerol	CBG	6
Cannabinidiol	CBND	2
Cannabinol	CBN	7
Cannabitriol	CBT	9
Miscellaneous types		11
Total		66

I Recettori per i Cannabinoidi (CB1 e CB2)

Struttura dei Recettori CB1● e CB2●



I Recettori **CB1** e **CB2** appartengono alla famiglia dei recettori con 7 domini transmembrana accoppiati a proteine G, responsabili dei meccanismi di trasduzione del segnale intracellulare

- Regolano numerosi pathways intracellulari
- Modulano la neurotrasmissione, regolando i canali ionici per K^+ e Ca^{2+}

Recettori Cannabinoidi (CB1, CB2)

Recettori CB1

Sono primariamente espressi nel SNC e nei neuroni periferici a livello sinaptico

Localizzazione	Effetti Biologici
Sistema Nervoso Centrale	Ridotta locomozione e temperatura corporea, catalessi, alterazione della memoria, sedazione, euforia
Sistema Nervoso Periferico	Analgesia, riduzione della tosse, inibizione del transito intestinale

Recettori CB2

Sono predominantemente espressi dalle cellule del sistema immune e da quelle ematiche

Localizzazione	Effetti Biologici
Sistema Immunitario	Azione Immunomodulatrice
Sistema Nervoso Periferico	Analgesia periferica e azione anti-infiammatoria

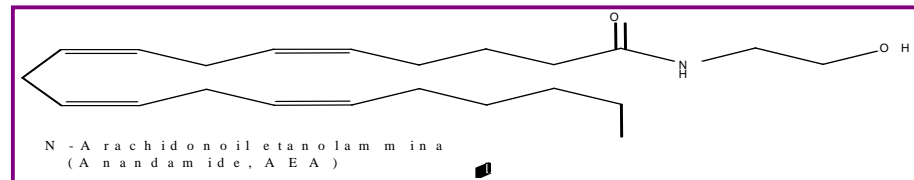
I Cannabinoidi Endogeni

O

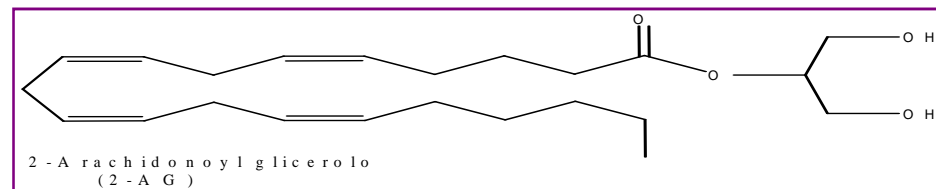
Endocannabinoidi



- **1992:** Devane e coll. isolano e caratterizzano dal cervello di maiale l' N-arachidonoyl etanolamina (N-ArE), a cui viene dato il nome di anandamide (dal sanscrito “ananda” cioè “portatore di illuminazione e tranquillità)



- **1994:** Mechoulam identifica un estere, il 2-Arachidonoyl glicerolo, capace di legare entrambi i recettori e avente anch'esso proprietà cannabimetiche



Gli Endocannabinoidi

2-Arachidonilglicerolo (2-AG)

È il più specifico ed abbondante agonista endogeno dei recettori CB1, CB2

N-Arachidoniletanolamina o Anandamide (AEA)

- È più selettiva verso i recettori CB1 rispetto ai CB2
- E' attiva anche sul recettore vanilloidi di tipo 1 (TRPV1: Transient Receptor Potential Vanilloid Type 1)

Il Sistema Endocannabinoide

La scoperta dei recettori per i cannabinoidi e dei loro ligandi lipidici endogeni (endocannabinoidi) ha fatto ipotizzare un enorme ed eccitante numero di applicazioni terapeutiche che potrebbero derivare dalla modulazione dell'attività del "Sistema Endocannabinoide":

- malattie del metabolismo (osso)
- dolore e infiammazione
- malattie del sistema nervoso centrale (schizofrenia, sclerosi multipla, ansietà, depressione)
- malattie cardiovascolari e respiratorie
- malattie oculari (glaucoma, degenerazioni retiniche, neuriti....)
- cancro
- malattie gastrointestinali ed epatiche
- malattie muscoloscheletriche e del sistema riproduttivo

Cannabinoid CB2 receptor in human inflammation diseases

Maresz K et al. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med.* 2007 Apr;13(4):492-7

Ashton JC, Glass M. The Cannabinoid CB2 Receptor as a Target for Inflammation-Dependent Neurodegeneration. *Curr Neuropharmacol.* 2007;5(2):73-80.

Iuvone T et al. Selective CB2 up-regulation in women affected by endometrial inflammation *J Cell Mol Med.* 2008 Apr;12(2):661-70.

Benito C et al. Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol.* 2008 Jan;153(2):277-85. Epub 2007 Oct 15.

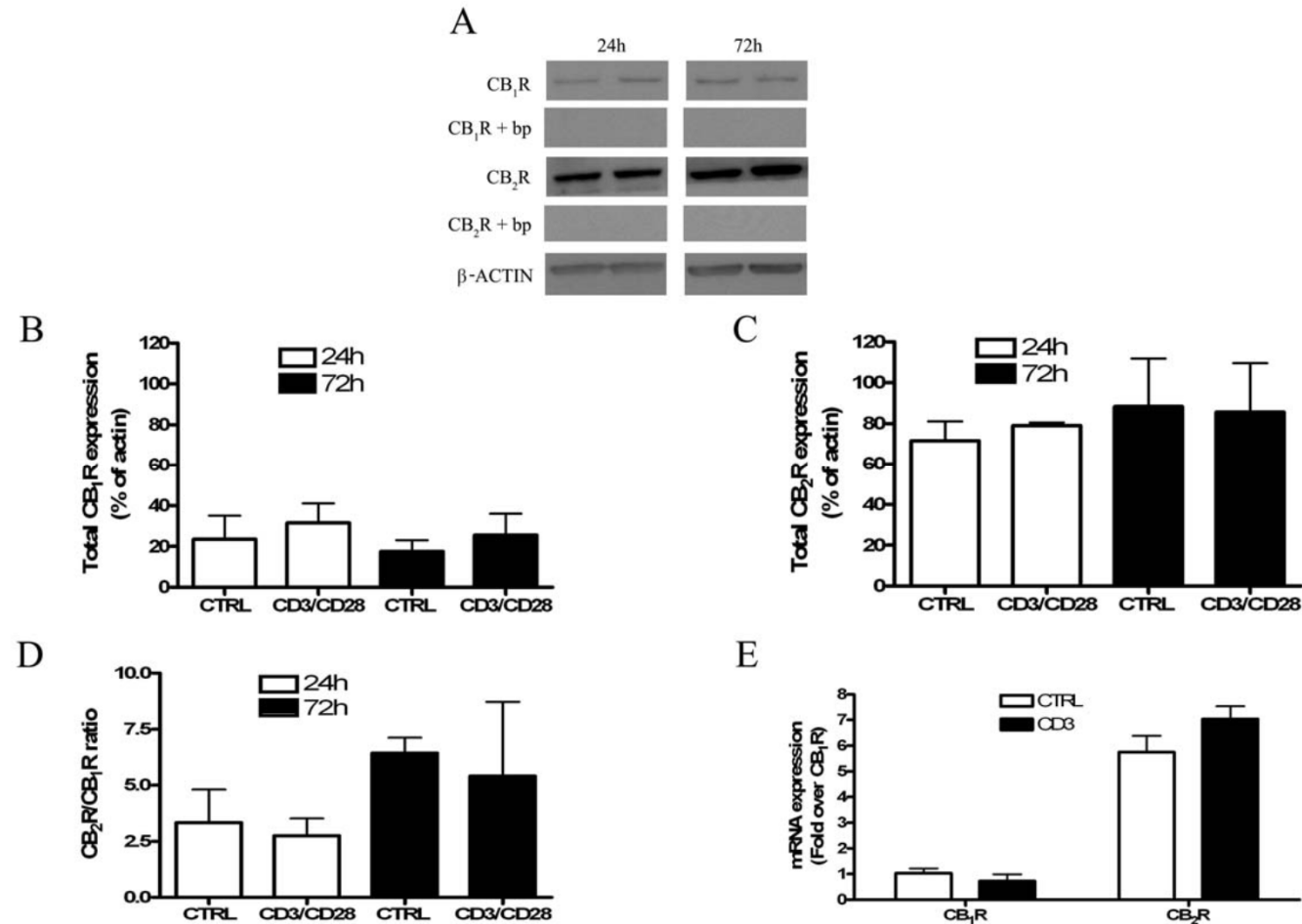
Muñoz-Luque J et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther.* 2008

Mimura T, Ueda Y, Watanabe Y, Sugiura T. The cannabinoid receptor-2 is involved in allergic inflammation. *Life Sci.* 2012 Apr 14

