# PREVENIRE LA CELIACHIA ?

CASERTA

19 MAGGIO 2012

luigi greco

# Fattori Causali di una Patologia

Varianza Totale di una Malattia Multifattoriale

| Genetica Ereditata dai Genitori | Esperienza Ambientale Condivisa | Esperienza non condivisa |
|---|---|---|

INFEZIONI

| Genetica Ereditata dai Genitori | Esperienza Ambientale Condivisa | Esperienza non condivisa |
|---|---|---|

MALATTIE GENETICHE

| Genetica Ereditata dai Genitori | Esperienza Ambientale Condivisa | Esperienza non condivisa |
|---|---|---|

*Gut. 2006 Jun;55(6):803-8.*

# Concordance, disease progression, and heritability of coeliac disease in Italian twins.

Nisticò L. Fagnani C., Coto I, Percopo S, Cotichini R, Limongelli MG, Paparo F, D'Alfonso S, Giordano M, Sferlazzas C, Magazzu G, Momigliano-Richiardi P, Greco L, Stazi MA

**Table 2** Concordance by zygosity and sex in twin pairs

| | | | | Concordance (%) | | | |
|---|---|---|---|---|---|---|---|
| | Concordant | Discordant | Total | Probandwise | 95% CI | Pairwise | 95% CI |
| MZ male | 5 | 1 | 6 | 90 | 70.6–100 | 81.8 | 49.7–100 |
| MZ female | 12 | 5 | 17 | 80.8 | 64.4–97.1 | 67.7 | 44.7–90.7 |
| All MZ | 17 | 6 | 23 | 83.3* | 70.3–96.4 | 71.4† | 52.3–90.6 |
| DZ male | 0 | 12 | 12 | 0 | | 0 | |
| DZ female | 1 | 14 | 15 | 12.5 | 0–34.7 | 6.7 | 0–19.3 |
| DZ opposite sex | 4 | 19 | 23 | 26.9 | 5.2–48.7 | 15.6 | 1–30.1 |
| All DZ | 5 | 45 | 50 | 16.7* | 3.6–29.8 | 9.1† | 1.3–16.9 |
| Total | 22 | 51 | 73 | | | | |

MZ, monozygotic, DZ, dizygotic; 95% CI, 95% confidence interval.

Test for difference between MZ and DZ twins: *$\chi^2 = 49.98$, $p = 1.55 \times 10^{-12}$; †$\chi^2 = 40.77$, $p = 1.71 \times 10^{-10}$.

# Geni + Esperienza Condivisa + Esperienza Individuale ?

(peso % di ciascun elemento)

| CASI ‘CLINICI’ 1:1000 | |
|---|---|
| GENI | 57% |
| Fattori comuni | 42% |
| Fattori Individuali | 1% |

| INCIDENZA 1:100 | |
|---|---|
| GENI | 87% |
| Fattori comuni | 12% |
| Fattori Individuali | 1% |

# La finestra della prevenzione ?

Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease.

**JAMA. 2005 May 18;293(19):2343-51.**

| ***Glutine a mesi*** | ***Celiaci*** | ***Controlli*** | ***Odds Ratio CI*** | ***Estimate OR*** |
|---|---|---|---|---|
| **1-3 mesi** | 3 (6%) | 40 | 0,83-10,4 | 2,9 |
| **4-6 mesi** | 12 (2%) | 574 | 1 | 1 |
| **> 7 mesi** | 36 (4%) | 895 | 0,92-3,42 | 1,78 |
| | **51** | **1509** | **Solo 25 casi** | **con biopsia** |

# PREVENT-CD PROTOCOL

ENROLLMENT

Families with at least one member affected by CD

BIRTH

HLA analysis on cordon blood

HLA DQ2/DQ8 positive

HLA DQ2/DQ8 negative

•Breastfeeding

•Interve[redacted]

Daily intake of 100 mg of gluten

•Gradual introduction of gluten after 6° month

•Clinical an[redacted] 3-6 months

AGA IgA, tTG IgA

Clinical follow up every year

Persistent positivity of serological tests

Clinical symptoms

INTESTINAL BIOPSY

**Basilio Malamisura**
Centro Diagnosi Celiachia - Cava de' Tirreni

# Incidence CD

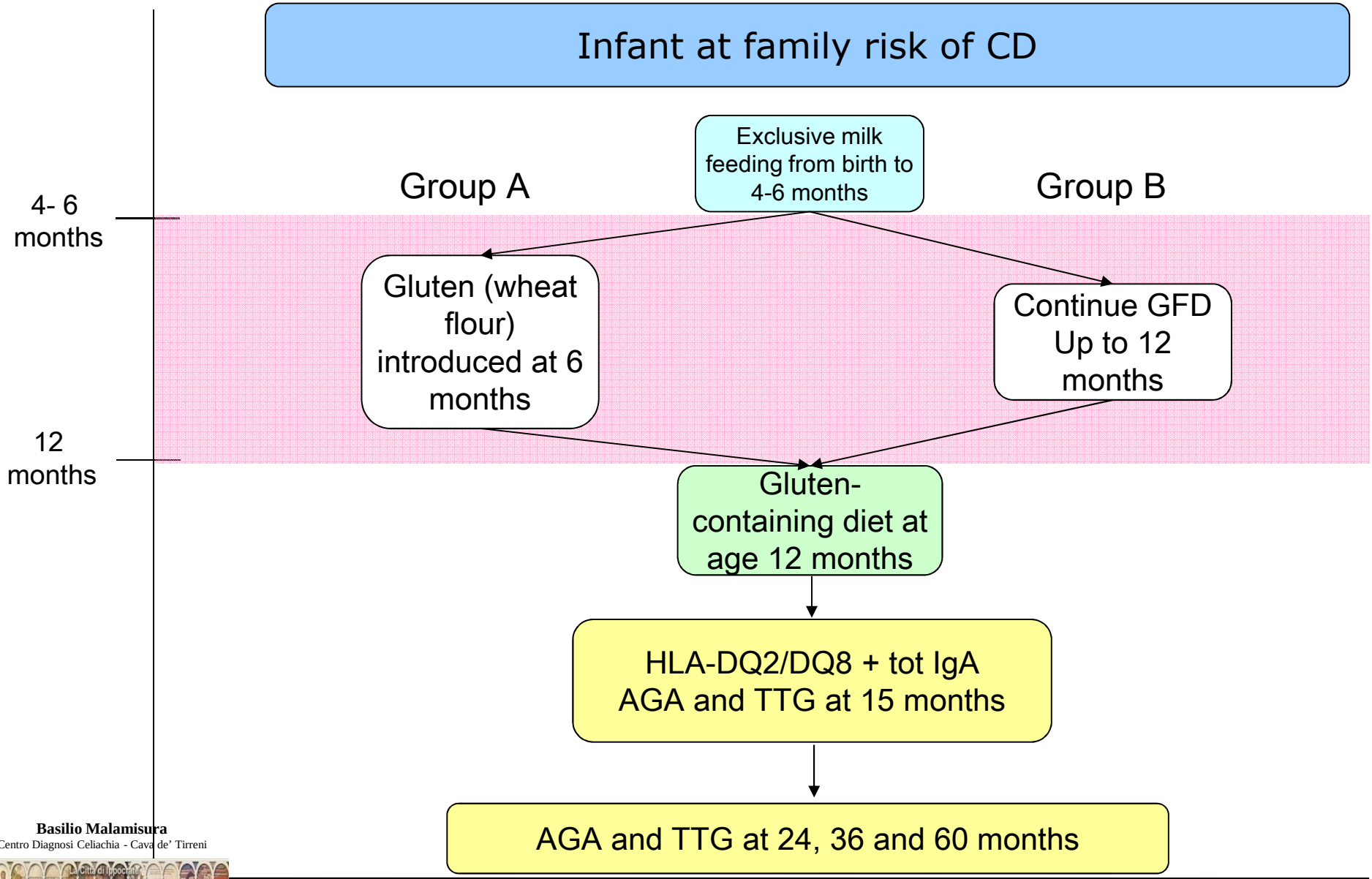
Recruited: 1348

Randomised: 952

| | |
|---|---|
| At 1 year | 0.6% |
| At 2 years | 4.6% |
| At 3 years | 7.6% |

**Basilio Malamisura**
Centro Diagnosi Celiachia - Cava de' Tirreni

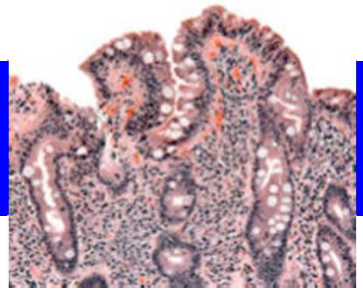
# Age at gluten introduction and risk of celiac disease (CD)




**Basilio Malamisura**
Centro Diagnosi Celiachia - Cava de' Tirreni

# Biopsy-proven CD

| | 15m | 24m | 36m | Tot |
|---|---|---|---|---|
| Gruppo A | 8 | 17 | 5 | 30 |
| *(group size)* | *(377)* | *(329)* | *(294)* | |
| Gruppo B | 1 | 6 | 10 | 17 |
| *(group size)* | *(345)* | *(325)* | *(283)* | |



