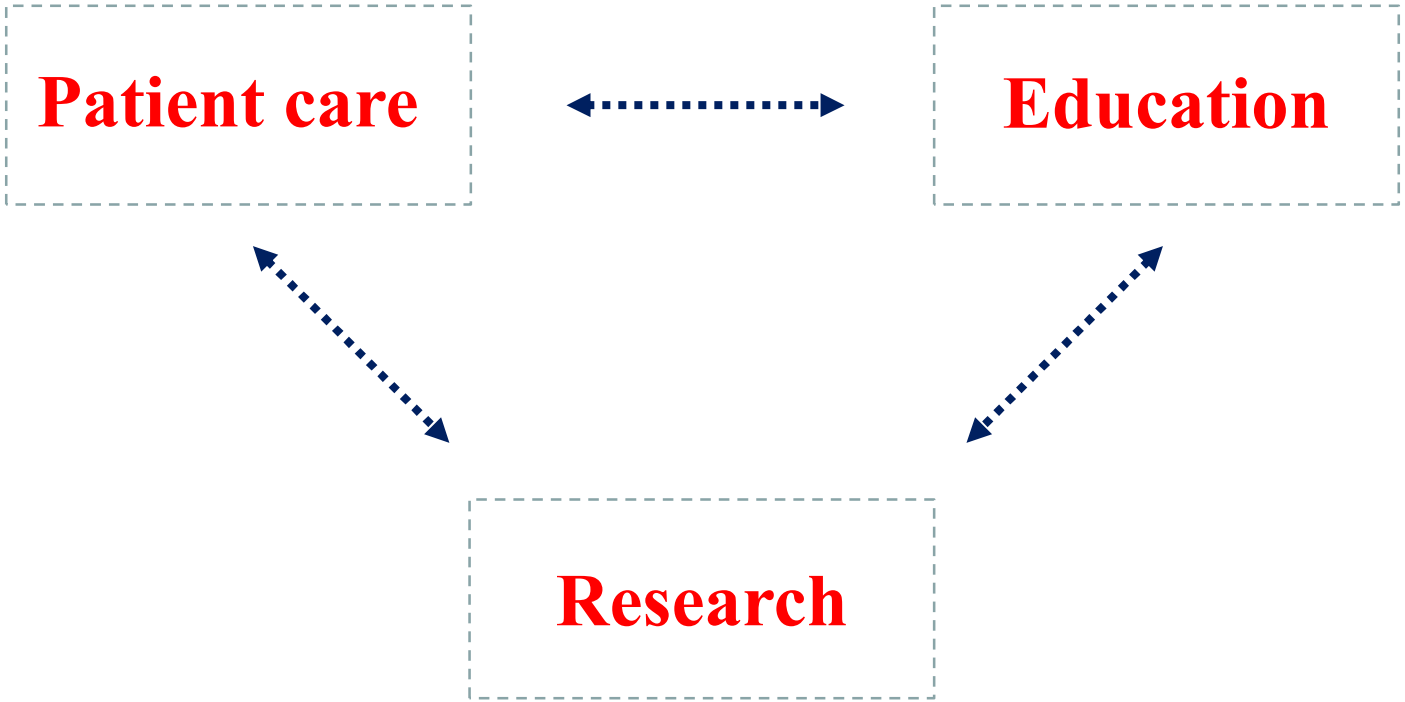
Benefits from molecular biology and genetic studies in the clinical work

Genetic and molecular studies to uncover the pathogenesis of acne

Kornélia Szabó, PhD

Different arms at the Department of Dermatology and Allergology



Genetic and molecular studies to uncover the pathogenesis of common multifactorial skin and allergic diseases (psoriasis, acne, rhinitis)

How can molecular biology and genetic studies help in the everyday clinical work?

Molecular studies

*identification of the exact molecular events playing a role in the pathogenesis of various diseases

⇨

*identification of new drug targets for the development of novel treatment modalities and therapeutic options

Genetic studies

*identification of the genetic inheritance of various diseases

⇨

*provide the better understanding of disease pathogenesis
*help the education of patients

How can molecular biology and genetic studies help the better understanding of acne pathogenesis?

Their use in the everyday clinical work and in the education

General introduction (skin, acne)

Classical genetic studies:

What do they teach us about the pathogenesis, clinical and demographical characteristics and population-related differences regarding this common skin disease?

How do they change our current understanding of the exact role of various pathogenic factors in the disease pathogenesis?

Molecular genetic studies:

What do they teach us about the molecular pathogenesis of acne?

Summary

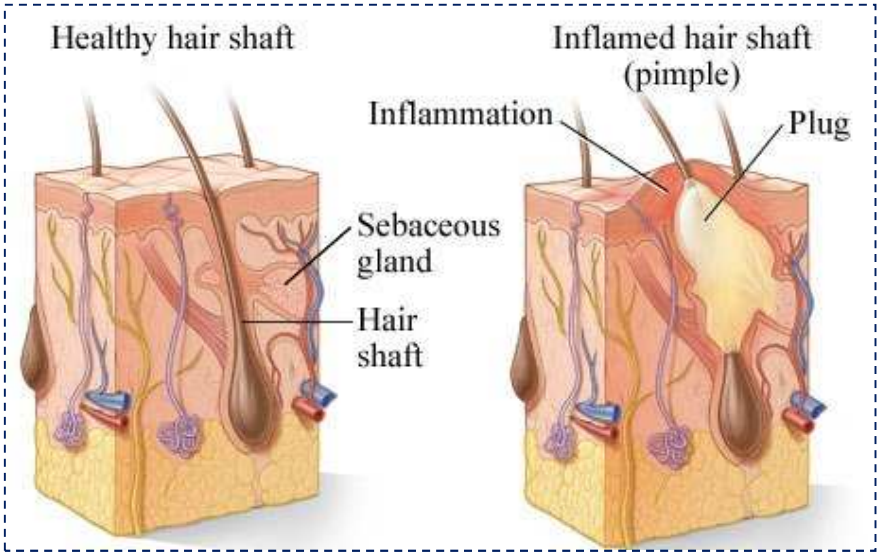
How these results can be used in the clinical work and in education?

Acne is a multifactorial inflammatory disease of the pilosebaceus unit

Environmental factors

hypercolonization of *Propionibacterium acnes *(P. acnes),* resulting an activation of innate immune events in the keratinocytes

***individual life style factors** (pl. diet, smoking, stress)



Self factors

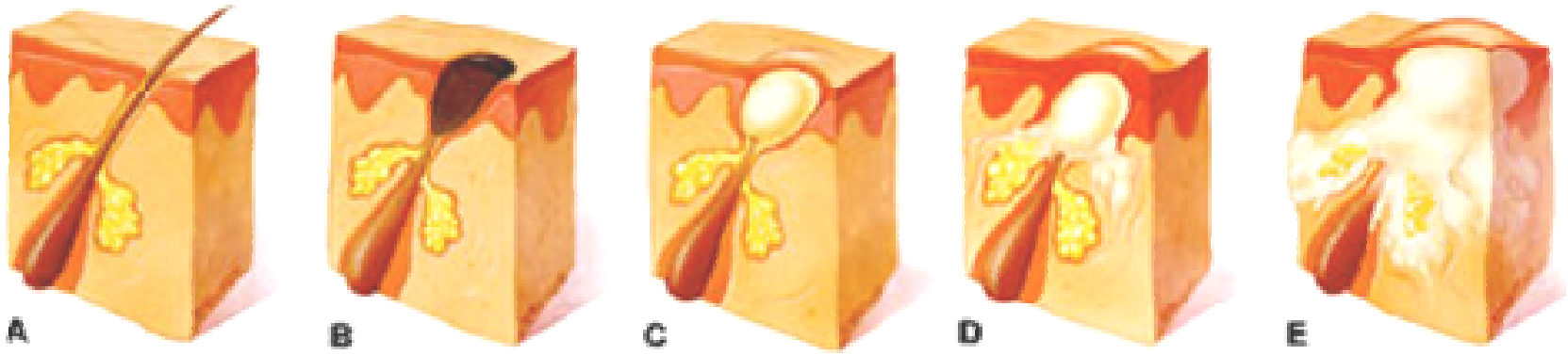
***hormonal changes around puberty**

***abnormal cellular properties of sebocytes** (increased sebum secretion)

***abnormal cellular properties of epidermal keratinocytes** (increased proliferation and differentiation properties leading to e.g. follicular hyperkeratosis)