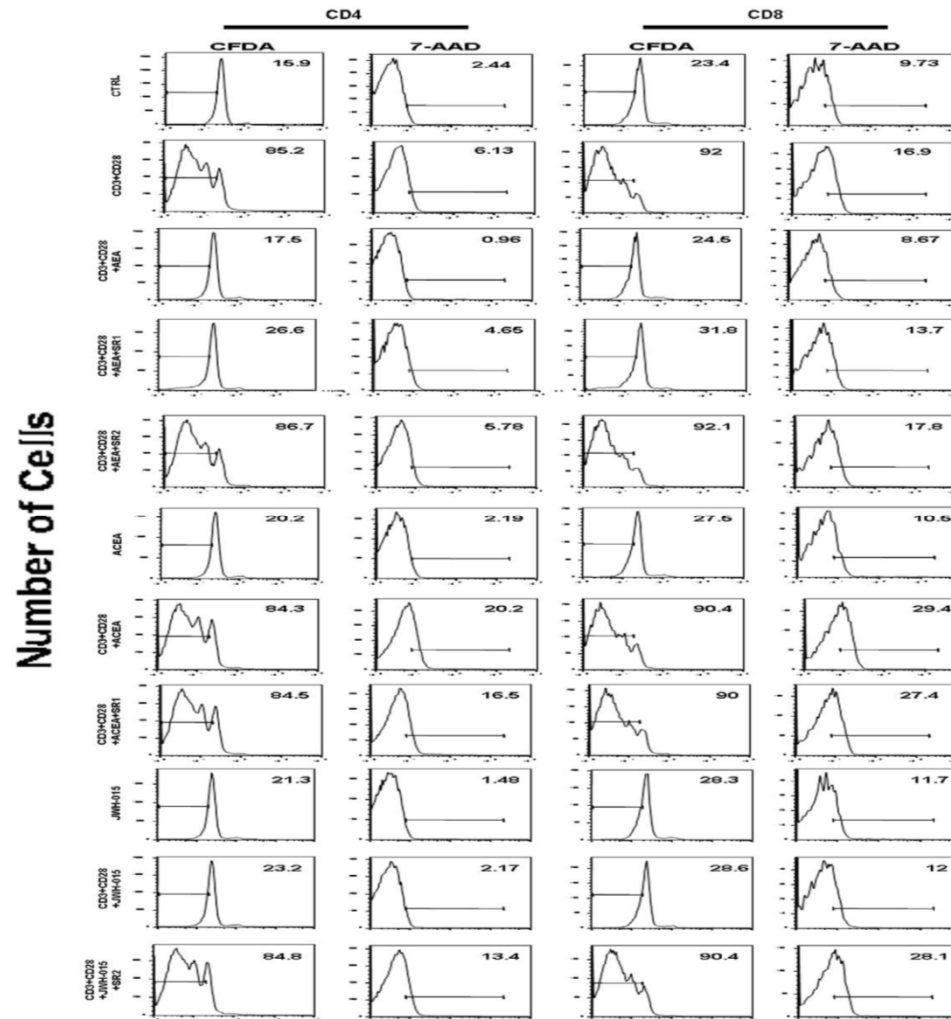
Louvet A et al. Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice. *Hepatology.* 2011 Oct;54(4):1217-26.

Kinsey SG et al. The CB2 cannabinoid receptor-selective agonist O-3223 reduces pain and inflammation without apparent cannabinoid behavioral effects. *Neuropharmacology.* 2011 Feb-Mar;60(2-3):244-51.

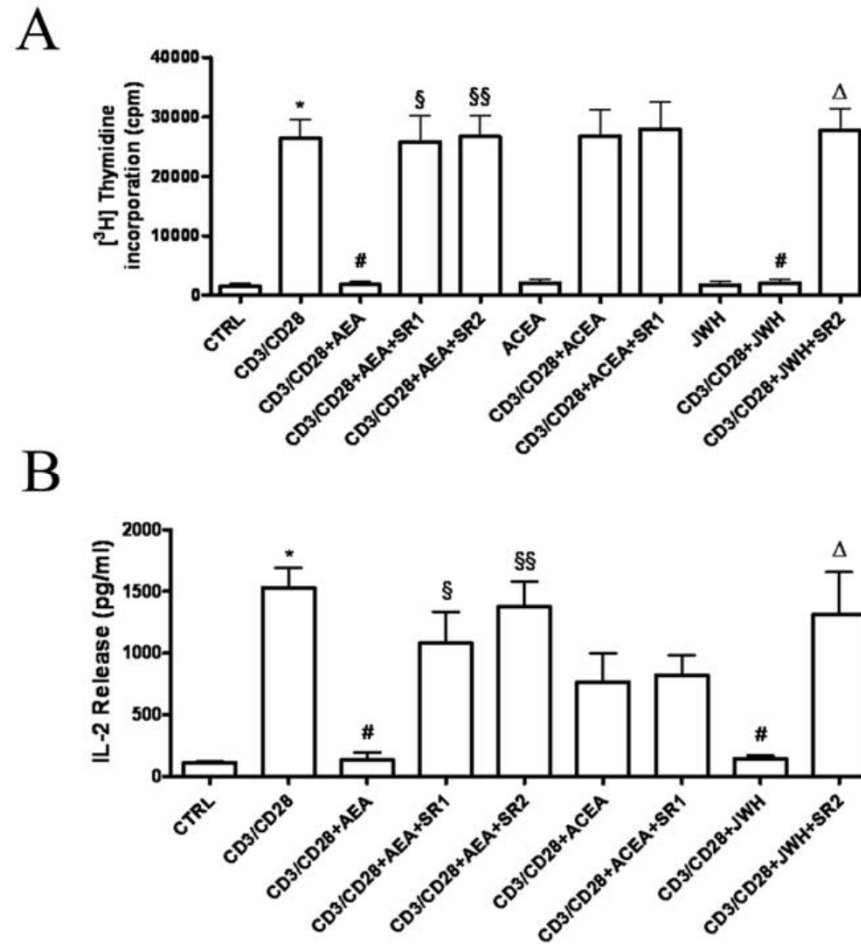
Analysis of CB1R and CB2R expression in resting and activated primary human T-lymphocytes



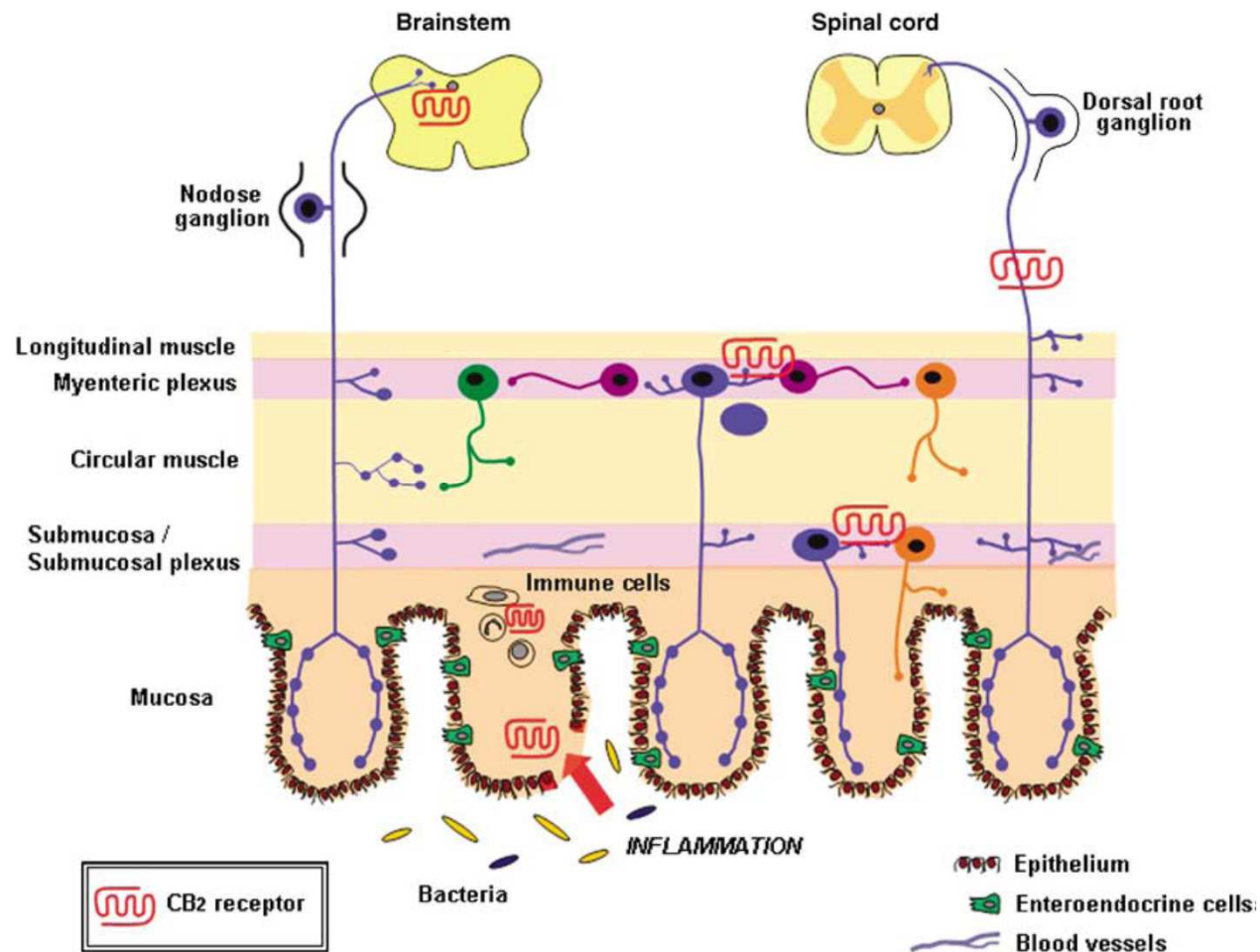
Effect of AEA on cell proliferation versus cell viability in anti-CD3/anti-CD28-stimulated T-lymphocytes