*Journal oJ Pediatric Gastroenterology and Nutrition*

*7:395-399 @ **1988** Raven Press, Ltd., New York*

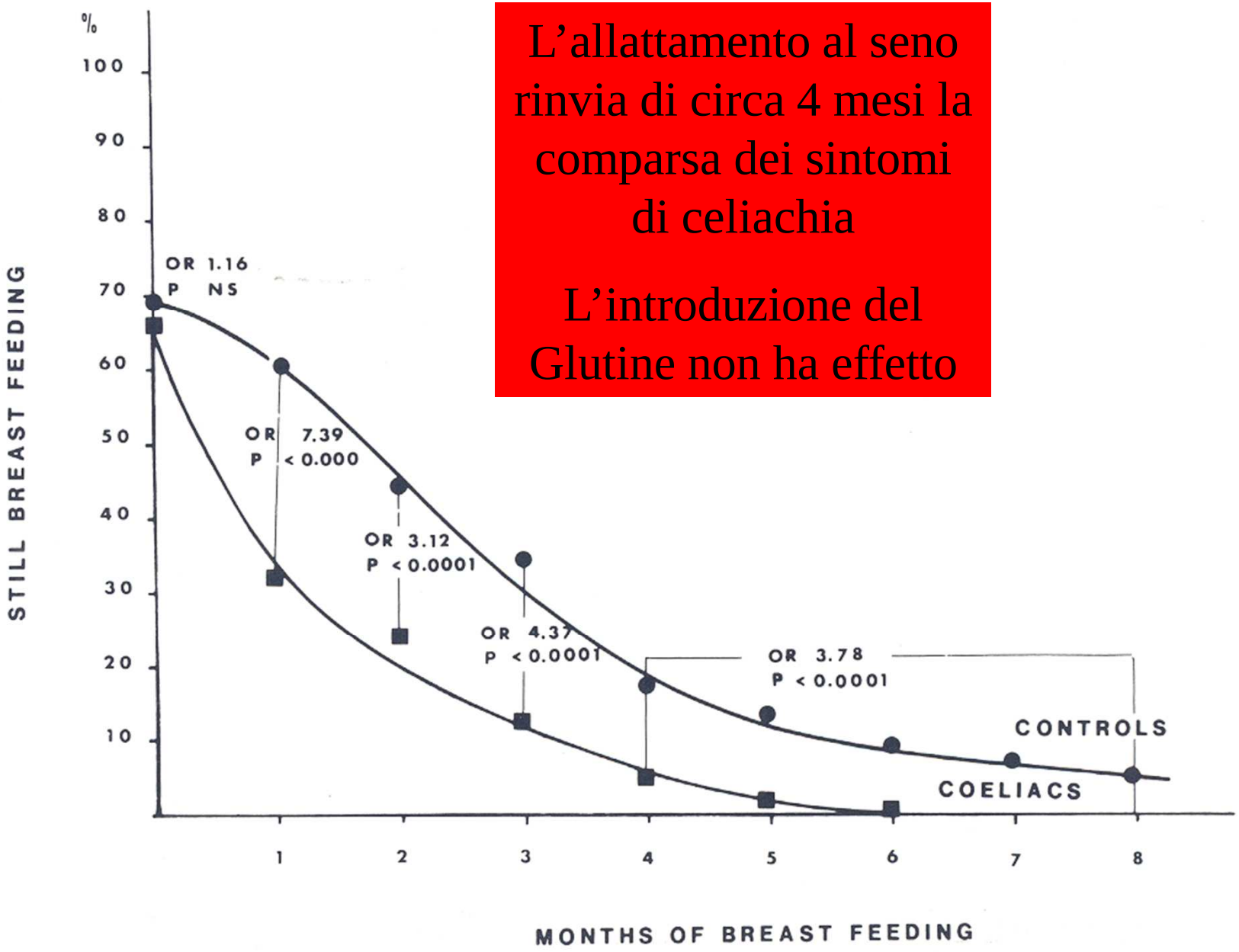
# Case Control Study on Nutritional Risk Factors in Celiac Disease

**L. Greco, S. Auricchio, M. Mayer, and M. Grimaldi**

***Department of Clinical Pediatrics, 2nd Faculty of Medicine, University of Naples, Naples, ltaly***

L'allattamento al seno rinvia di circa 4 mesi la comparsa dei sintomi di celiachia

L'introduzione del Glutine non ha effetto



# Abbiamo lavorato già 20 anni fa !

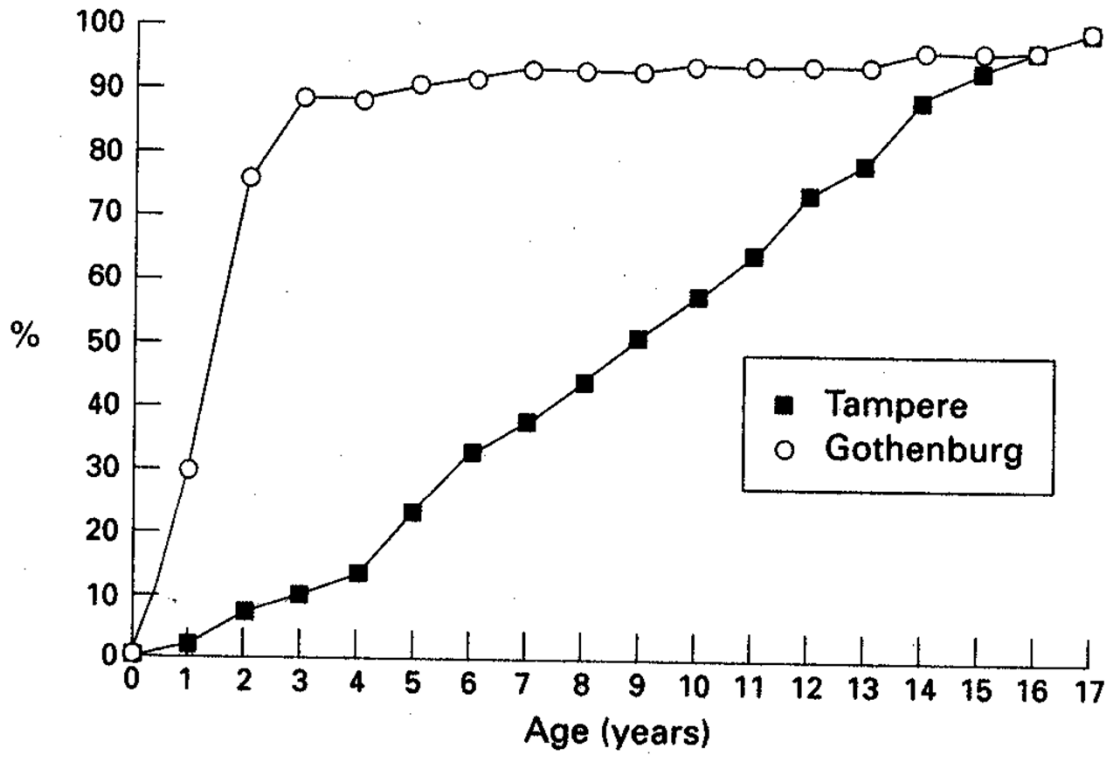
**Changing pattern of childhood coeliac disease in Finland** Acta Paediatr Scand. 1988 May;77(3):408-12

Long breast-feeding seemed to postpone the symptoms but the introduction of gluten was of no significance

We do not believe that coeliac disease has disappeared but that it will be found during the next decade

- **Different features of coeliac disease in two neighbouring countries** Arch Dis Child. 1993 Sep;69(3):375-80

- It is concluded that the intake of infant cereal protein might influence when and how clinical coeliac disease appears




| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tampere | 1 | 3 | 2 | 2 | 6 | 6 | 3 | 4 | 4 | 4 | 4 | 6 | 3 | 6 | 3 | 2 | 2 | = 61 |
| Gothenburg | 25 | 40 | 11 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | = 86 |

*Cumulative distribution of age at diagnosis in children with diagnosed coeliac disease by centre.*

# HLA ASSOCIATED RISK

| | | | ***DQ*** | ***Risk%*** | |
|---|---|---|---|---|---|
| **General population** | | | **Any** | **1%** | |
| General population | | | DQ2/8+ | 2% | |
| General population | | | DQ2/8- | 0% | |
| **First Degree Relatives** | | | **Any** | **9%** | |
| First Degree Relatives | | | DQ2/8- | 0% | |
| First Degree Relatives | | | DQ2/8+ | **>15%** | |

# Genotypic Relative Risk groups

| DQ genotypes | DR genotype* | GR | Italy | France | Scandinavia |
|---|---|---|---|---|---|
| $\alpha_5 \beta_2 / \alpha_5 \beta_2$ | **DR3/DR3** | $G_1$ | **1** | **1** | **1** |
| $\alpha_5 \beta_2 / \alpha \beta_2$ | **DR3/DR7** | | | | |
| $\alpha_5 \beta / \alpha \beta_2$ | **DR5/DR7** | $G_2$ | **0.68** | **0.28** | **0.23** |
| $\alpha_5 \beta_2 / \alpha_5 \beta$ | DR3/DRX | $G_3$ | **0.23** | **0.09** | |
| $\alpha_5 \beta_2 / \alpha_3 \beta_3$ | | | | | |
| $\alpha_5 \beta_2 / \alpha \beta$ | | | | | |
| $\alpha \beta_2 / \alpha \beta_2$ | DR7/DR7 | $G_4$ | **0.27** | **0.10** | **0.08** |
| $\alpha \beta_2 / \alpha_3 \beta_3$ | DR7/DR4 | | | | |
| $\alpha_3 \beta_3 / \alpha_3 \beta_3$ | DR4/DR4 | | | | |
| others | others | $G_5$ | **0.02** | **0.01** | **0.01** |

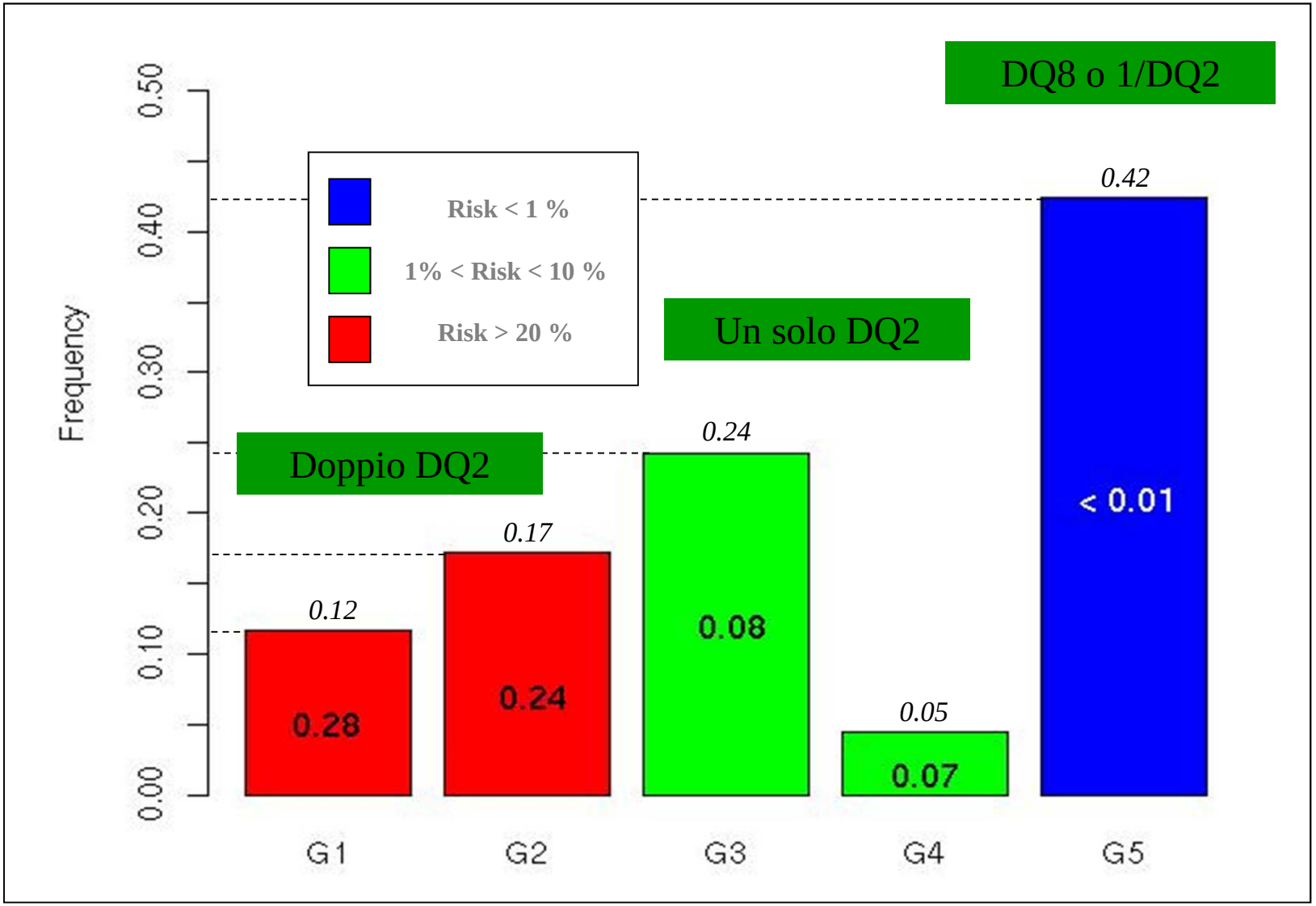
# Rischio per un fratello di un probando in accordo al genotipo DQ dei genitori