***individual genetic susceptibility and protective factors**

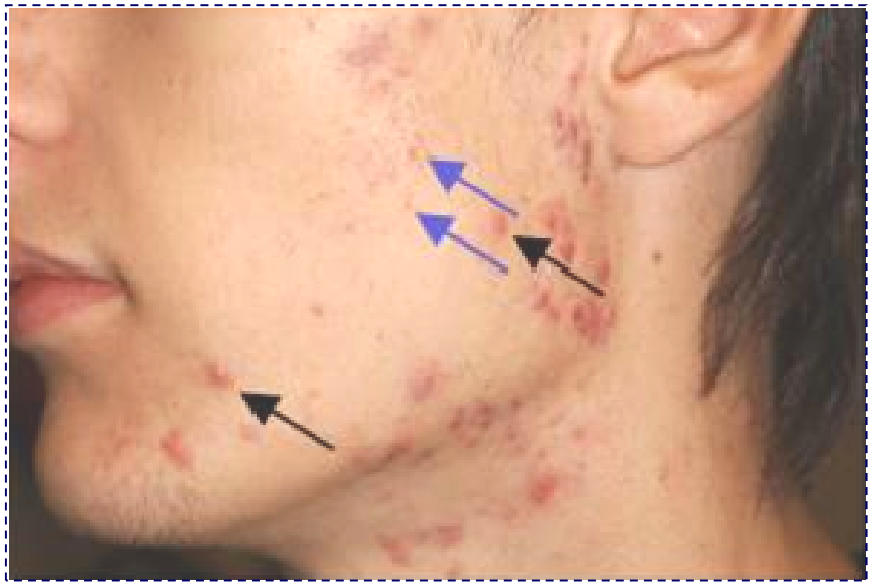
Acne is a multifactorial inflammatory disease of the pilosebaceus unit

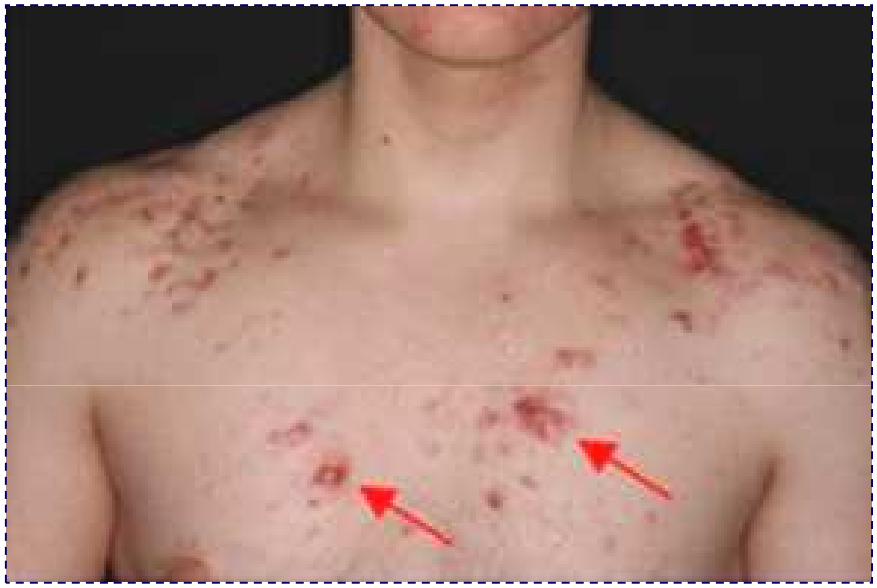


healthy skin	open comedo (blackhead)	closed comedo (whitehead)	papules and pustules	nodules and cysts

INFLAMMATION

Acne – clinical picture



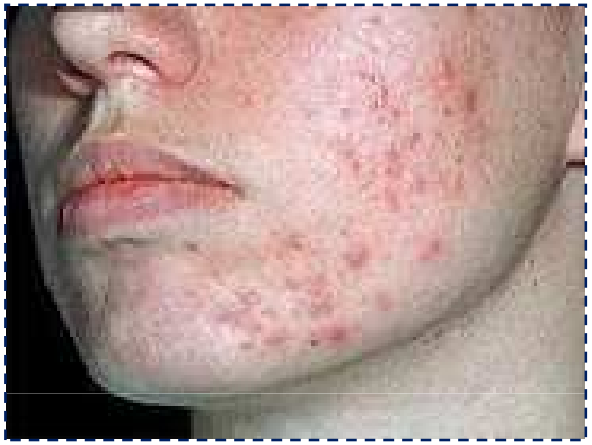


- comedos (blackheads and whiteheads)
- papules and pustules
- nodules and cysts

Acne – clinical types

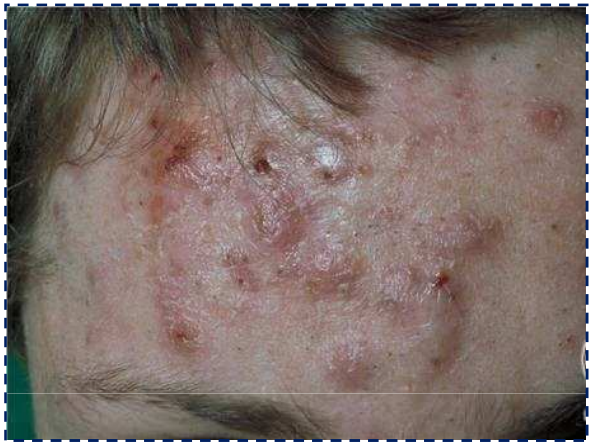
acne codedonica

main lesions are open and closed comedos, usually appear on the face



acne papulopustulosa

next to the comedos inflamed papules and pustules are also present, on the face, trunk and chest



acne nodulocystica

comedos, papules, pustules, painful nodules and cyst are present together on the face, trunk and chest (scaring)

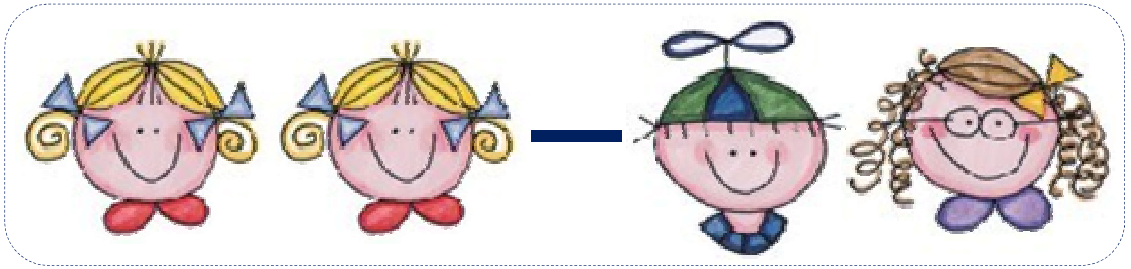
INFLAMMATION

Classical genetic studies

Hermann Werner Siemens

1891. 08. 20 (Charlottenburg) – 1969. 11. 21 (Leiden)

- First systematic twin studies to find out if genetic factors play a role in the pathogenesis of acne (1926.)
- Worked out the rules of twin studies
- Distinction between mono- and dizygotic twins

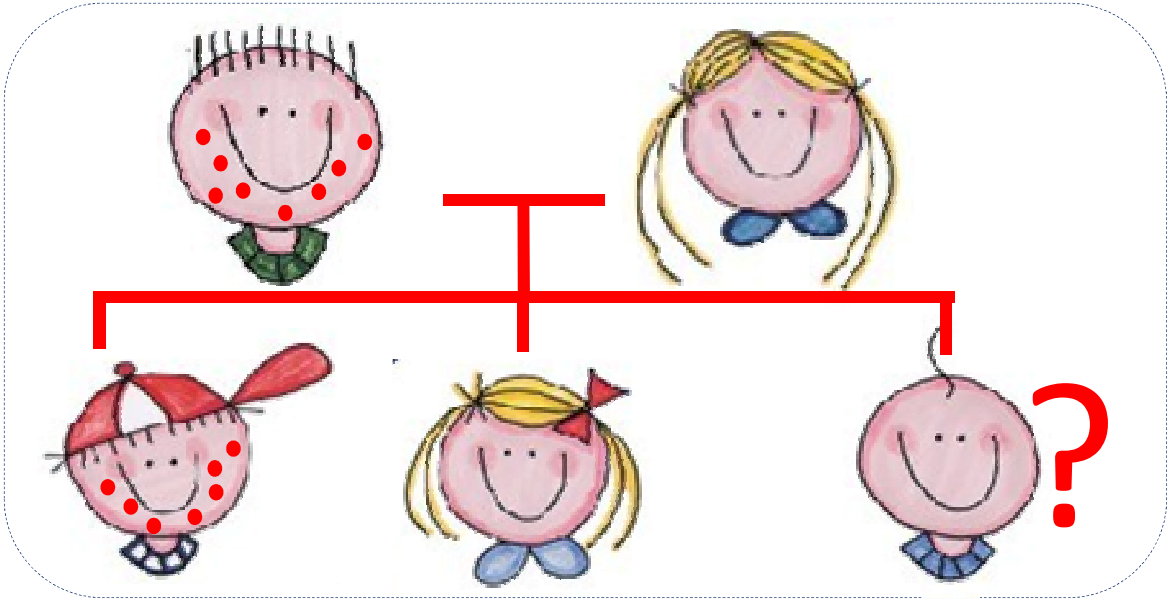


His results suggest that inherited factors play an important role in the pathogenesis of acne

The first systematic family studies

Hugo Hecht (1960.)