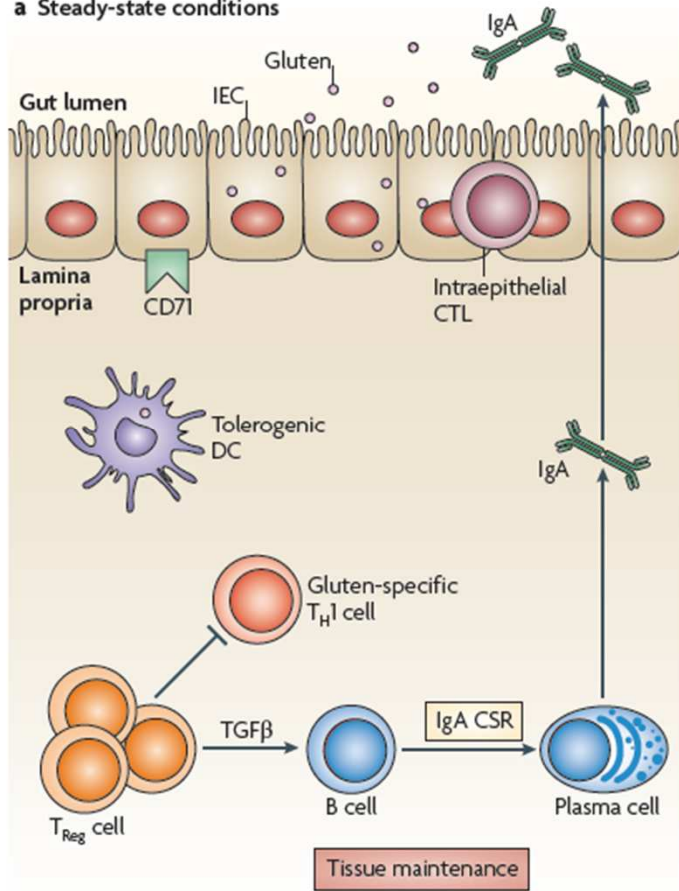
Effect of AEA on IL-2 dependent anti-CD3/anti-CD28-stimulated T-cell proliferation



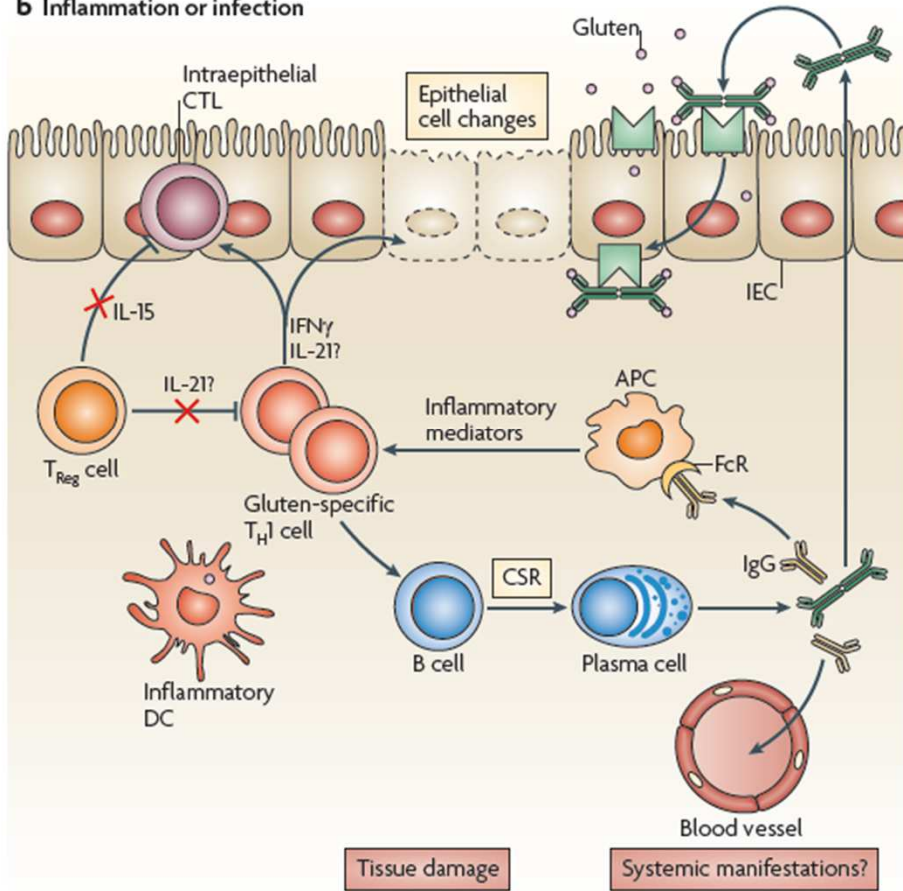
Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation



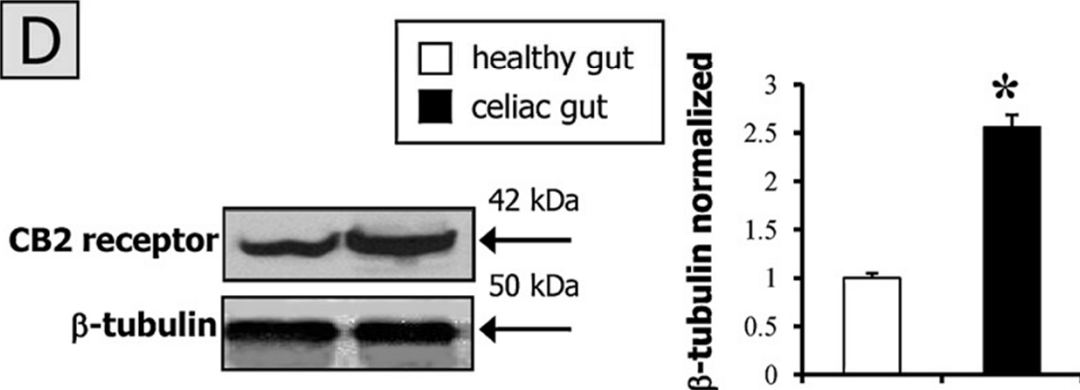
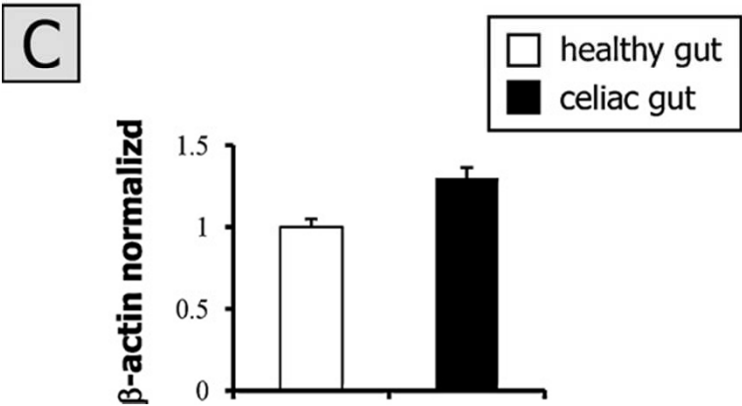
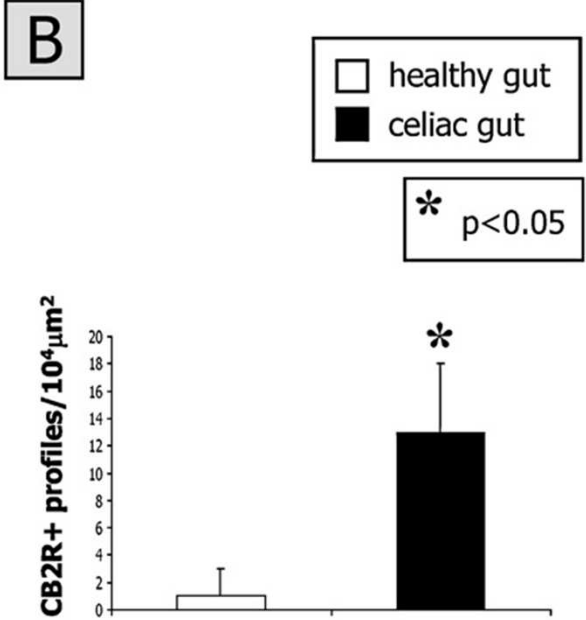
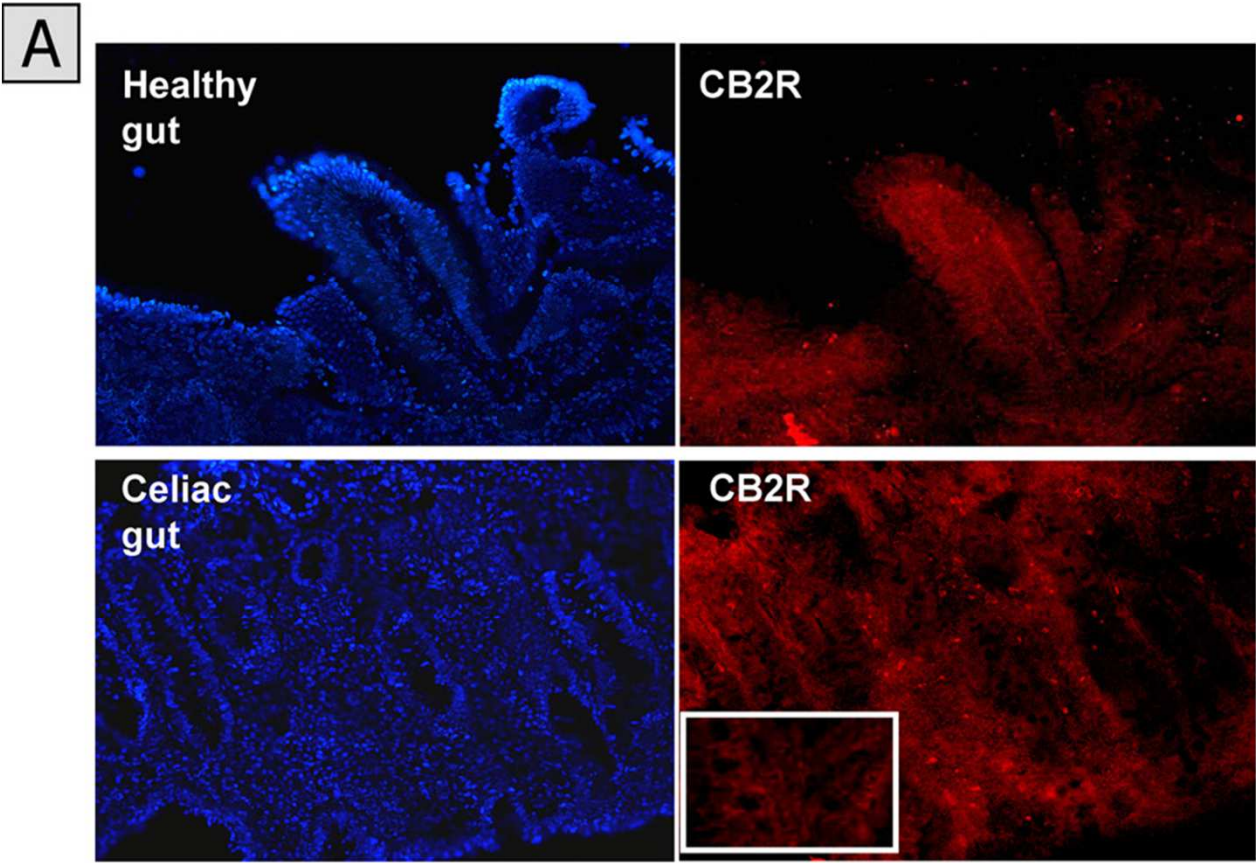
a Steady-state conditions



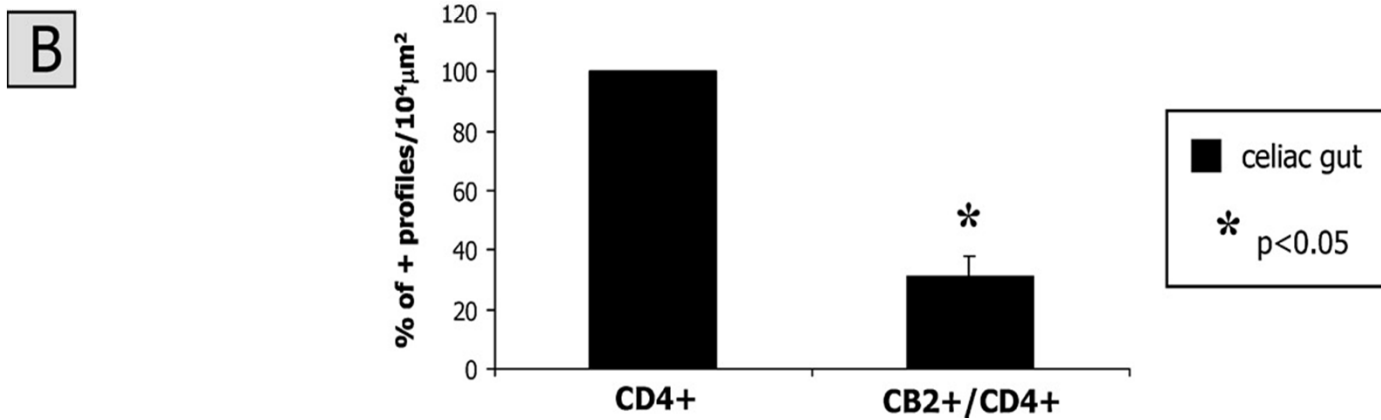
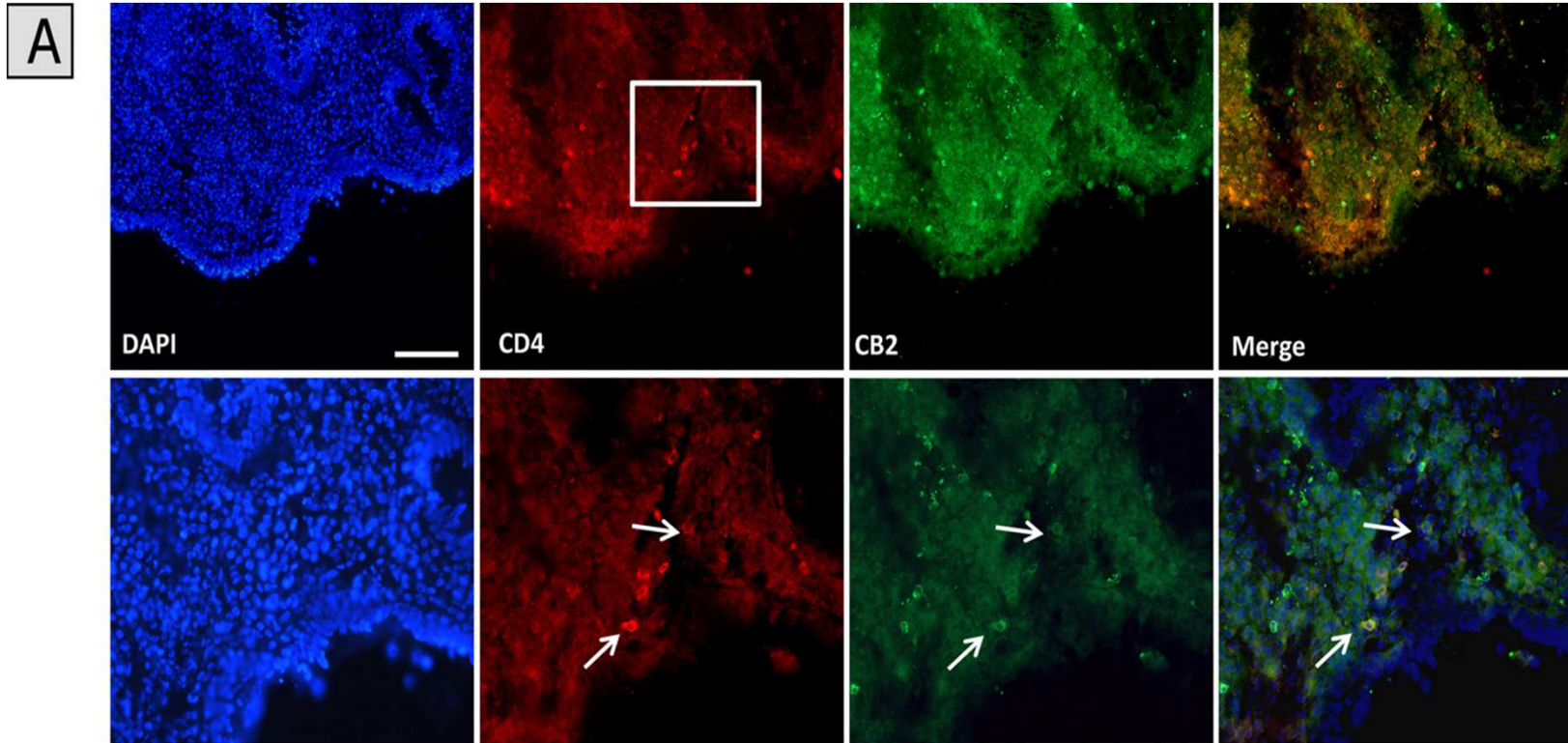
b Inflammation or infection