| | H1H1 | H1H2 | H1H3 | H1H4 | H1H5 | H2H2 | H2H3 | H2H4 | H2H5 | H3H3 | H3H4 | H3H5 | H4H4 | H4H5 | H5H5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H1H1 | | | [8;29] | [8;29] | [8;29] | | [8;29] | [8;29] | [8;29] | | | | | | |
| H1H2 | | [7;29] | [8;29] | [7;29] | [1;29] | [7;29] | [7;29] | [7;29] | [1;29] | [8;24] | [7;24] | [1;24] | | | |
| H1H3 | | | [1;29] | [1;29] | [1;29] | | [1;29] | [1;29] | [1;29] | | | | | | |
| H1H4 | | | | [7;29] | [1;29] | [7;29] | [1;29] | [7;2 | [1;29] | | | | | | |
| H1H5 | | | | | [1;29] | [1;29] | [1;29] | [1;29] | [ | | | | | | |
| H2H2 | | | | | | | [7;24] | | | | [7;24] | [1;24] | | | |
| H2H3 | | | | | | | [1;24] | [1;24] | [1;24] | [1;24] | [1;24] | [1;24] | | | |
| H2H4 | | | | | | | | | | [1;24] | [1;24] | [1;24] | | | |
| H2H5 | | | | | | | | | | [1;24] | [1;24] | [1;24] | | | |
| H3H3 | | | | | | | | | | | | | | | |
| H3H4 | | | | | | | | | | | | | | | |
| H3H5 | | | | | | | | | | | | | | | |
| H4H4 | | | | | | | | | | | | | | | |
| H4H5 | | | | | | | | | | | | | | | |
| H5H5 | | | | | | | | | | | | | | | |

**Stima del rischio in base al genotipo dei genitori**

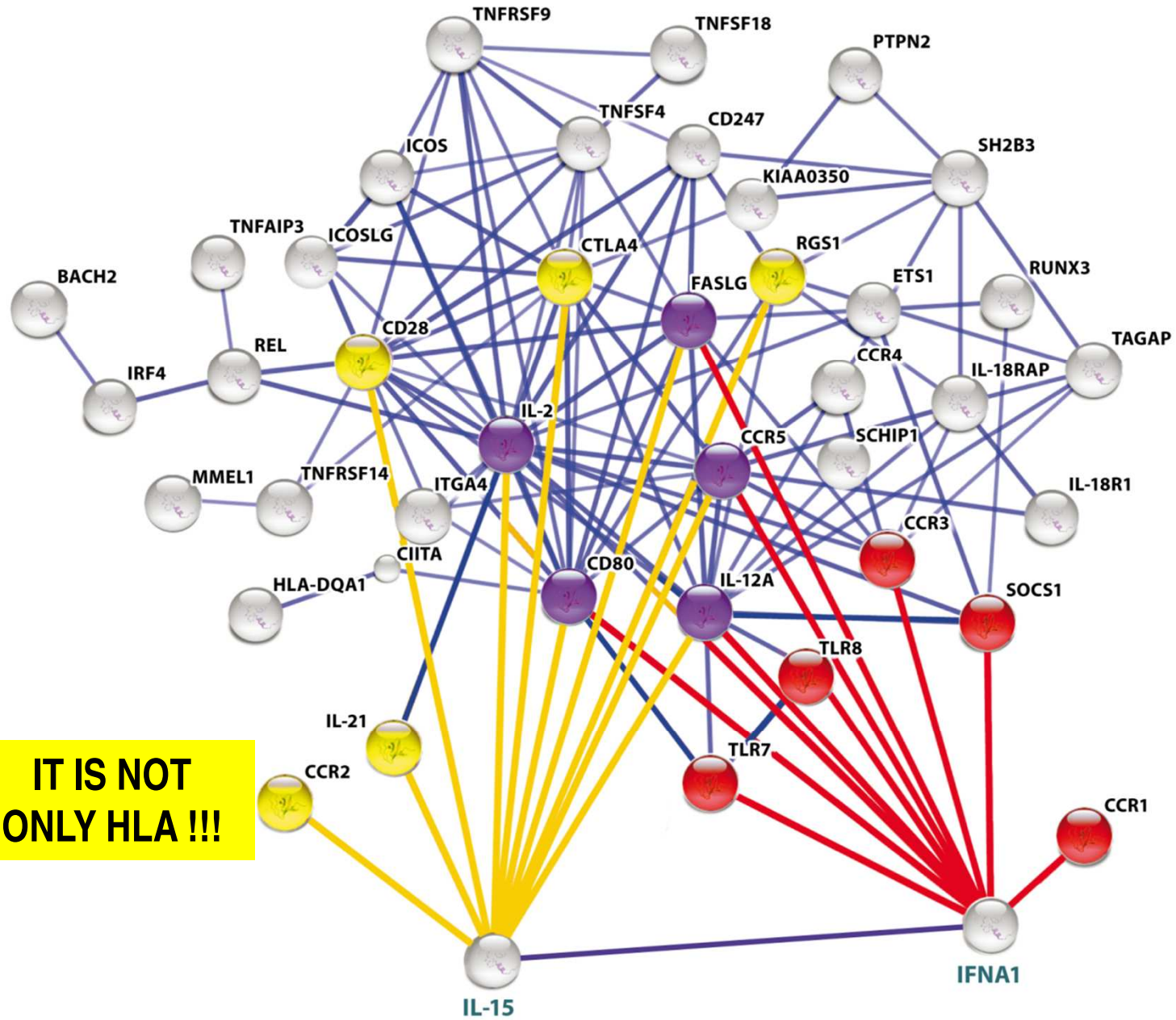
- Rischio > 20%
- 15% < Rischio < 20%
- 10% < Rischio < 15%
- 1% < Rischio < 10%
- Rischio < 1%

# PROBABILITA' PER UN FRATELLO DI UN PROBANDO DI APPARTENERE A Gi E RISCHIO CORRISPONDENTE



# Che dobbiamo fare ?

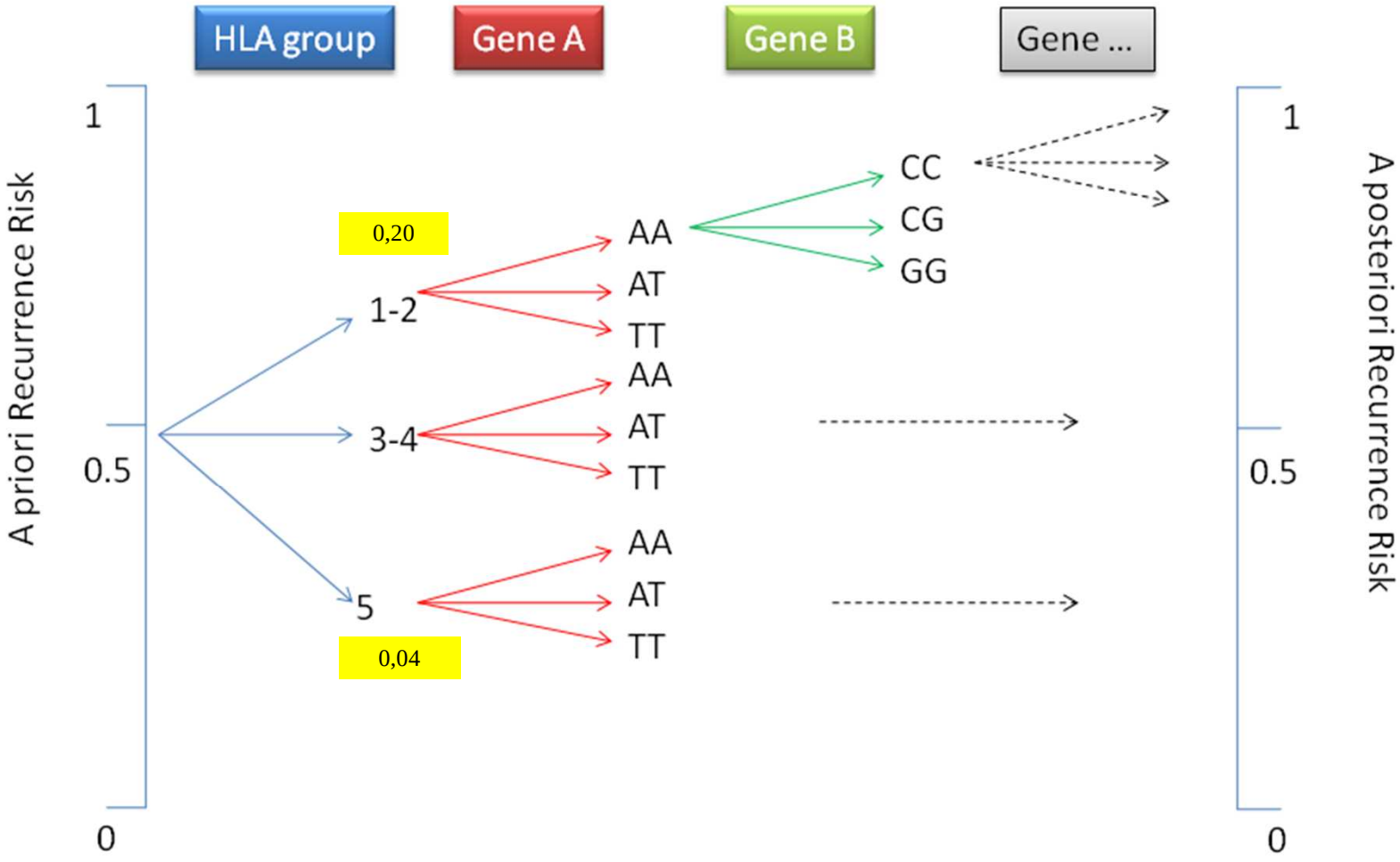
Scoraggiare un matrimonio ?