- Systematic family studies by analyzing a database established from questionnaires (gender, relatives, acne status, inherited physical features within the family)
- Whoever the kid (sufferring form acne) resembles in appearance, he/she will also resemble in his/her acne severity too.

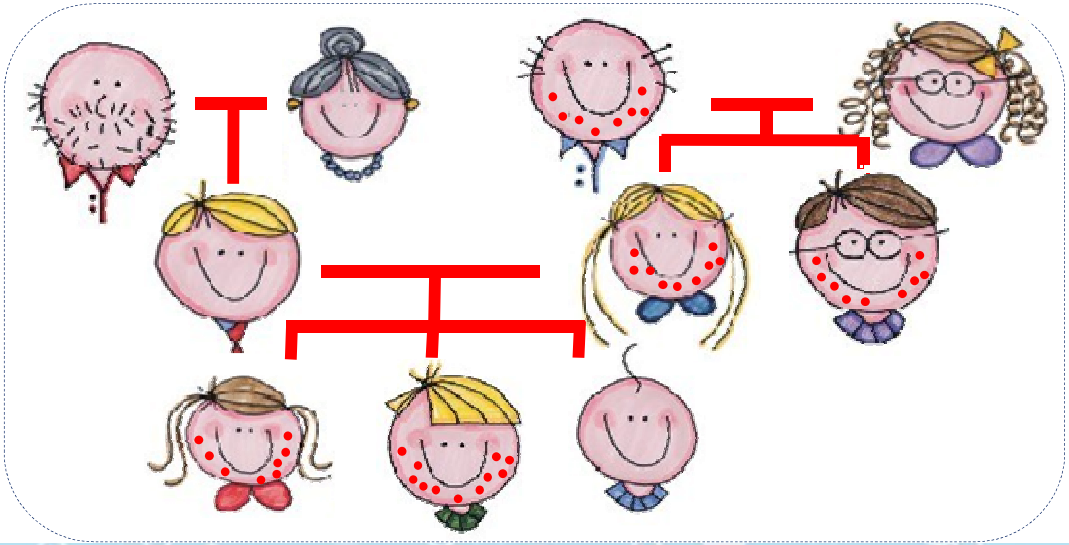


Systematic twin studies I.

(From 1950.)

- There is a strong concordance in the occurrence and severity of acne in identical twins.

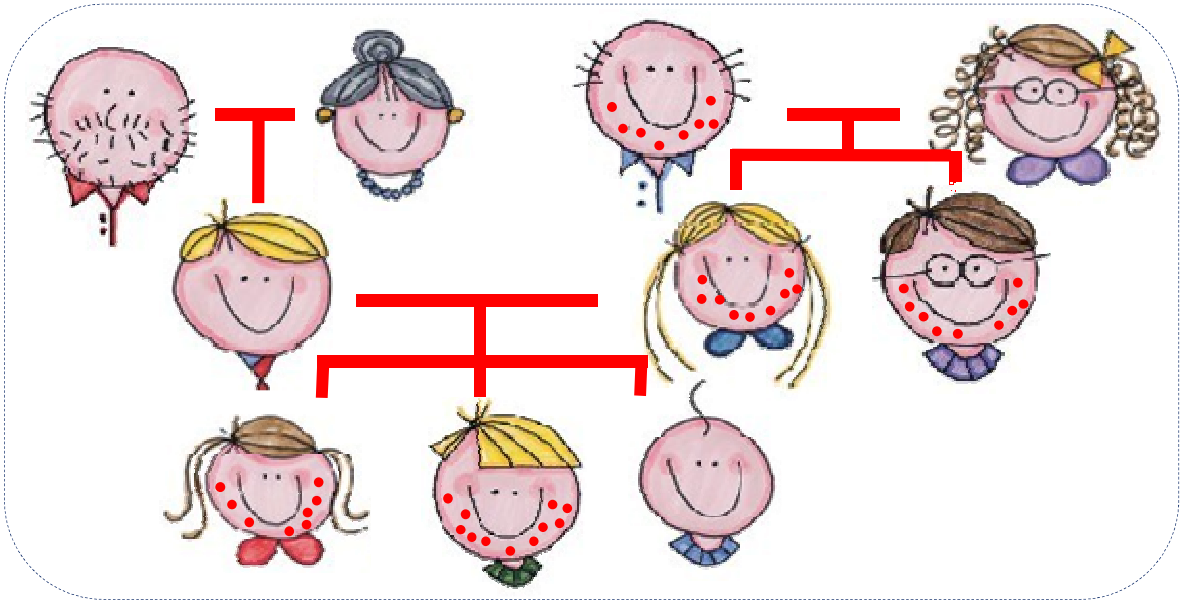
- There are families where the family members often suffer from severe acne symptoms. A person, whose parents had acne in their teenage years has a higher chance to suffer from such skin symptoms too (especially the mother's acne status is an important determinant).



Systematic twin studies II.

(From 1950.)

- The inheritance of acne does not follow the Mendelian rules, and these results suggests that acne is a multifactorial disease (some elements of the hormonal system and sebum secretion can be genetically determined.)



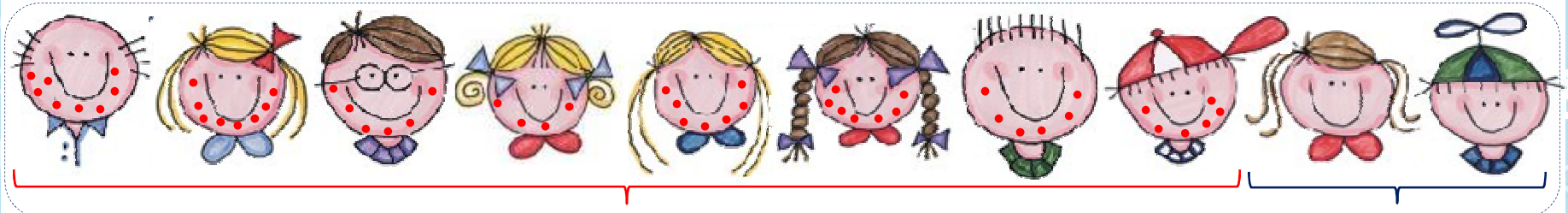
?

acne– polygenic, multifactorial skin disease

Population and cross-sectional studies I.

Westernized countries

- The prevalence of acne is 80-90%



- The symptoms first appear at the age of 10-12.The severity of symptoms gradually increases till the age of 16-18 and then they disappear by the mid 20s.

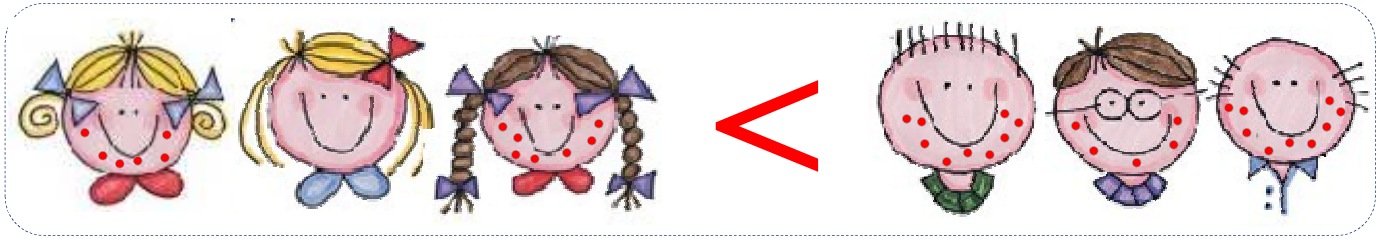
age in years

Population and cross-sectional studies II.

Westernized countries

- In 12 year olds the ratio of girls suffering from acne is higher, but in older age groups this trend reverses.

12 year old 

18 year old 

What about the natural populations?

Natural populations – acne I.

Schaefer, O; Nutr Today; 2010.

Findlay, GH; Br J Dermatol; 1967.
Park, RG; Br J Dermatol; 1968.

Steiner, PE; Arch Pathol. 2010.