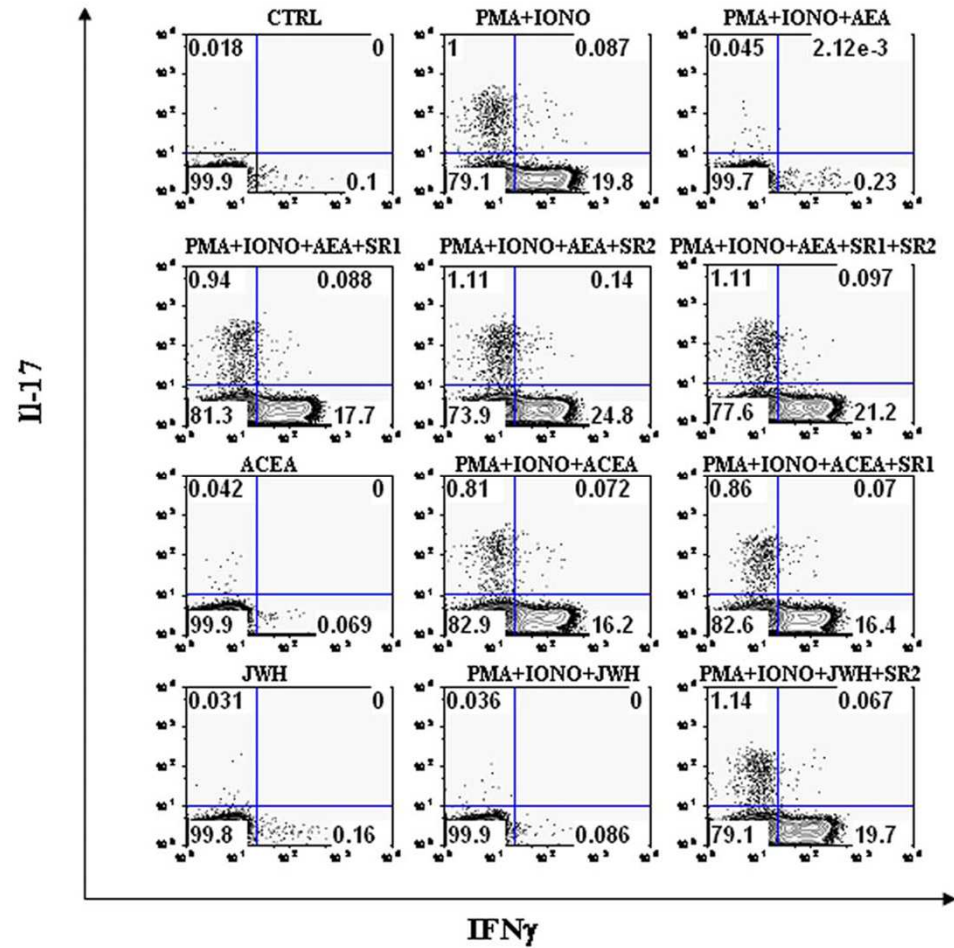
CB2 receptor over expression in the celiac derived bowel biopsies



CB2 positive staining merges with positive CD4 profiles in the celiac derived bowel biopsies



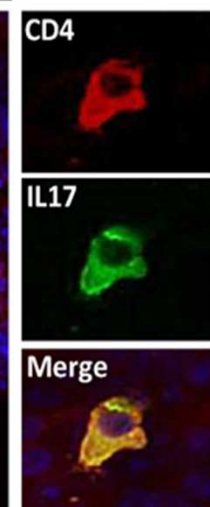
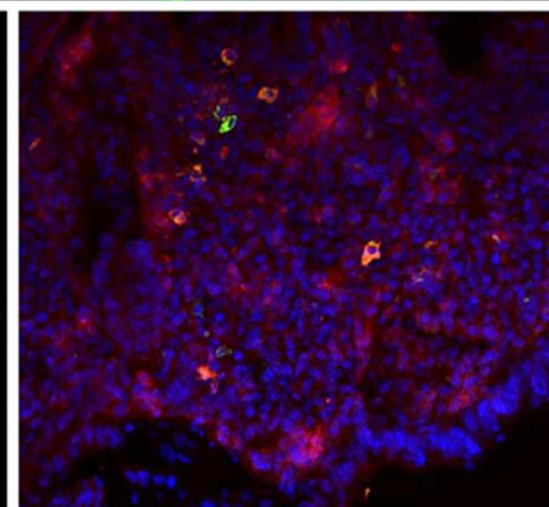
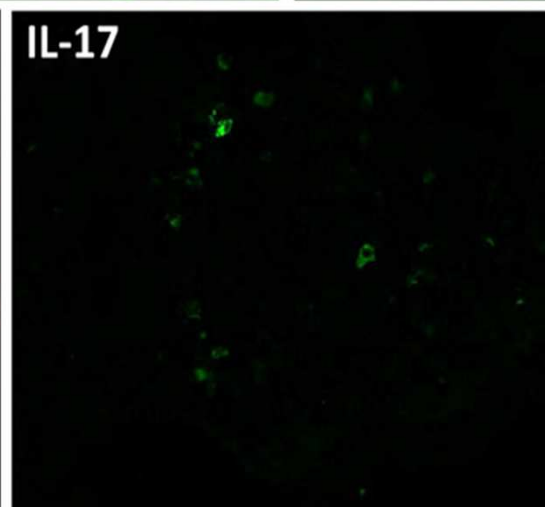
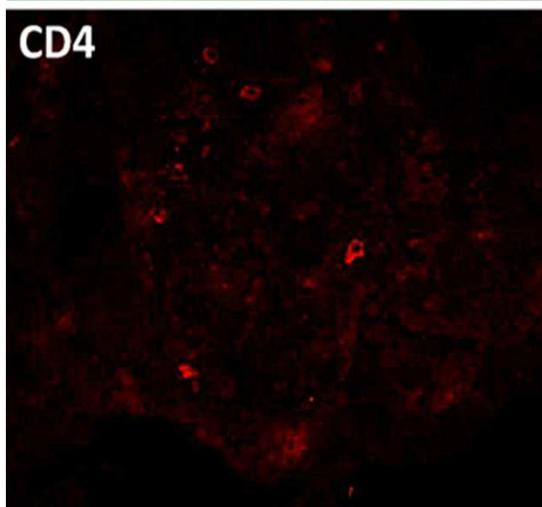
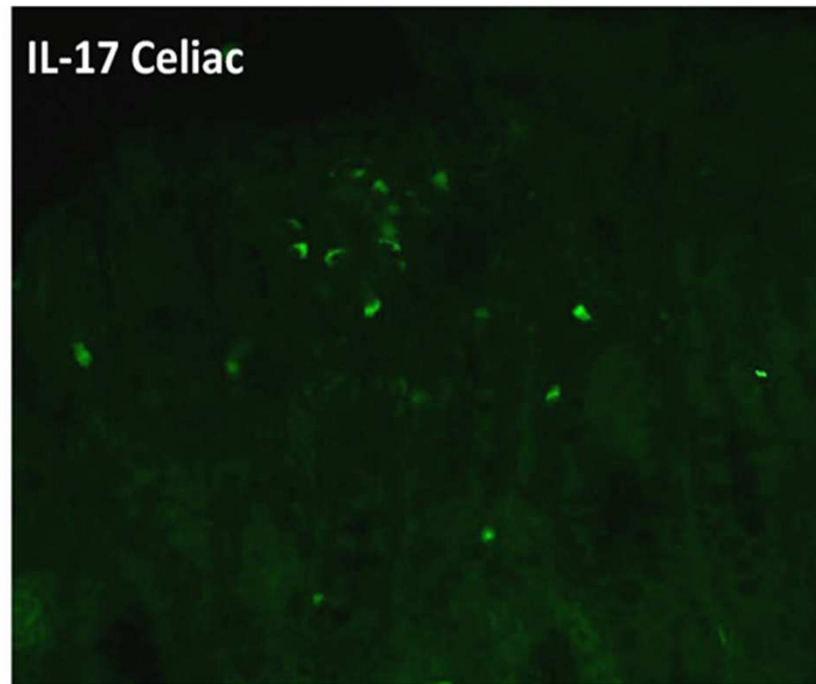
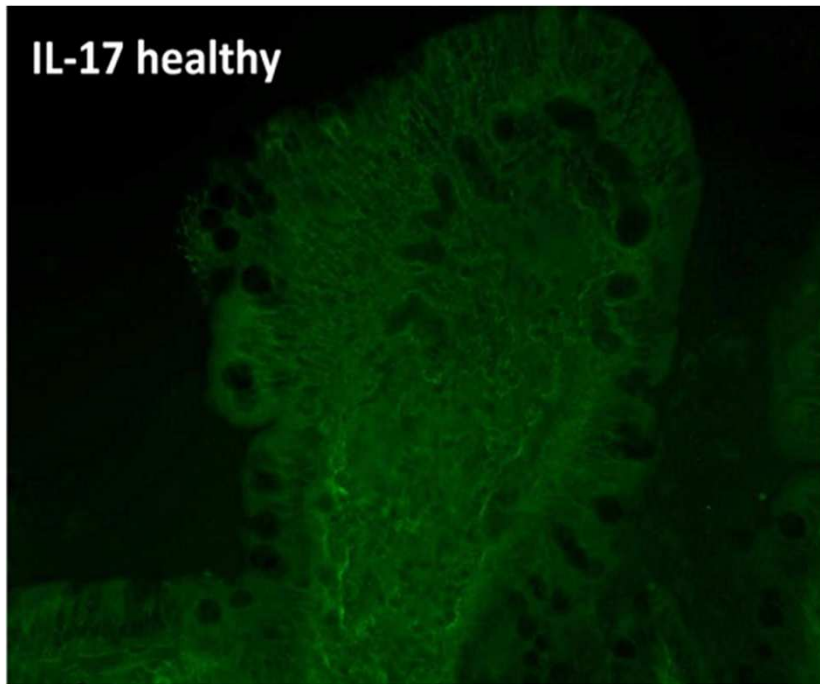
Effect of AEA on IL-17 versus IFN- γ production in activated T-lymphocytes