**IT IS NOT ONLY HLA !!!**

Abadie V, et al. 2011.
Annu. Rev. Immunol. 29:493–525

# Bayesian Approach



# Case-control association analyses at the single SNP level

Subunit of NF-kB

Regulator of G-protein signalling

Chemokine receptor

| Chr | SNP | Locus | A1 | A2 | CD cases, Controls [illegible] | MAF CD | MAF Controls | $\chi^2$ | p- value | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **rs2816316** | **RGS1** | **A** | **C** | **1[illegible]82,1169:253** | **0.14** | **0.18** | **6.10** | **0.0135** | **1.299 (1.055-1.598)** |
| 2 | rs917997 | IL18RAP | [illegible] | A | 965:309, 1092:330 | 0.24 | 0.23 | 0.408 | 0.5231 | 0.9438 (0.7901-1.127) |
| 2 | rs842647 | REL | A | G | 967:307, 1080:342 | 0.24 | 0.24 | 0.001 | 0.9774 | 0.9974 (0.8357-1.190) |
| **3** | **rs[illegible]** | **CCR3** | **G** | **A** | **741:533, 906:516** | **0.41** | **0.36** | **8.707** | **0.0032** | **0.7918 (0.6780-0.9247)** |
| 3 | rs17810546 | SCHIP1 | A | G | 1158:116, 1312:110 | 0.09 | 0.07 | 1.641 | 0.2002 | 0.8370 (0.6373-1.099) |
| **3** | **rs1464510** | **LPP** | **C** | **A** | **646:628, 844:578** | **0.49** | **0.40** | **20.32** | **6.5E-06** | **0.7045 (0.6048-0.8206)** |
| **4** | **rs1311972[illegible]** | **[illegible]IAA1109** | **A** | **G** | **1135:139, 1220:196** | **0.11** | **0.14** | **5.285** | **0.0215** | **1.312 (1.040-1.654)** |
| **4** | **rs13151[illegible]** | **KIAA1109** | **A** | **G** | **1139:135, 1225:195** | **0.10** | **0.14** | **6.143** | **0.0132** | **1.343 (1.063-1.697)** |
| 6 | rs2[illegible]832 | OLIG3 | [illegible] | G | 1008:248,1162:256 | 0.19 | 0.23 | 1.246 | 0.2643 | 0.8955 (0.7376-1.087) |
| **6** | **[illegible]738074** | **TAGAP** | **G** | **[illegible]** | **681:593, 816:604** | **0.46** | **0.42** | **4.376** | **0.0365** | **0.8500 (0.7299-0.9899)** |
| 19 | rs3760746 | TNFSF[illegible] | T | C | [illegible]276, 461:375 | 0.42 | 0.41 | 0.362 | 0.5473 | 0.9372 (0.7588-1.158) |

Chr: chromosom[illegible]2: minor allele; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval; $\chi^2$: $\chi$-square test;
[illegible]ic p-value was calculated by 2x2 two-sided $\chi$-square test; bold indicates p-value < 0.05

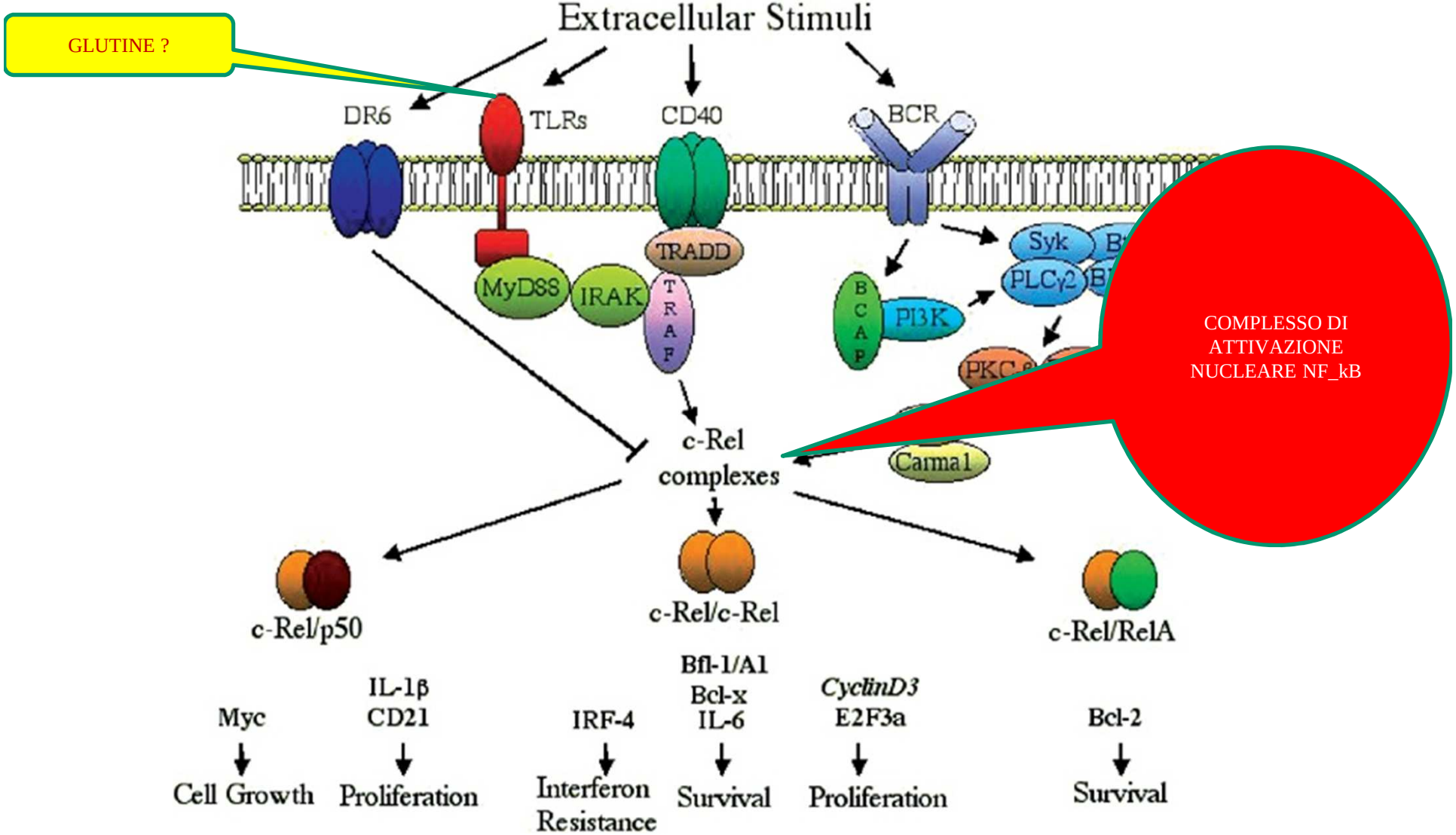
Structural role in maintaining cell shape and motility; it may be involved in signal trasduction and activation of gene transcription