Freyre, EA; J Adolescent Health; 19

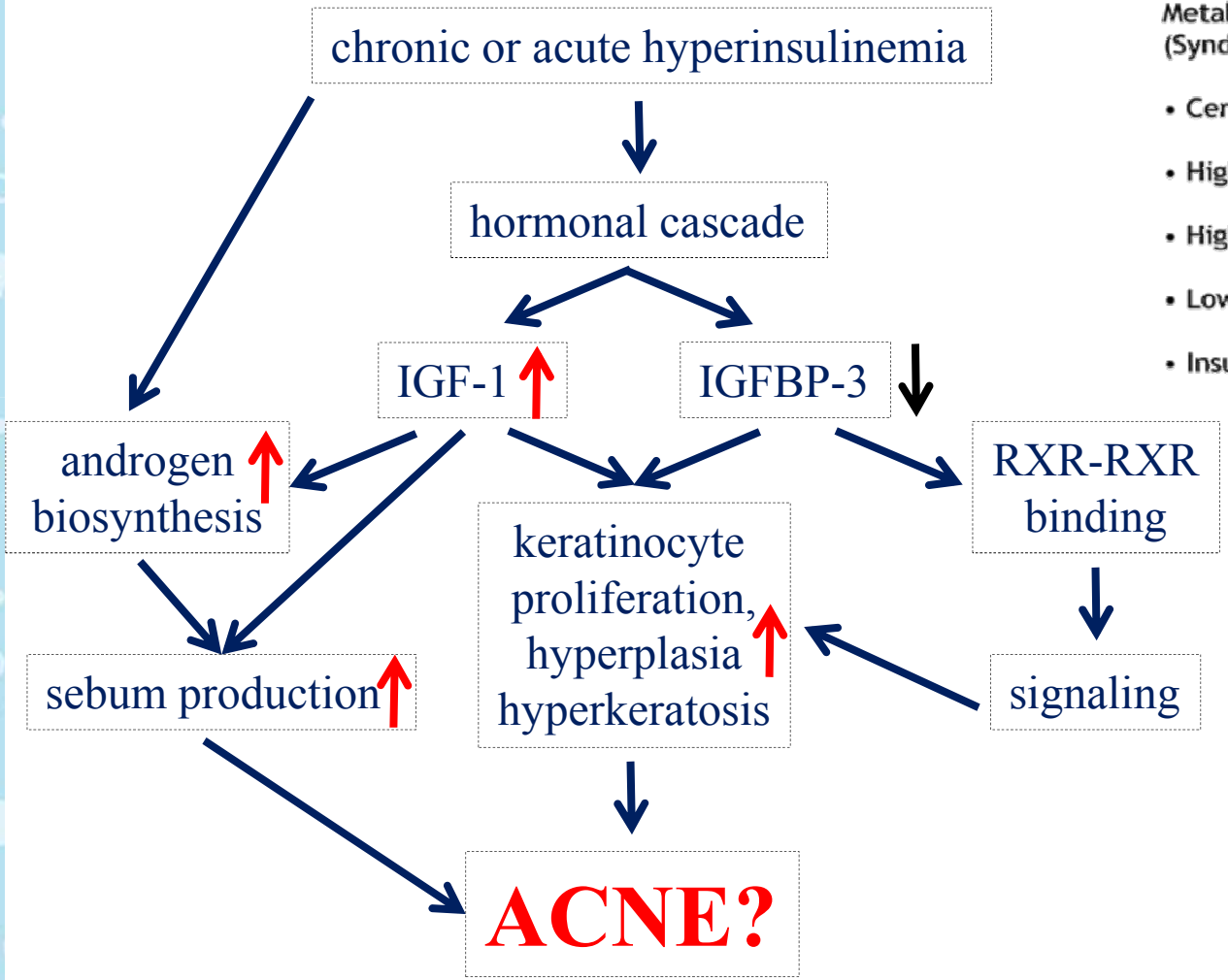
Cordain, L; Arch Dermatol; 2002.

Westernized countries – natural populations

Westernized countries	Individual and environmental factors	Natural populations
MAJOR PROBLEM	diabetes, arthritis, heart and coronaria diseases)	NOT SIGNIFICANT

Is acne a result of Westernized life style?

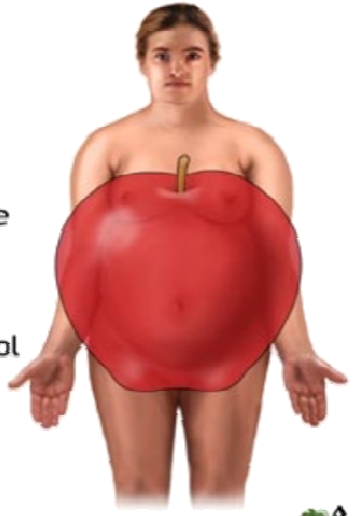
Acne and metabolic syndrome?

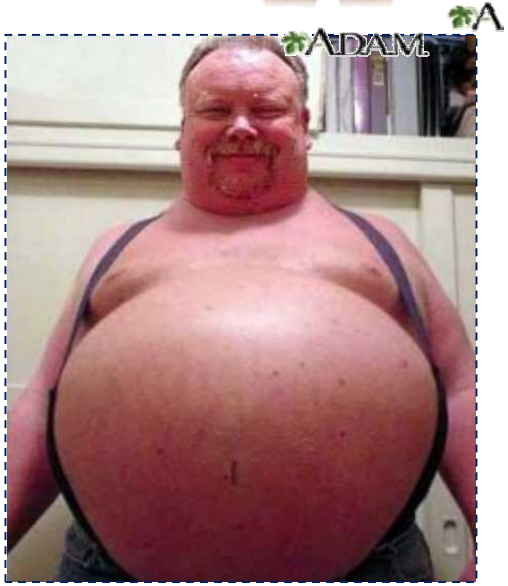


Based on Berra B. Clin in Dermatol. 2009.

Metabolic syndrome
(Syndrome X)

- Central obesity
- High blood pressure
- High triglycerides
- Low HDL-cholesterol
- Insulin resistance





Molecular genetic studies

Acne is a multifactorial inflammatory disease of the pilosebaceus unit

Environmental factors

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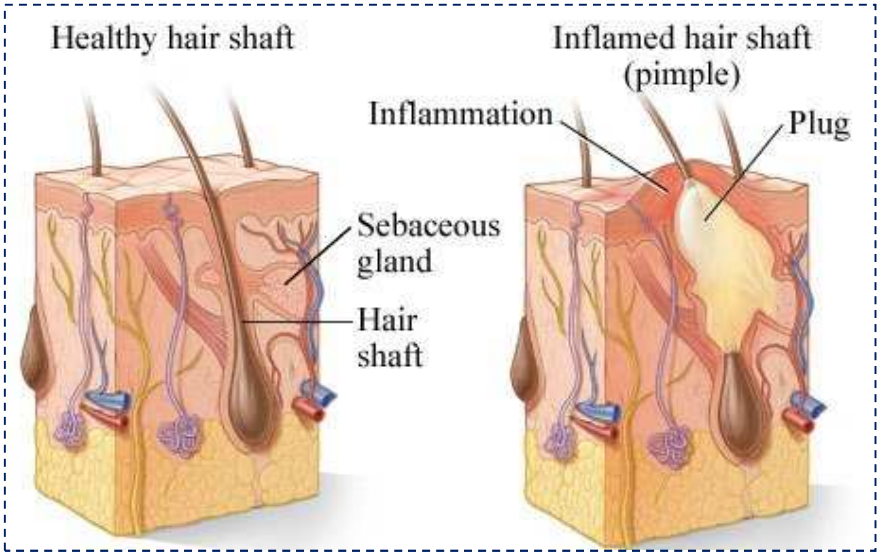
***individual life style factors** (pl. diet, smoking, stress)



Self factors

***hormonal changes around puberty**

***abnormal cellular properties of sebocytes** (increased sebum secretion)

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***individual genetic susceptibility and protective factors**

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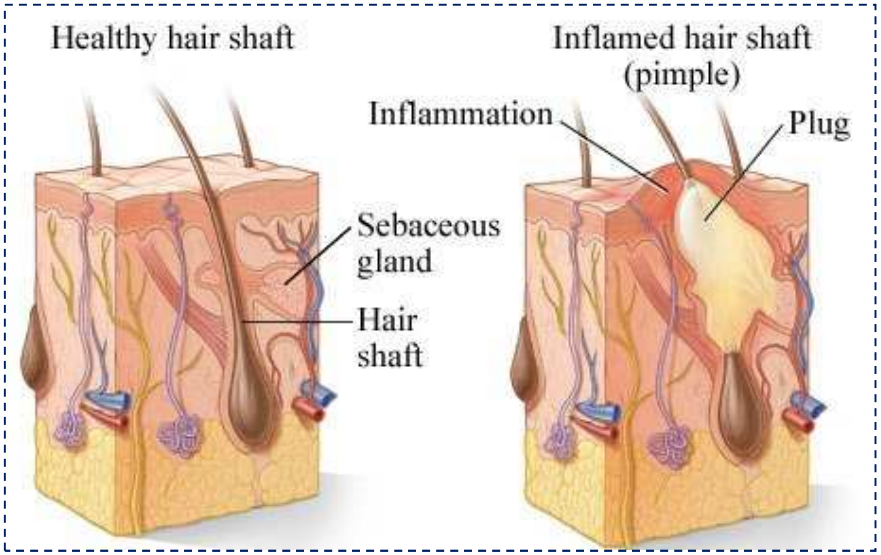

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***individual genetic susceptibility and protective factors**

Molecular genetic studies

(From 1989.)