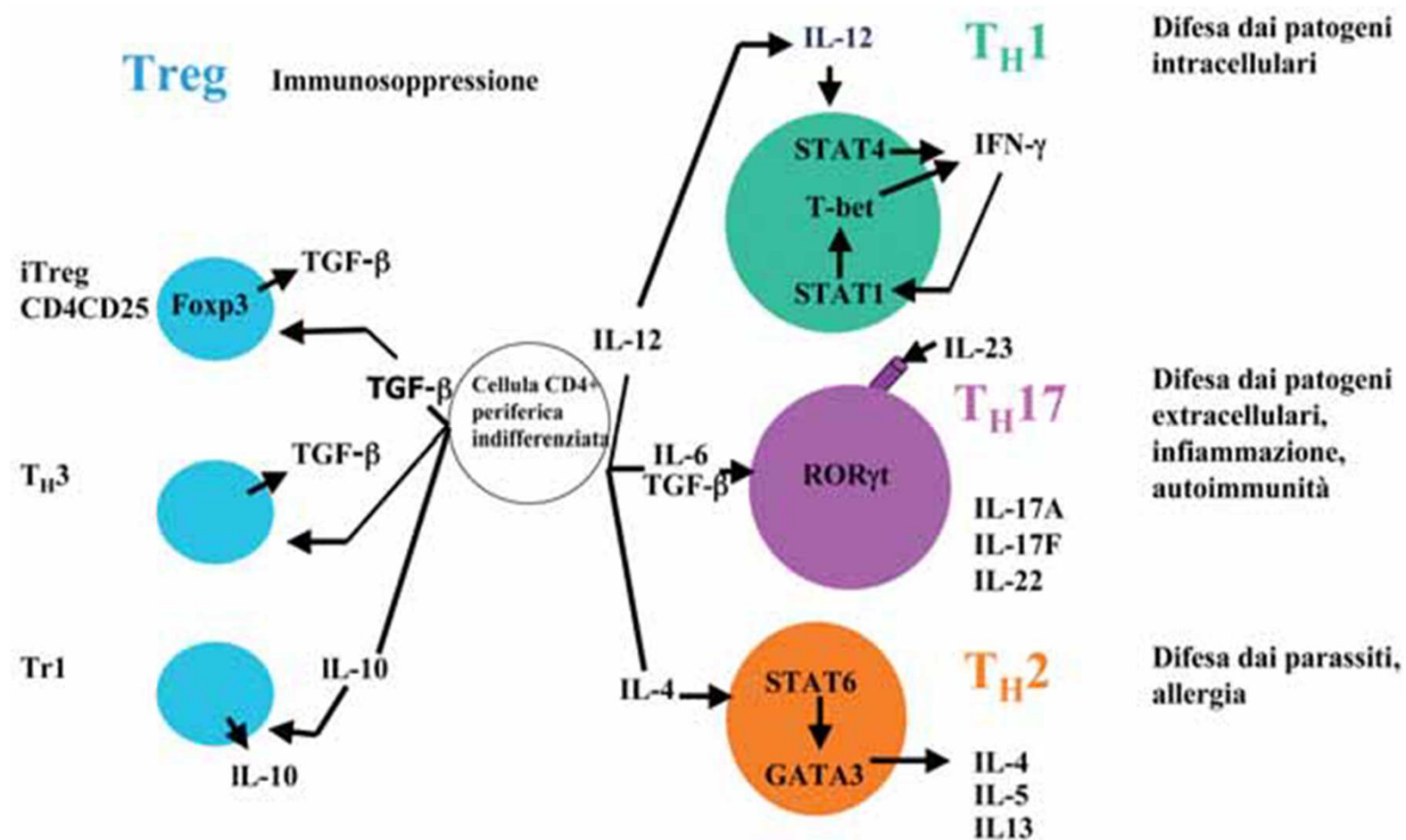


IL-17 nella malattia celiaca

- Fernández S et al. Characterization of gliadin-specific Th17 cells from the mucosa of celiac disease patients. *Am J Gastroenterol.* 2011 Mar;106(3):528-38.
- Harris KM, Fasano A, Mann DL. Monocytes differentiated with IL-15 support Th17 and Th1 responses to wheat gliadin: implications for celiac disease. *Clin Immunol.* 2010 Jun;135(3):430-9.
- Monteleone I et al. Characterization of IL-17A-producing cells in celiac disease mucosa. *J Immunol.* 2010 Feb 15;184(4):2211-8.
- Sapone A et al. Differential mucosal IL-17 expression in two gliadin-induced disorders: gluten sensitivity and the autoimmune enteropathy celiac disease. *Int Arch Allergy Immunol.* 2010;152(1):75-80.

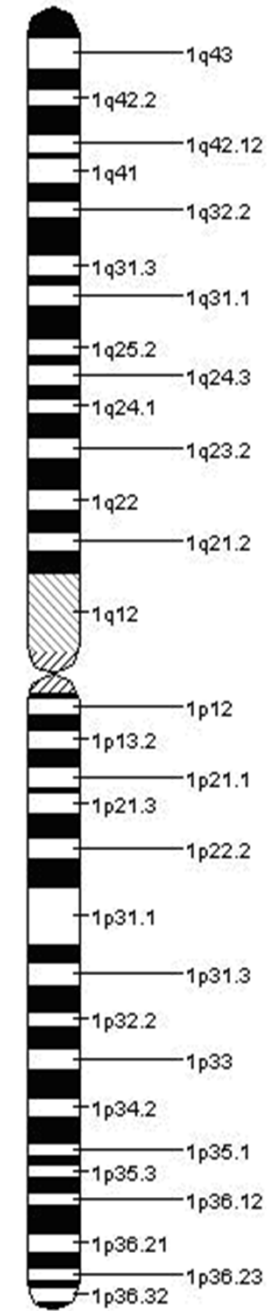
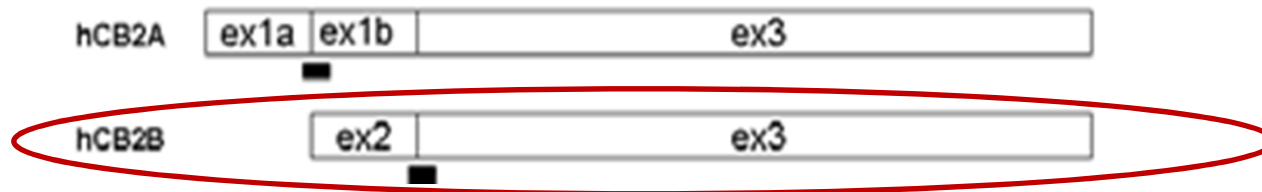
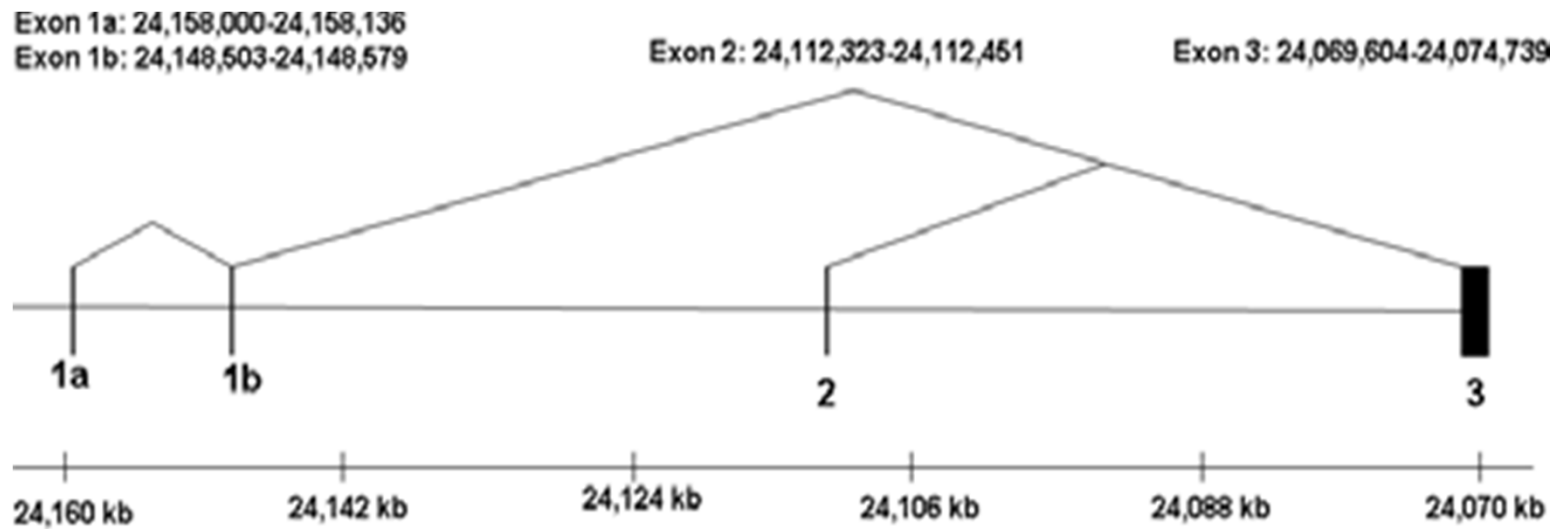
IL-17 positive staining increases in the celiac derived bowel biopsies