Rho GTPase activatin protein

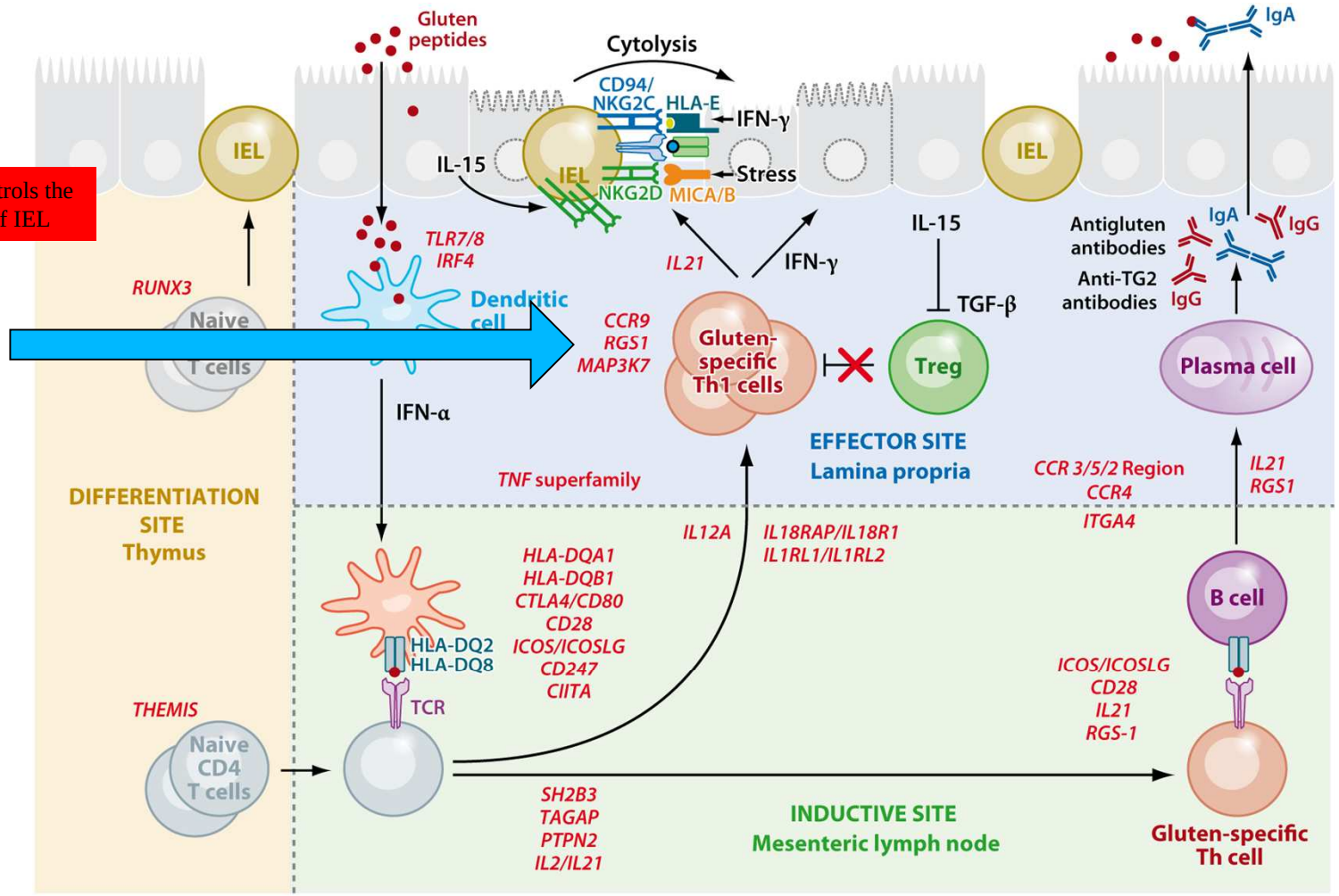
Endopeptidase

372 CD-Cases vs 451 Controls

# Pathway of c-Rel in NF-KB



RGS1 : controls the homing of IEL



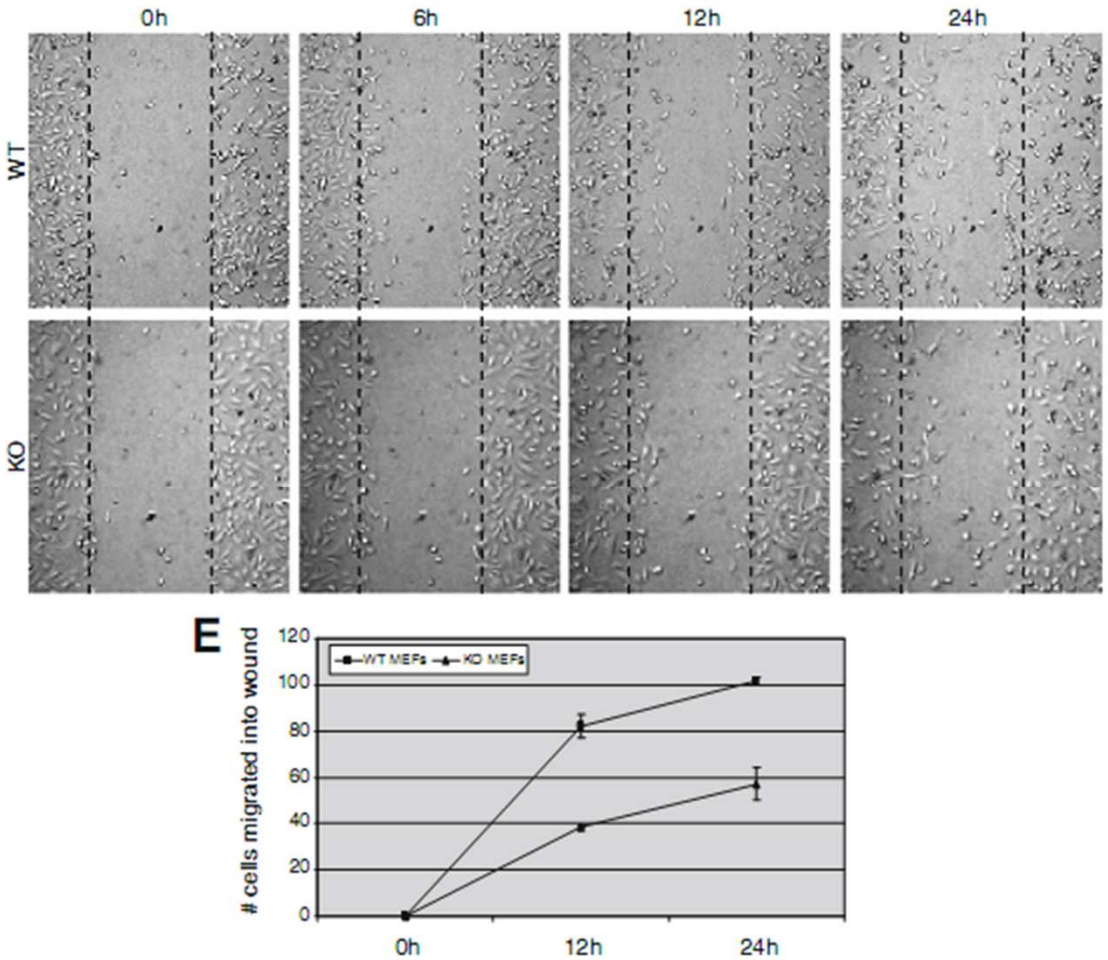

Abadie V, et al. 2011.
Annu. Rev. Immunol. 29:493–525

Integration of immunological pathways and celiac disease (CD)-associated genes into a model of CD pathogenesis. The figure is subdivided into three distinct anatomical regions in which T cell differentiation (thymus), T cell polarization (inductive site), and effector immune response (effector site) take place. Genes associated with CD by genome-wide association studies are listed in red according to their potential implication in distinct immunological pathways. *THEMIS* and *RUNX3* are involved in the thymic differentiation of CD4 and CD8 T cells, respectively. Dendritic cells located in the lamina propria acquire a proinflammatory phenotype upon viral recognition (*TLR7/8* and *IRF4*) and migrate to the mesenteric lymph nodes (inductive site). There, they present gluten peptides (*HLA-DQA1*, *HLA-DQB1*, and *CIITA*) to naive CD4 T cells and promote T cell activation (e.g., *CD28*, *CD80*, *CTLA4*, *CD247*, *PTPN2*, *SH2B3*, *TAGAP*, *IL2*, and *FASLG*) and differentiation into inflammatory effector T cells (*IL12A*, *IL18R1*, *IL18RAP*, *IL1RL1*, and *IL1RL2*). In addition, transglutaminase 2 (TG2) and gluten-specific B cells (that have internalized gluten-TG2 complexes) receive help from gluten-specific T cells, become activated, and differentiate into immunoglobulin (Ig)A- and IgG-producing plasma cells (*ICOS*, *ICOSLG*, *IL21*, and *RGS1*). Other genes regulate activation and migration of cytotoxic intraepithelial lymphocytes (IELs) (*MAP3K7*, *IL-21*, *CCR9*, and *RGS1*). Finally, some genes are involved in cell migration [e.g., genes coding for chemokine receptors (*CCR*s) and *ITGA4*], and others regulate tumor necrosis factor (TNF)-dependent pathways (*TNFAIP3*, *TNFSF4*, *TNFSF18*, *TNFRSF9*, and *TNFRSF14*). Even though their genes have not been identified by genetic studies, interleukin (IL)-15 and interferon (IFN)-α play a critical role in orchestrating the immune responses that lead to CD pathogenesis. IL-15 upregulates activating natural killer cell (NK) receptors and licenses IELs to kill epithelial cells, whereas IFN-α promotes the differentiation of proinflammatory dendritic cells. Abbreviations: HLA, human leukocyte antigen; TGF, transforming growth factor; Th, T helper cell.

# Lpp -/- mouse model

Lpp -/- mouse embryonic fibroblasts exhibited reduced migration capacity, reduced viability, and reduced expression of some Lpp interaction partners.