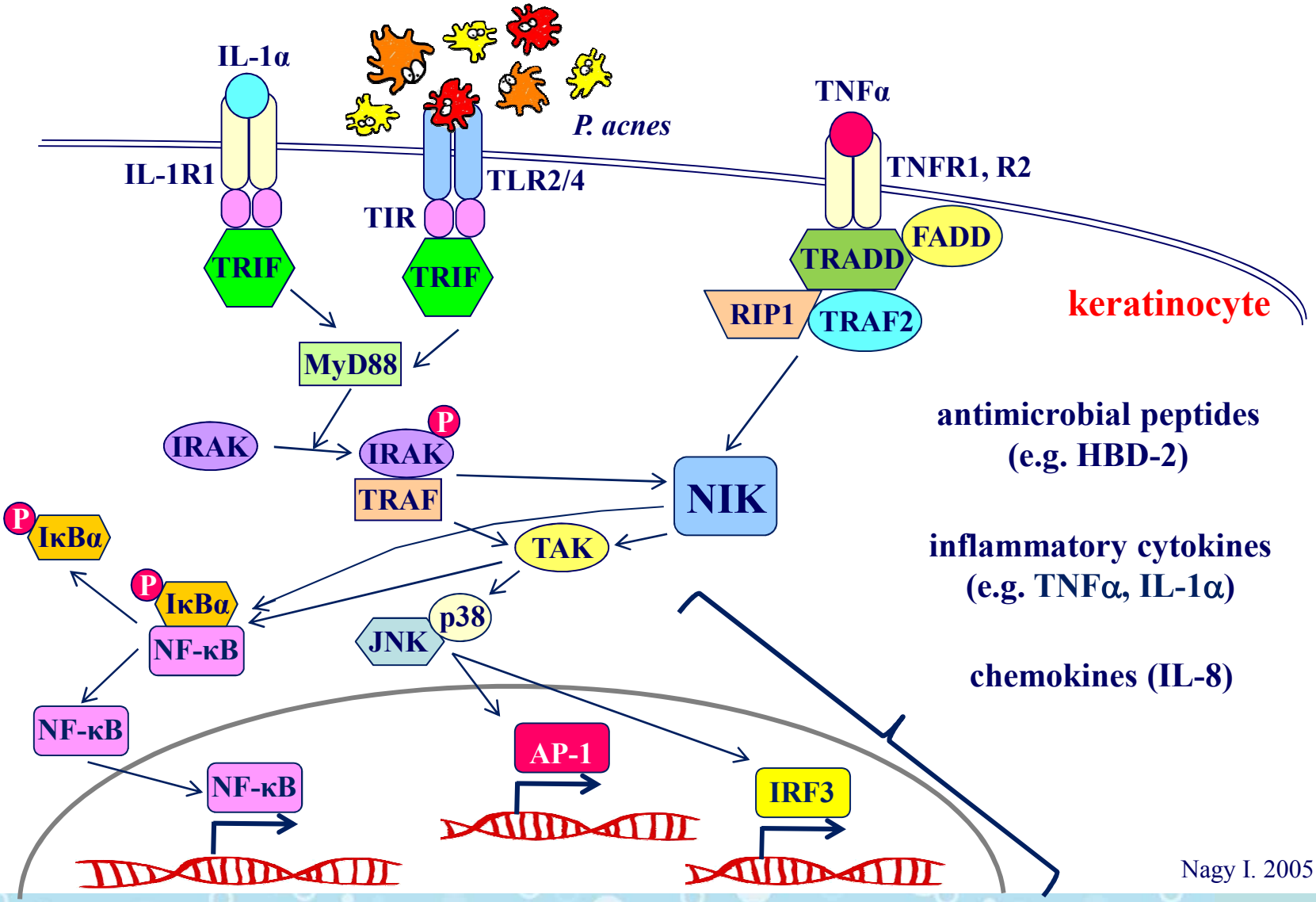
genes playing a role in the regulation of the innate immune function of keratinocytes

TLR2	Koreck, 2006.
TLR4	Koreck, 2006.
TNFA	Baz,. 2008. Sobjanek, 2009. Szabó, 2010.
IL-1A	Szabó, 2010.
IL1RN	Szabó, 2010.
MUC1	Ando, 1999.
TNFR2	Tian, 2010.

regulating the metabolism of steroid hormones

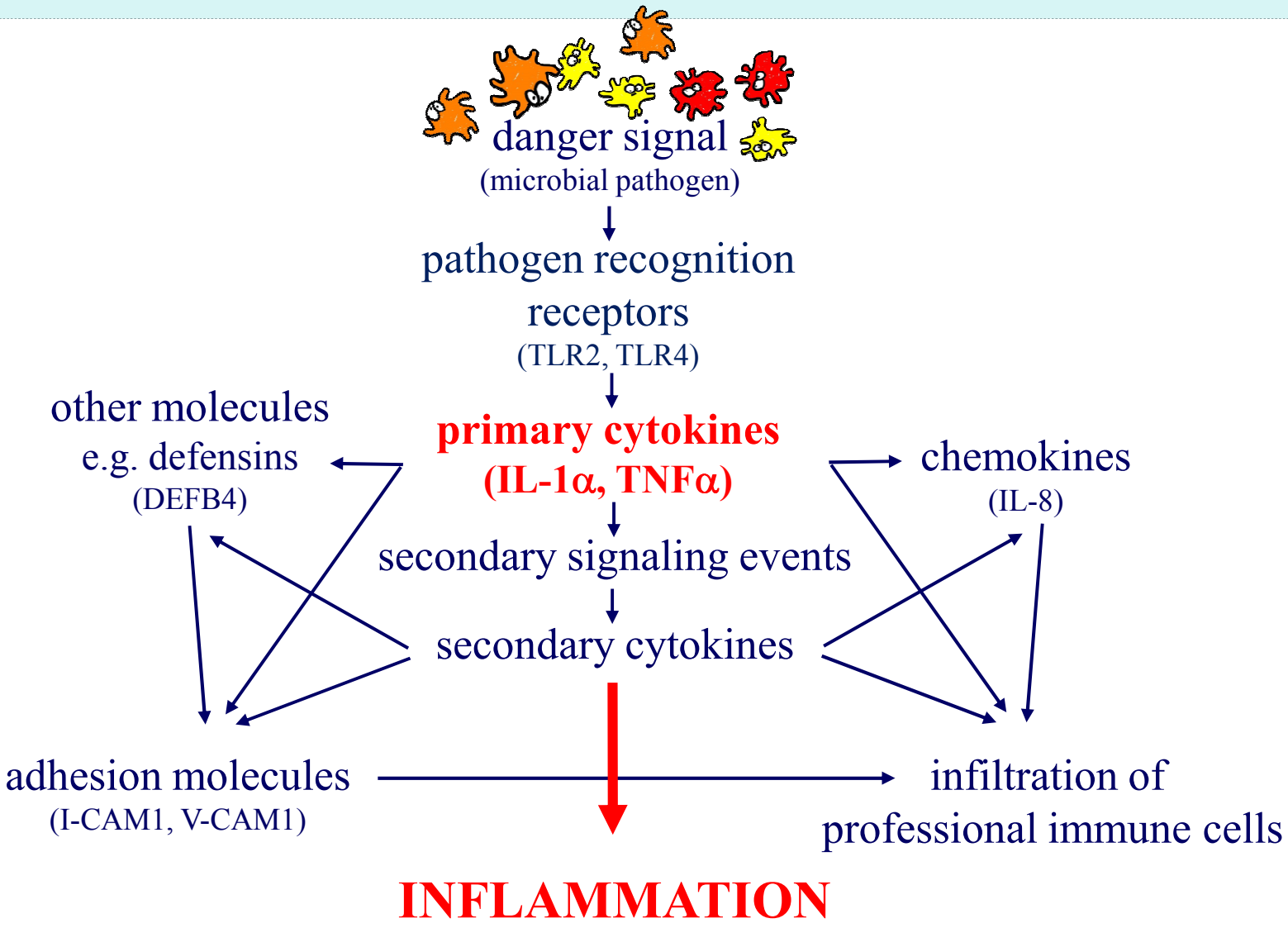
AR	Sawaya, 1999. Yang, 2009. Pang, 2008.
CYP1A1	Paraskevaidis, 1998.
CYP17	He, 2006.

Innate immune function of keratinocytes



Nagy I. 2005

Innate immune function of keratinocytes II.