Il gene CNR2

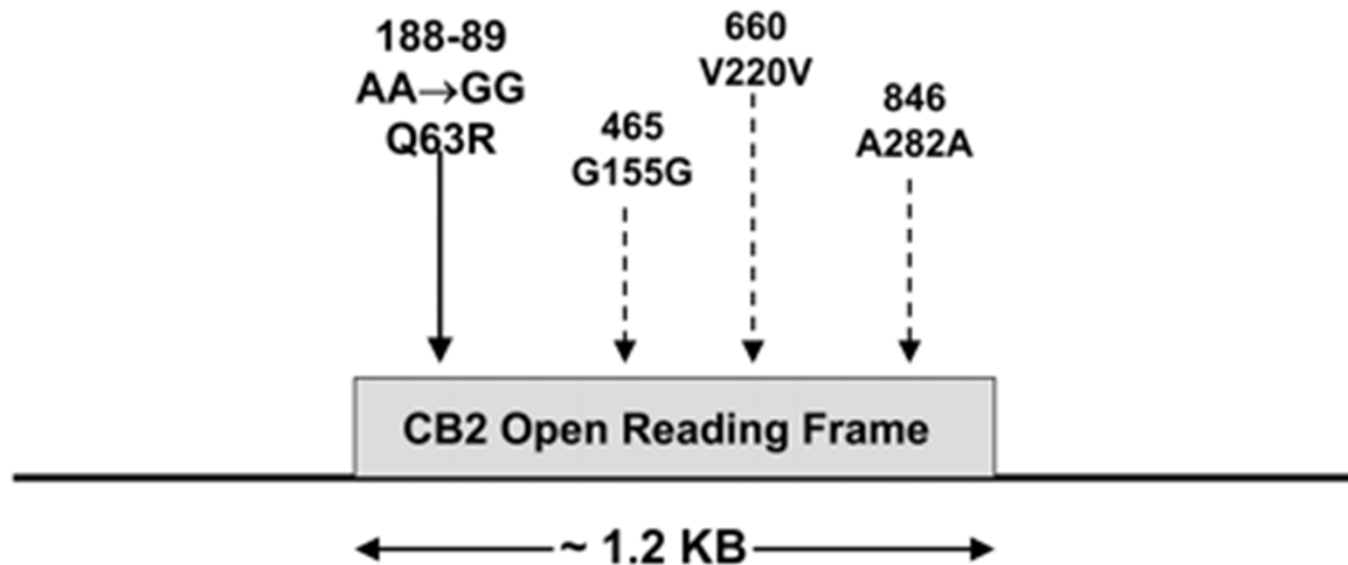


1p36 e malattie autoimmuni

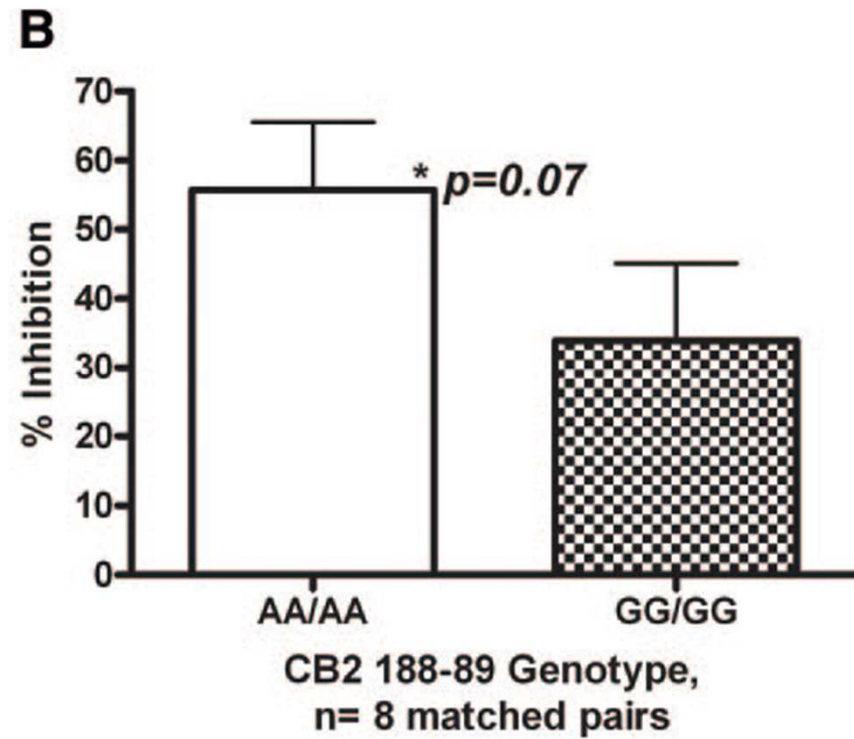
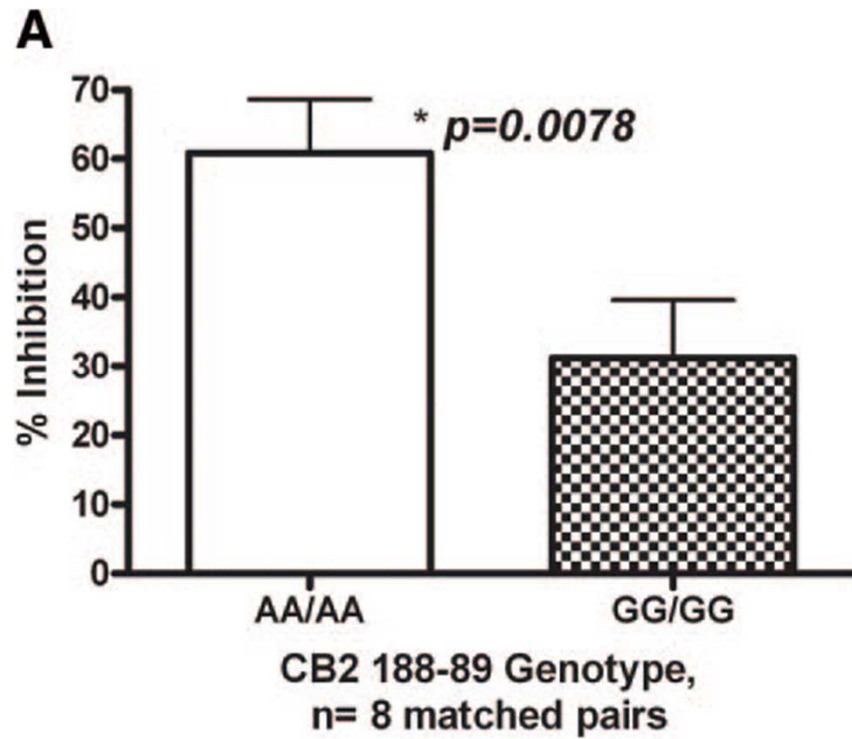
- Osawa K, Takami N, Shiozawa K, Hashiramoto A, Shiozawa S. Death receptor 3 (DR3) gene duplication in a chromosome region 1p36.3: gene duplication is more prevalent in **rheumatoid arthritis**. *Genes Immun* 2004;5:439–43.
- Shai R, Quismorio Jr FP, Li L, Kwon OJ, Morrison J, Wallace DJ, et al. Genome wide screen for **systemic lupus erythemathosus** susceptibility in multiplex families. *Hum Mol Genet* 1999;8:639–44.
- Nishimura M, Obayashi H, Mizuta I, Hara H, Adachi T, Ohta M, et al. TNF, TNF receptor type 1, and allograft inflammatory factor-1 gene polymorphisms in Japanese patients with **type 1. Diab** *Hum Immunol* 2003;64:302–9.
- Liu J, Juo SH, Holopainen P, Terwilliger J, Tong X, Grunn A, et al. Genomewide linkage analysis of celiac in Finnish families. *Am J Hum Genet* 2002;70: 51–9.
- Halpern AV, Bansal A, Heymann WR. Pemphigus vulgaris in a patient with 1p36 deletion syndrome. *J Am Acad Dermatol* 2005;55:98–9.
- Rossi F, Mancusi S, Bellini G, Roberti D, Punzo F, Vetrella S, et al. CNR2 functional variant (Q63R) influences childhood immune thrombocytopenic purpura. *Haematologica* 2011;96:1883–5.