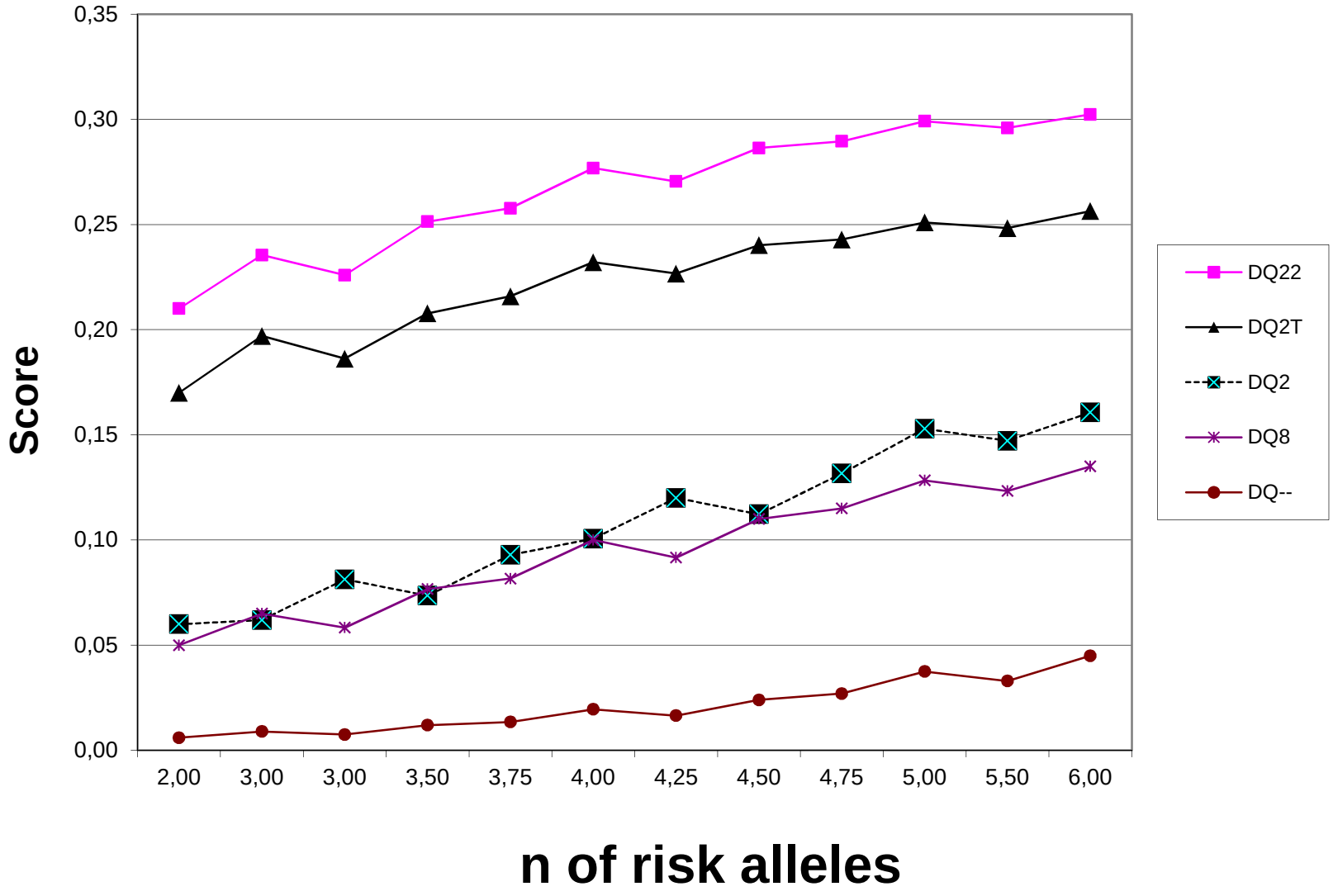
Vervenne et al., 2009

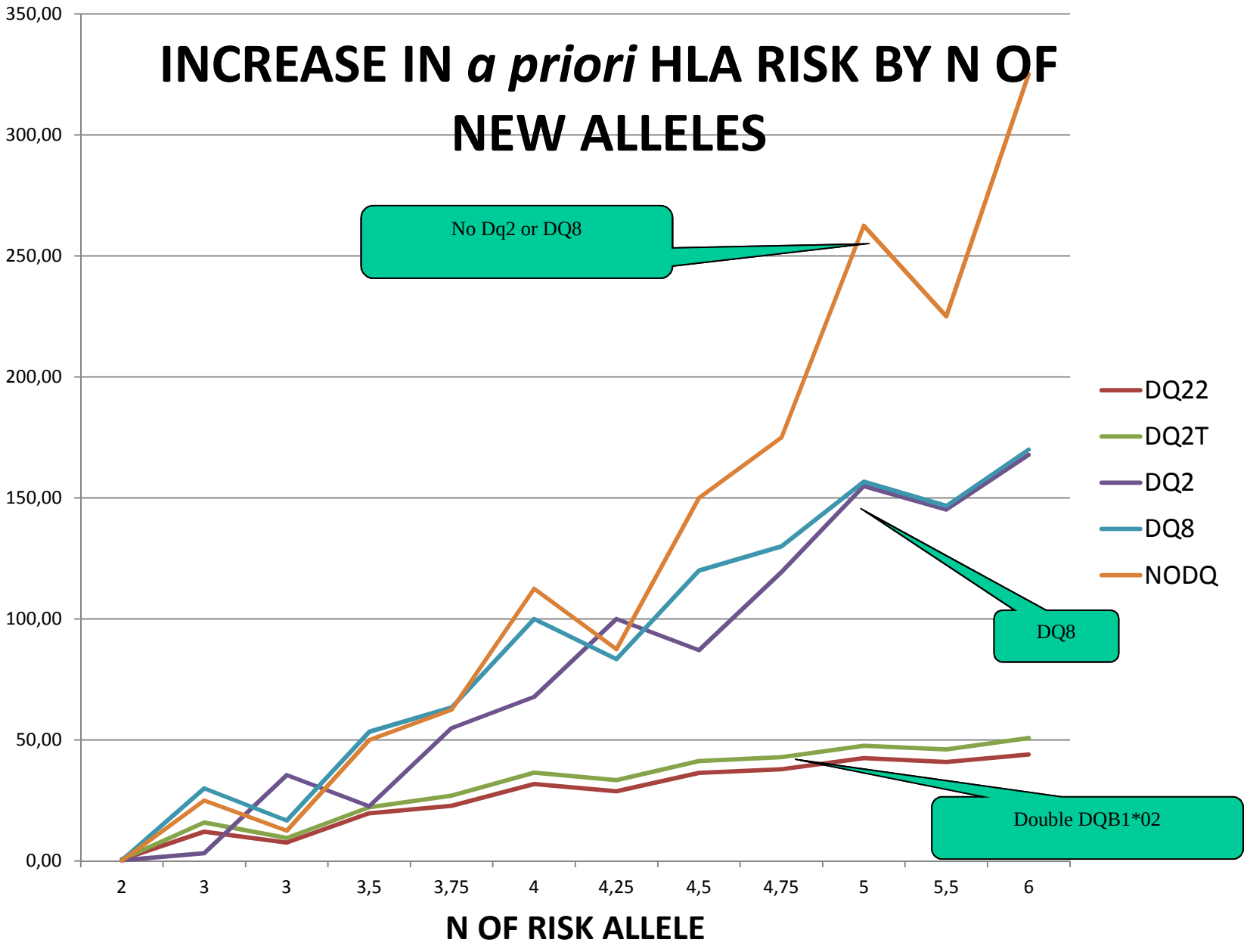


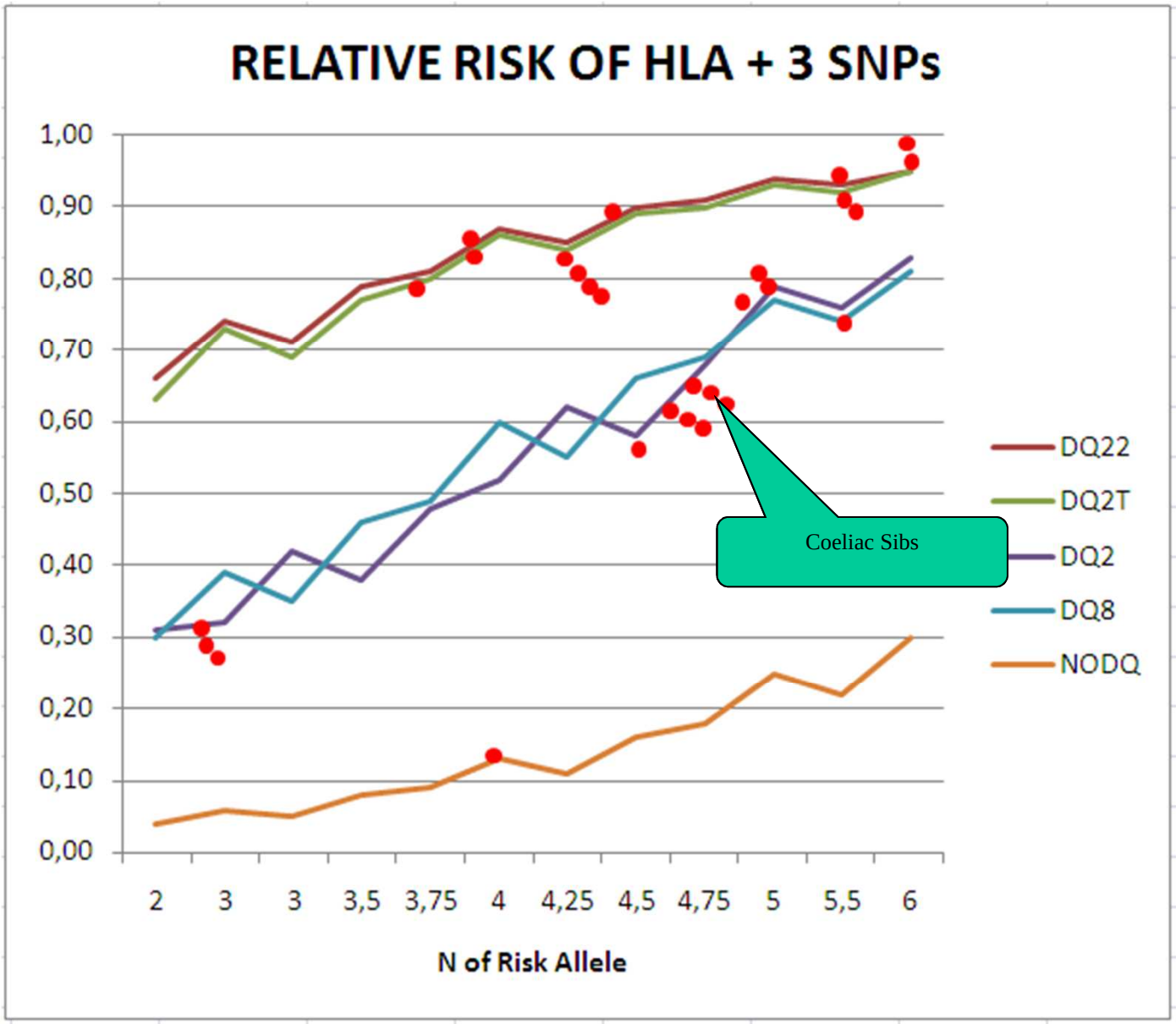
# Increase of the a priori HLA Risk by adding LPP, c-REL, RGS1

| LPP | c-REL | RGS1 | DQ22 | DQ2T | DQ2 | DQ8 | DQ-- |
|---|---|---|---|---|---|---|---|
| CC | AG+GG | AC+CC | 0,21 | 0,17 | 0,06 | 0,05 | 0,01 |
| CC | AA | AC+CC | 0,24 | 0,20 | 0,06 | 0,07 | 0,01 |
| CC | AG+GG | AA | 0,23 | 0,19 | 0,08 | 0,06 | 0,01 |
| CC | AA | AA | 0,25 | 0,21 | 0,07 | 0,08 | 0,01 |
| AC | AG+GG | AC+CC | 0,26 | 0,22 | 0,09 | 0,08 | 0,01 |
| AC | AA | AC+CC | 0,28 | 0,23 | 0,10 | 0,10 | 0,02 |
| AC | AG+GG | AA | 0,27 | 0,23 | 0,12 | 0,09 | 0,02 |
| AC | AA | AA | 0,29 | 0,24 | 0,11 | 0,11 | 0,02 |
| AA | AG+GG | AC+CC | 0,29 | 0,24 | 0,13 | 0,12 | 0,03 |
| AA | AA | AC+CC | 0,30 | 0,25 | 0,15 | 0,13 | 0,04 |
| AA | AG+GG | AA | 0,30 | 0,25 | 0,15 | 0,12 | 0,03 |
| AA | AA | AA | 0,30 | 0,26 | 0,16 | 0,14 | 0,05 |

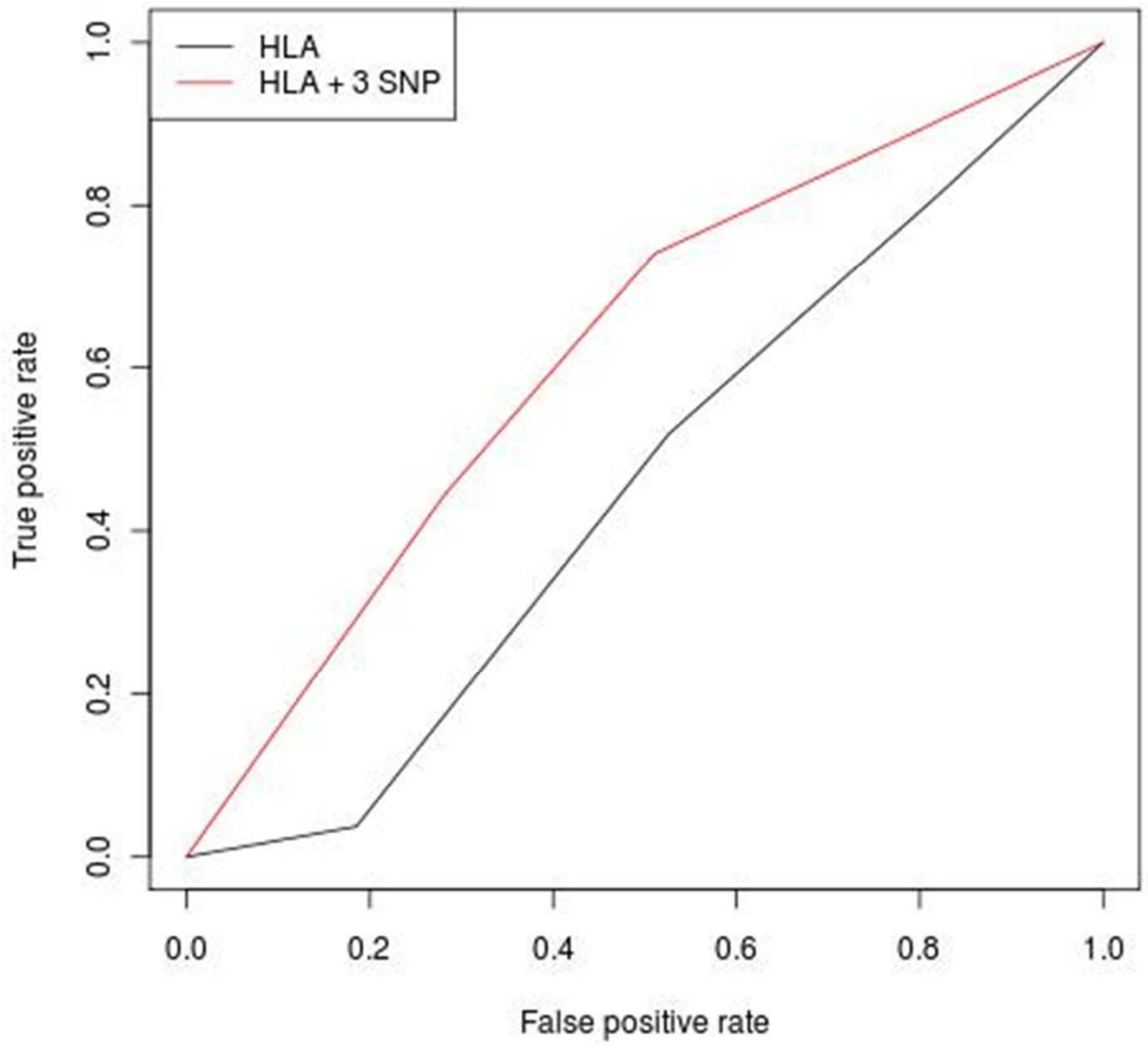
# A posteriori Risk adding each new allele to HLA