Methods I.

(Retrospective case-control study)

Acne patients: **229** (female/male = 136/93)
Controls: **126** (female/male = 91/35)

Stratification of the patient group based on the severity of inflammatory acne symptoms:

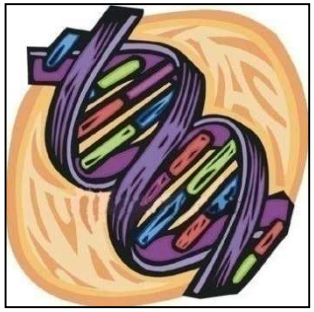
1. Acne comedonica group (acne 1)
2. Acne papulopustulosa group (acne 2)
3. Acne nodulocystica group (acne 3)

Statistical analysis: Pearson's χ^2 test
χ^2 for linear trend test

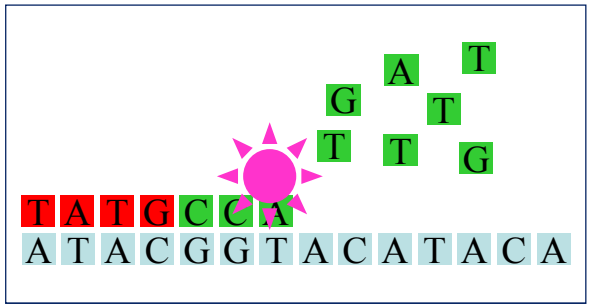
Methods II.

(Restriction Fragment Lenght Polymorphism analysis)

peripheral blood collection



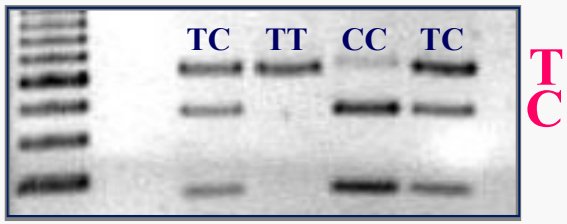
genomic DNS isolation



PCR amplification

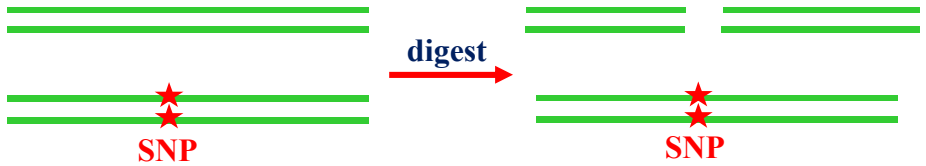
VNTR

SNP



running of the digested PCR fragments on an agarose gel

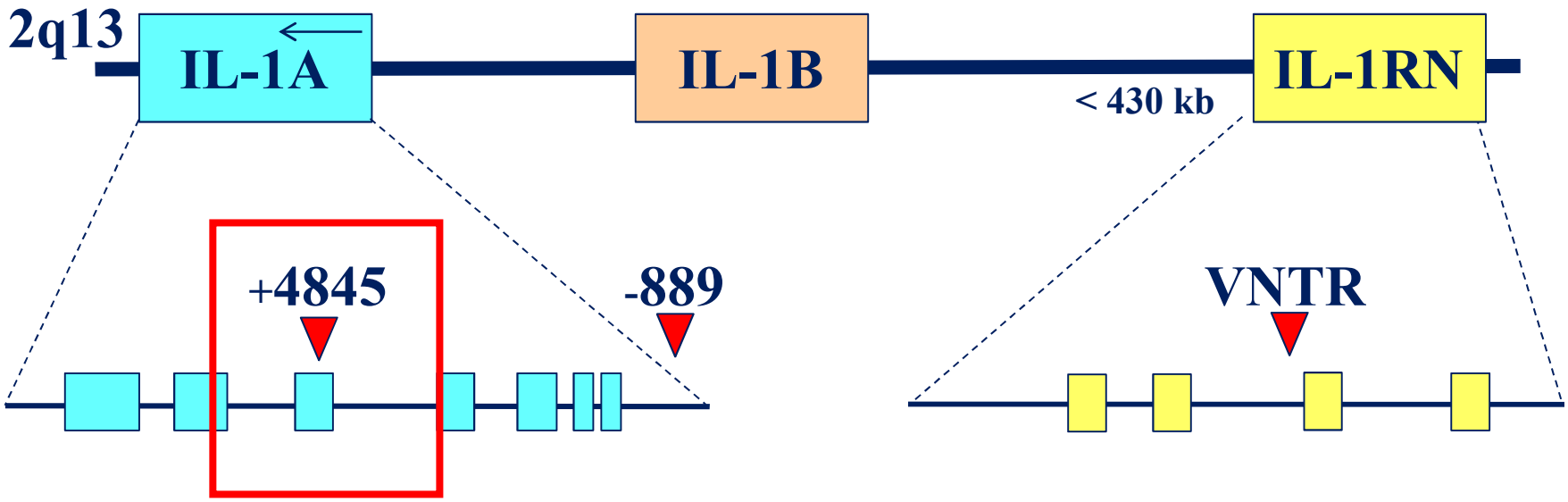
TT: homozygote wild type
CT: heterozygote
CC: homozygote minor



differential cleavage of the ampified PCR fragments carrying the minor and major alleles

The IL-1A +4845 G>T SNP

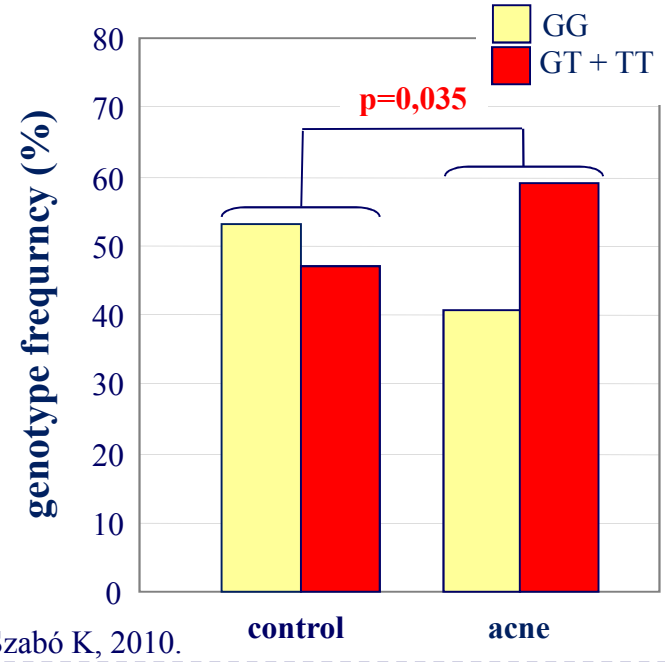
(rs17561)



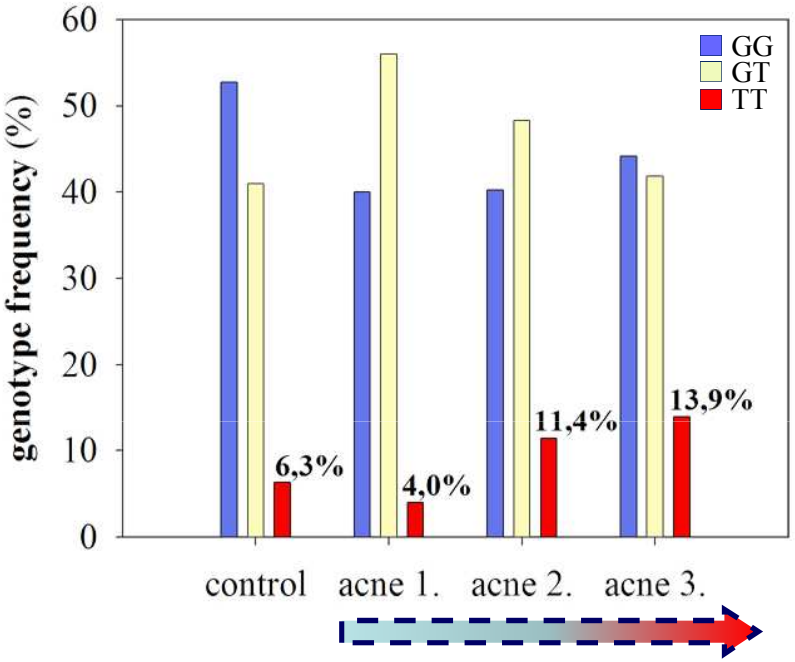
The IL-1α pro-inflammatory cytokine is encoded by the IL-1A gene