Varianti del gene CNR2

CB2 Gene Coding Region Polymorphisms



Mean percent inhibition of lymphocyte proliferation by fatty acid amides at 107 M; analysis of eight matched donor subject pairs with CB2 188–189 AA/AA and GG/GG genotypes



Allele and genotype frequencies in Caucasian autoimmune disease subjects compared with Caucasian controls

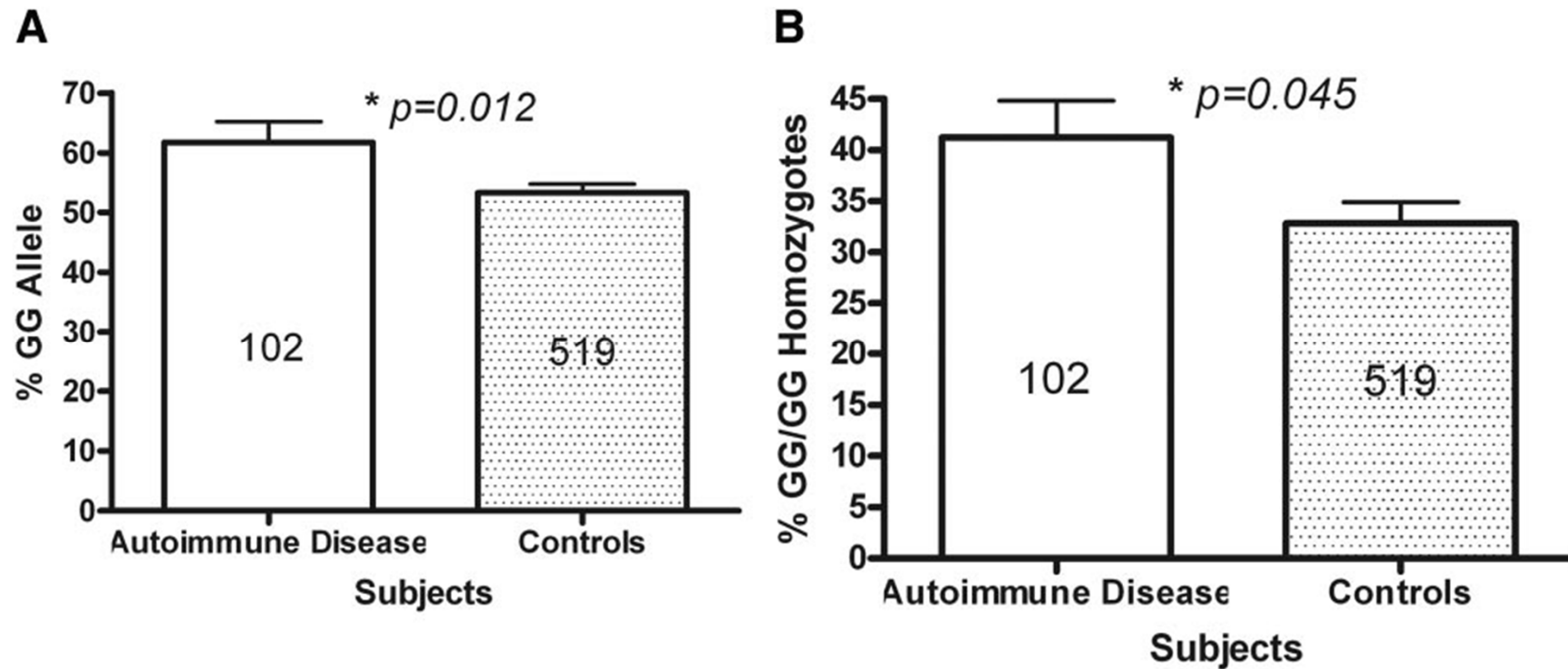


Table 1

Case-control association study of *CNR2* rs35761398 polymorphism in South Italian CD affected children

	CD patients	Controls	PubMed controls (Ornati et al., 2008)
Individuals (n)	327	600	487
Female (%)	57	54	-
median age and range (years)	11.5 1-17	10.7 1.8-16.8	-
AAAA Homozygous (%)	5%	16%	17%
AAGG Heterozygous (%)	34.5%	52%	53%
GGGG Homozygous (%)	60.5%	32%	30%
Minor allelic AA frequency (%)	22%	42%	43.5%
<p>The difference between AA and GG allelic frequencies (%) in celiac patients respect to controls was statistically evaluated by a chi-square test*</p> <p>$\chi^2 = 37.064$, df 1, $p=1.14 \times 10^{-9}$</p> <p>$\chi^2 = 43.975$, df 1, $p=3.32 \times 10^{-11}$</p> <p>The presence of the minor allele was associated to a protective effect:</p> <p>OR=0.396 C.L95% 0.319-0.492; $p=1.02 \times 10^{-17}$</p> <p>OR=0.392 C.L95% 0.320-0.480; $p=4.05 \times 10^{-20}$</p>			

*p values still significant after correcting for multiple testing

^ respect to relative controls

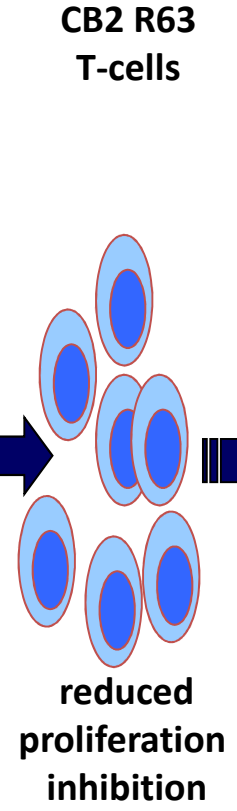
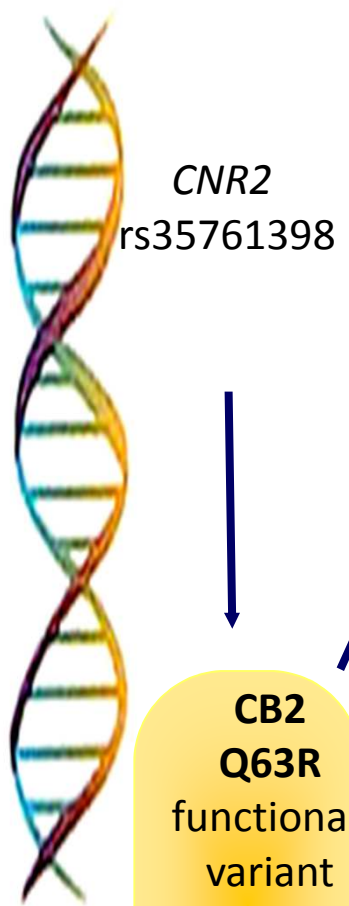
^^ respect to relative controls plus PubMed controls

Table 2**Genotype distribution for Q63R CB2 variant**

	CD		Controls	
<i>Genotype</i>	<i>Frequency</i>	<i>Relative Frequency</i>	<i>Frequency</i>	<i>Relative Frequency</i>
QQ	16	0.0489	96	0.1600
QR	113	0.3455	310	0.5167
RR	198	0.6055	194	0.3233
Summary Statistic*				
Tests of Independence	<i>Chi-squared</i>		<i>Df</i>	<i>p</i>
<i>CD vs Controls</i>	75.41		2	5.07×10^{-17}
Odds Ratios				
	<i>Odds Ratio</i>		<i>CI 95%</i>	<i>p</i>
<i>QR vs QQ</i>	2.187		1.242-3.848	0.006
<i>RR vs QQ</i>	6.124		3.499-10.708	0.000
<i>RR vs (QQ+QR)</i>	3.212		2.428-4.249	0.000
<i>(RR+QR) vs QQ</i>	3.702		2.153-6.364	0.000

**p*-values still significant after correcting for multiple testing.

This table shows the distribution of the three class of genotype in South Italian Celiac Patients (327) and unrelated



Ringraziamenti

Dip. Pediatria F.Fede- - SUN

Prof. E.Miraglia del Giudice

Prof. Bruno Nobili

Prof. Laura Perrone

Prof. Papparella

Prof. Carlo Tolone

Dip Medicina Sperimentale – SUN:

Prof. Sabatino Maione

Dott.ssa Giulia Bellini

Dott. Livio Luongo

Dott.ssa Silvia Mancusi

Mucosal Biology Research Center ,University of
Maryland School of Medicine, Baltimore, Md. ,
USA:

Alessio Fasano

Craig Sturgeon

Grazie per l'attenzione!!!!