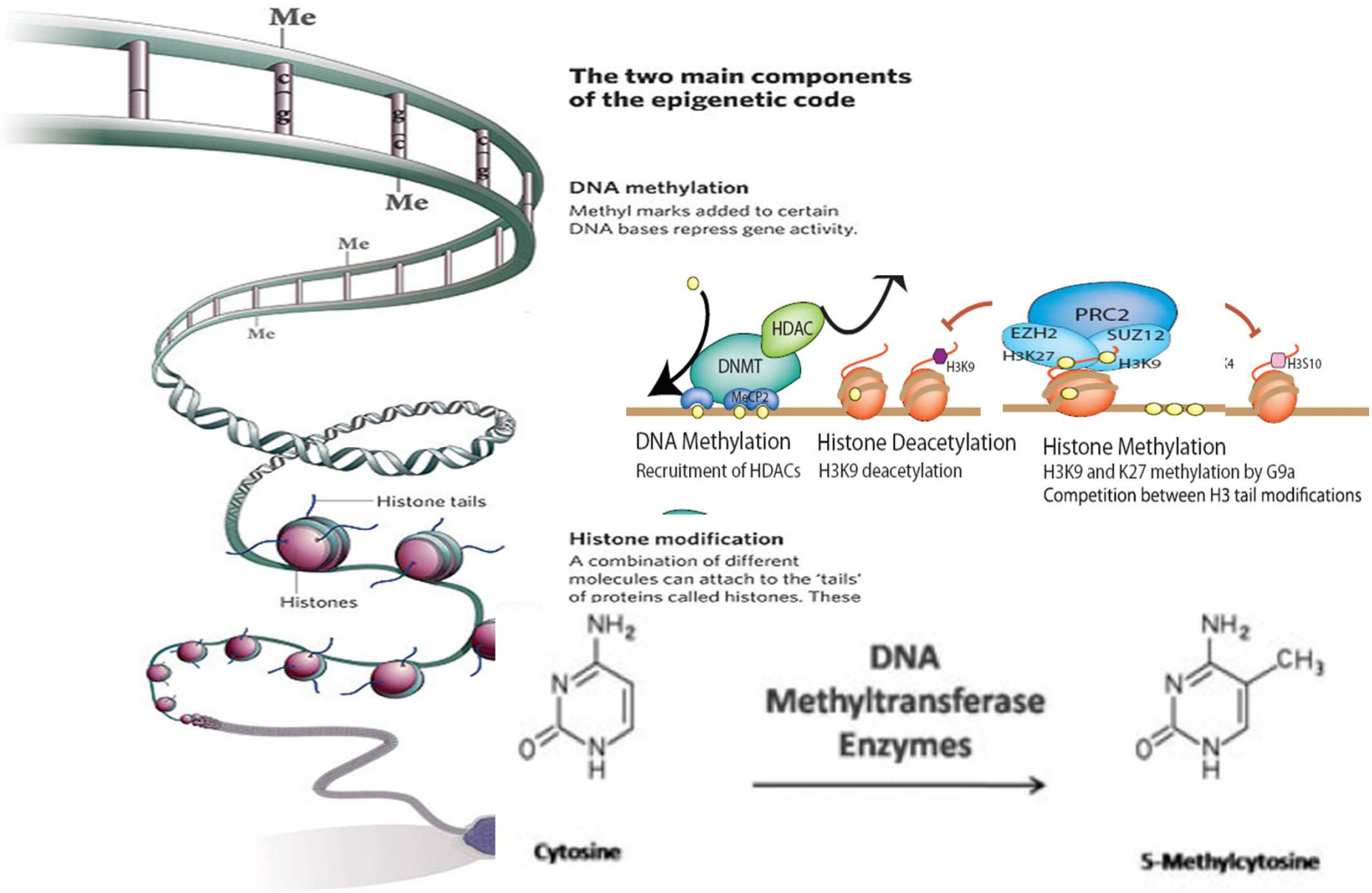



INCREASE IN *a priori* HLA RISK BY N OF NEW ALLELES
350,00
300,00
250,00
200,00
150,00
100,00
50,00
0,00
2
3
3
3,5
3,75
4
4,25
4,5
4,75
5
5,5
6
N OF RISK ALLELE
DQ22
DQ2T
DQ2
DQ8
NODQ
No Dq2 or DQ8
DQ8
Double DQB1*02


RELATIVE RISK OF HLA + 3 SNPs
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
2
3
3
3,5
3,75
4
4,25
4,5
4,75
5
5,5
6
N of Risk Allele
DQ22
DQ2T
DQ2
DQ8
NODQ
Coeliac Sibs

# ROC - Performance of the predicting model

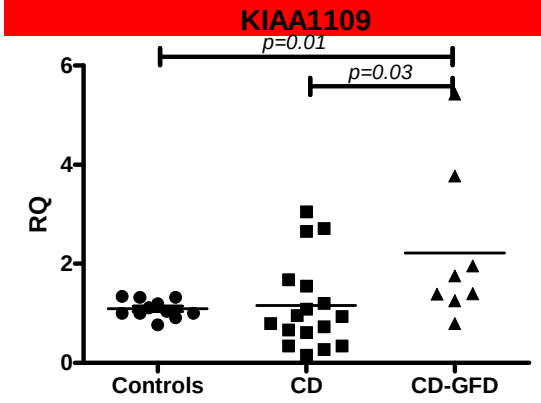


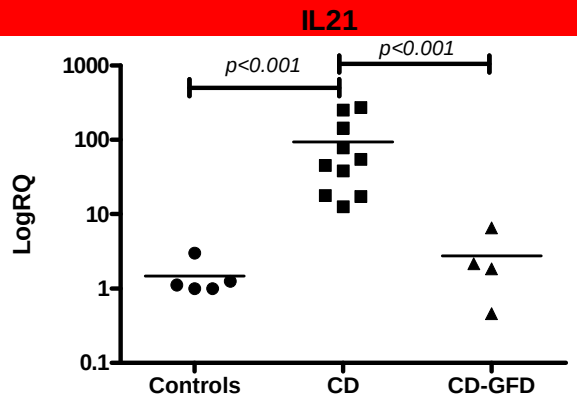



But the GENOMA is a written book : Life is the EXPRESSION of Genes

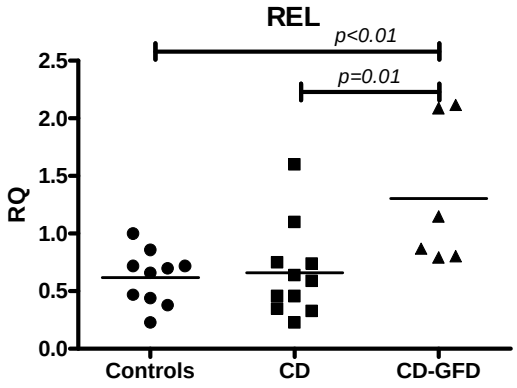
*di Napoli "Federico II"*

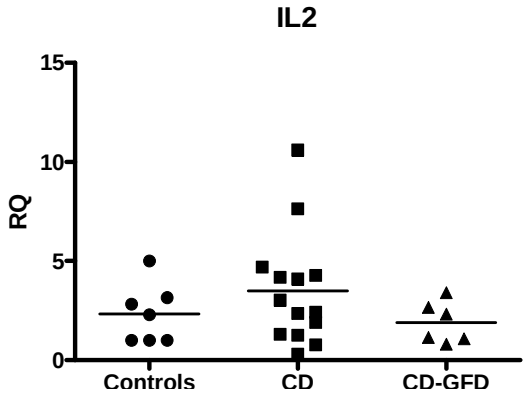
# EXPRESSION Biopsie intestinali - 1




4q27




*Sperandeo et al, 2011*

# Discriminant Analysis between Coeliacs and Controls by the Expression of Candidate Genes in Intestinal Mucosa

| | | Wilks' Lambda | df1 | Exact F | Sig. |
|---|---|---|---|---|---|
| 1 | TNFAIP3 | ,404 | 1 | 59,002 | ,000 |
| 2 | IL21 | ,300 | 2 | 45,521 | ,000 |
| 3 | REL | ,261 | 3 | 35,809 | ,000 |
| 4 | RGS1 | ,235 | 4 | 30,143 | ,000 |
| 5 | | ,222 | 5 | | ,000 |