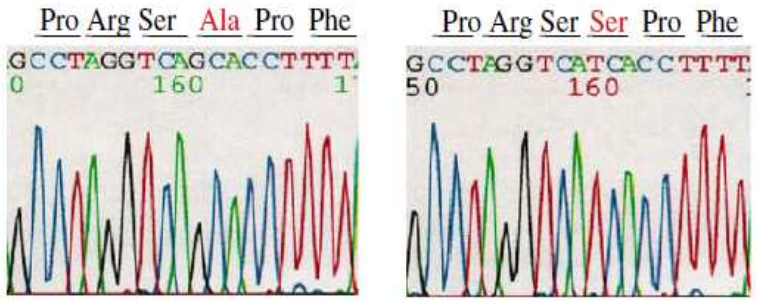
This cytokine possesses a wide spectrum of metabolic, hysiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. It binds to the interleukin-1 receptor

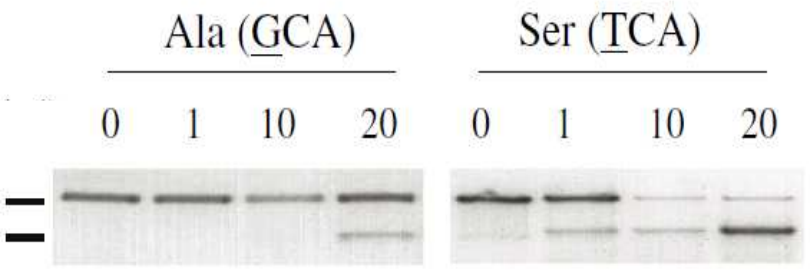
The role of the IL-1A +4845G>T SNP in the pathogenesis of acne I.



Szabó K, 2010.

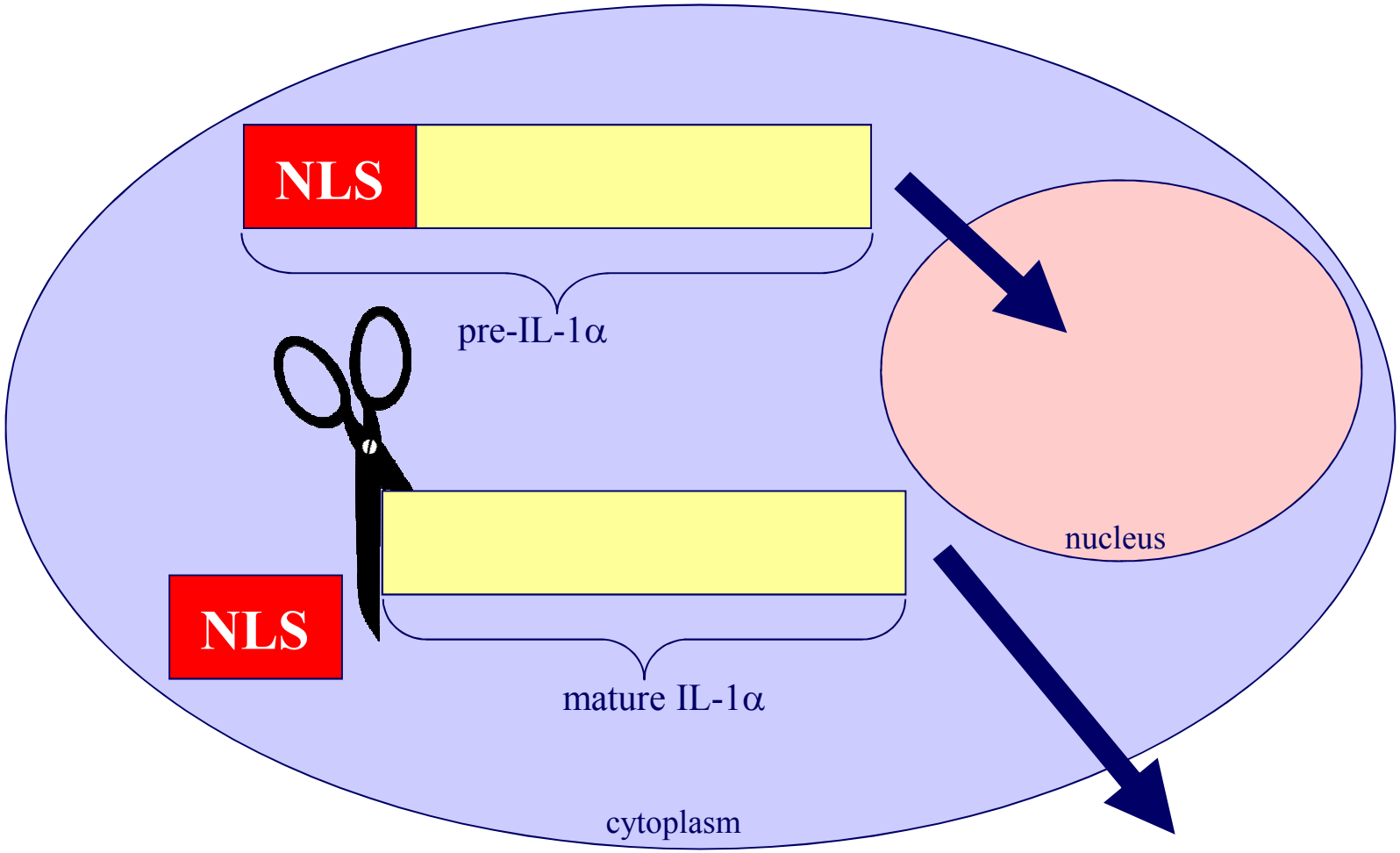




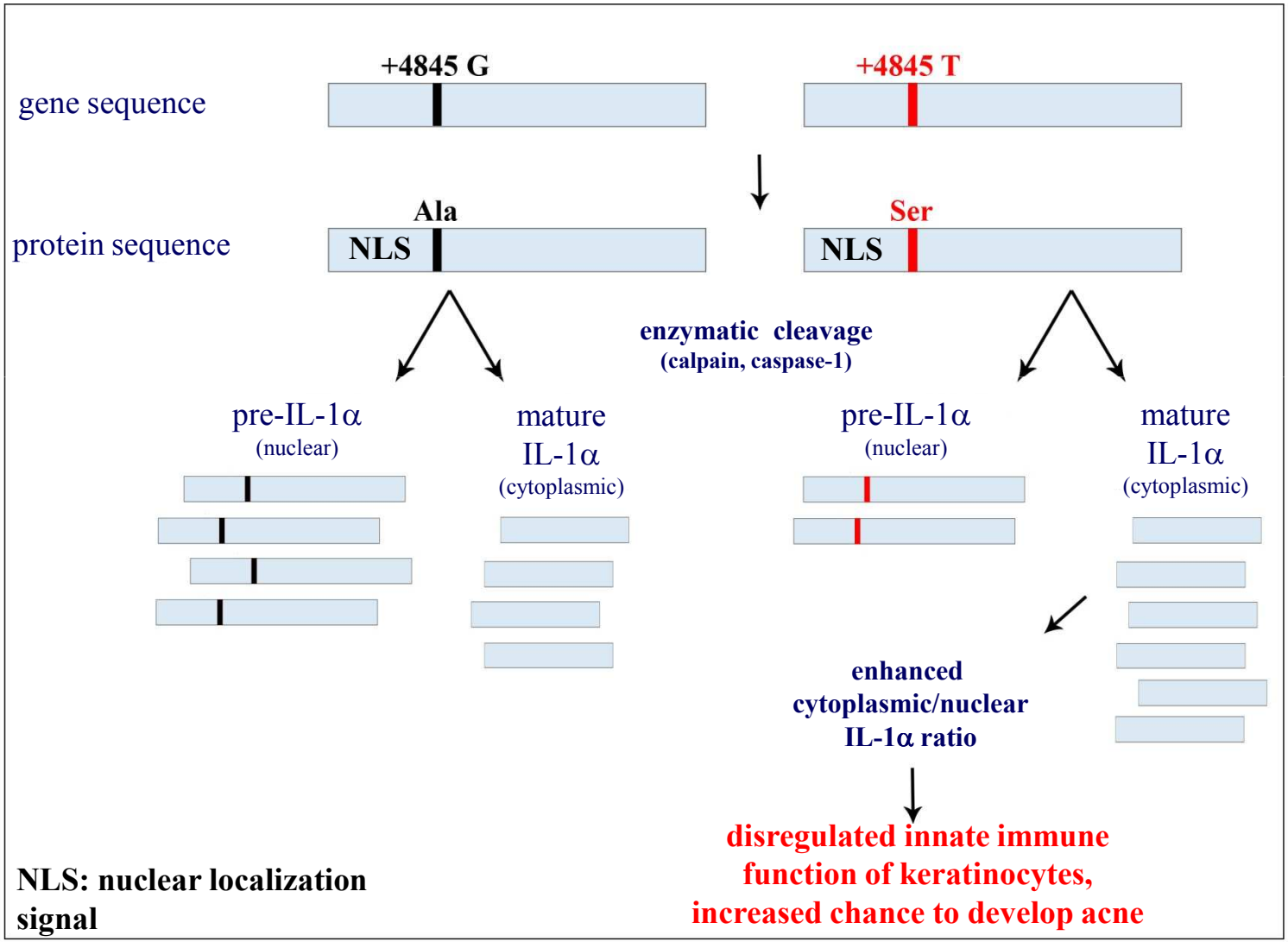


Kawaguchi, 2006.