Wilk's Lambda shows the ability to discriminate between Coeliac and Controls

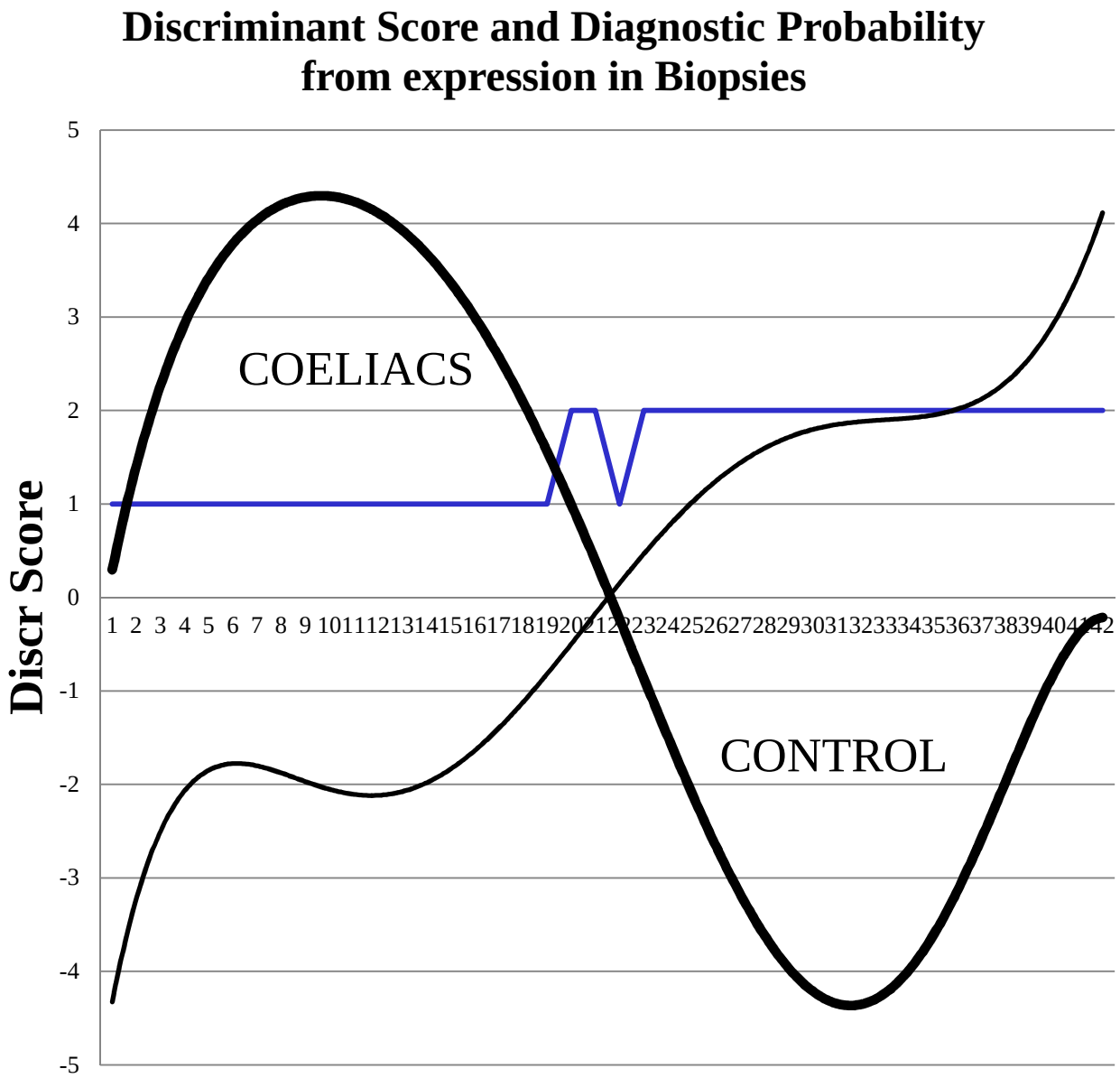
1----------0

**Wilks' Lambda**

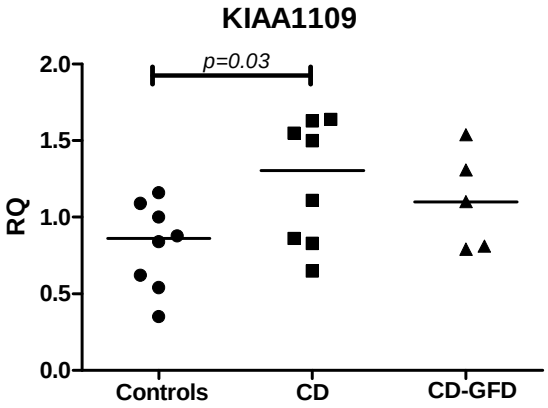
# Classification results

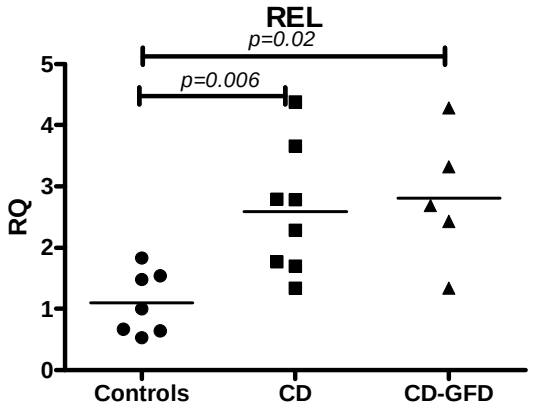
| | | Predicted Group Membership | | Total |
|---|---|---|---|---|
| | STATUS | Control | Celiac | |
| Count | Control | 19 | 1 | 20 |
| | Celiac | 2 | 20 | 22 |
| | | | | |
| % | Control | 95 | 5 | 100 |
| | Celiac | 10 | 90 | 100 |
| | | | | |

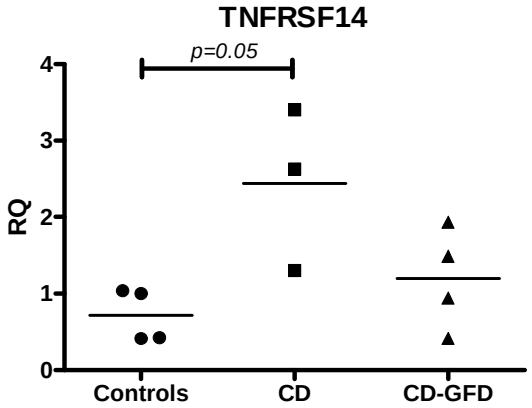
**92,9% of original grouped cases correctly classified.**


Discriminant Score and Diagnostic Probability
from expression in Biopsies
Discr Score
5
4
3
2
1
0
-1
-2
-3
-4
-5
COELIACS
CONTROL

# EXPRESSION Monociti da Sangue Periferico




- Endopeptidasi
- Si suppone che nei mammiferi abbia un ruolo nella regolazione della crescita delle cellule epiteliali e nella differenziazione e sviluppo tumorale

(Kuo et al., 2006)

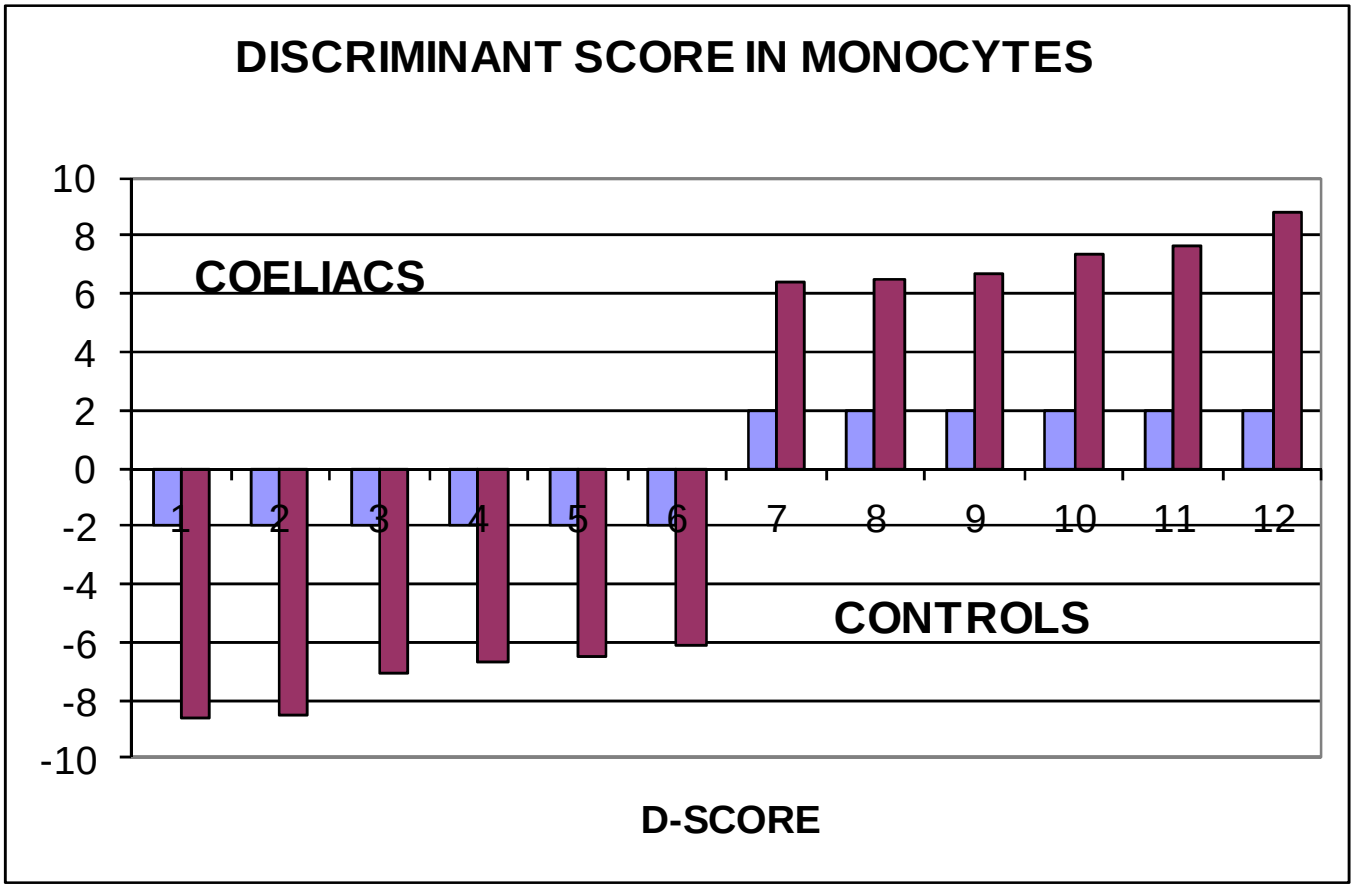
- Subunità del complesso NF-kB
- Può innescare ma anche terminare (feedback - ) i meccanismi di azione pro-infiammatoria di NF-kB

(Bonizzi et al, 2004, Lawrence et al, 2005).

- Conosciuto anche come Herpesvirus Entry Mediator (HVEM).

**Interessamento infiammatorio a livello periferico**

# Classification based on the expression of 4 gene in blood leucocytes



# Diseases in symptomatic cases

| Regions | GI Symptoms | Anaemia | Osteopenia | Abnormal Liver | Short Stature Children |
|---|---|---|---|---|---|
| *North* | 214.069 | 100.738 | 75.554 | 25.185 | 10.047 |
| *N.East* | 18.557 | 8.733 | 6.550 | 2.183 | 927 |
| *M.East* | 123.447 | 58.093 | 43.570 | 14.523 | 8.849 |
| *East* | 52.903 | 24.896 | 18.672 | 6.224 | 4.535 |
| *South* | 252.846 | 118.986 | 89.240 | 29.747 | 21.125 |
| MED tot | 657.119 | 309.232 | 231.924 | 77.308 | 41.609 |

Possiamo prevenirne almeno una parte ????