Proposed model of how the IL-1A +4845G>T SNP functions in keratinocytes I.

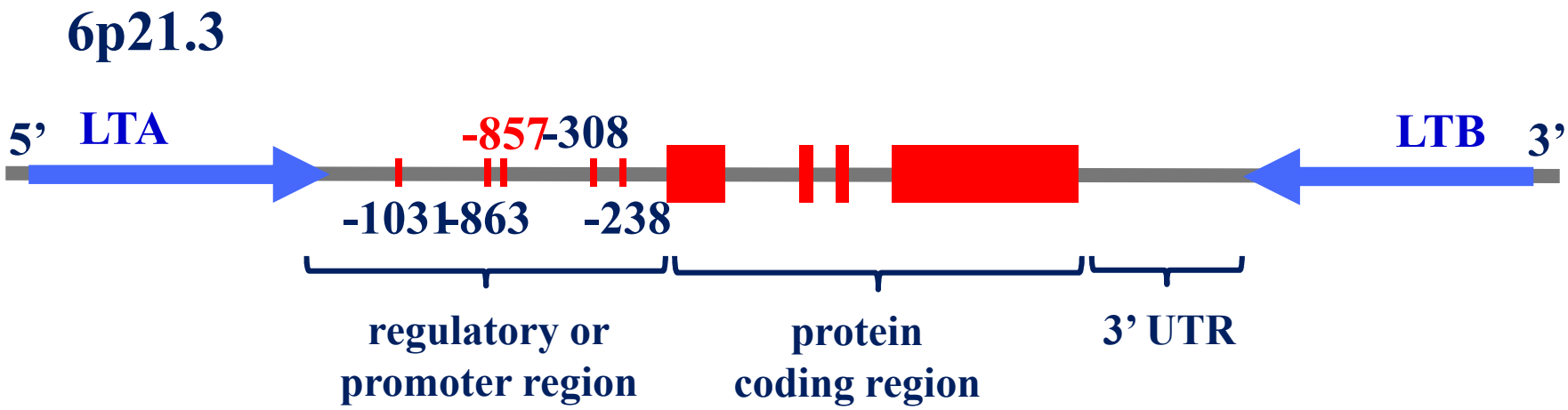


Proposed model of how the IL-1A +4845G>T SNP functions in keratinocytes II.



The TNFA -857C>T SNP

(rs1799724)

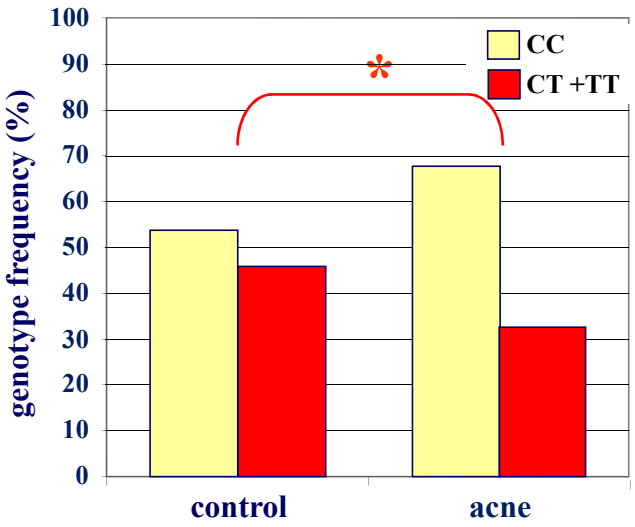


The TNFα pro-inflammatory cytokine is encoded by the TNFA gene

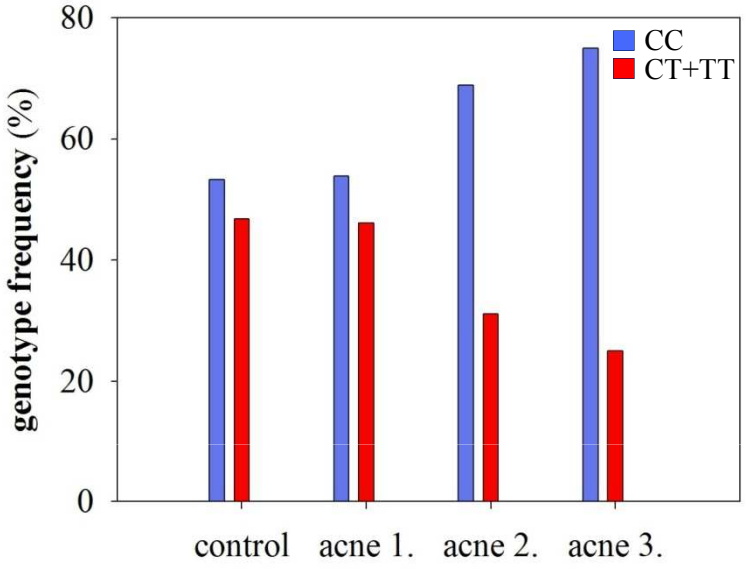
It is able to induce apoptotic cell death, inflammation, inhibits tumorigenesis and viral replication.

Dysregulation of TNF production has been implicated in a variety of human diseases, including Alzheimer's disease, cancer and a variety of other chronic inflammatory diseases.

The role of the TNFA -857C>T promoter SNP in the pathogenesis of acne



Szabó K, 2010.



The percentage of minor T allele containing genotypes (CT+TT) decreases as the inflammatory acne symptoms are increaseing in the different acne patient subgroups

Major allele (C):

AAGTCGAGTATGGGGACCCCCCCTTAA**C**GAAGACAGGGCCATG

-857

Minor allele (T) :

NF-κB

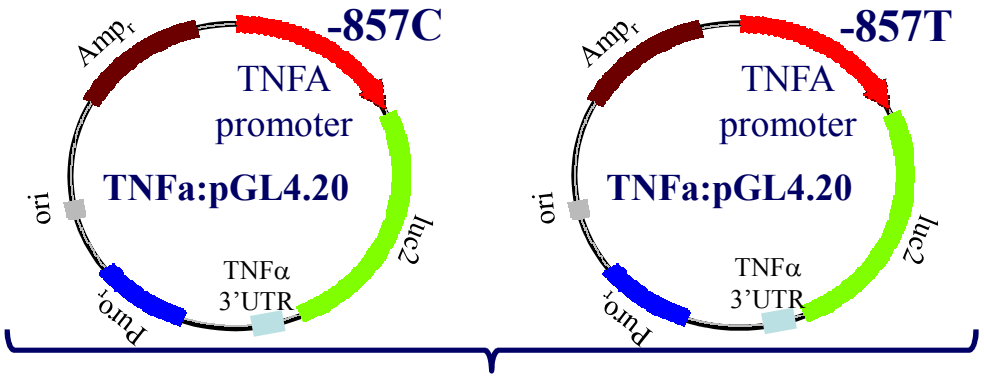
Oct-1

AAGTCGAGTATGGGGACCCCCCCTTAA**T**GAAGACAGGGCCATG

-857

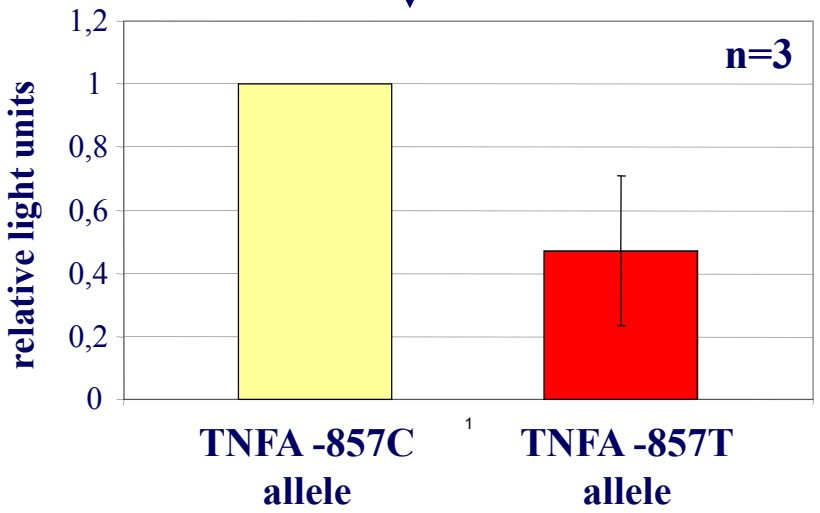
TF search: www.cbrc.jp/research/db/TFSEARCH.html

In vitro luciferase riporter assay

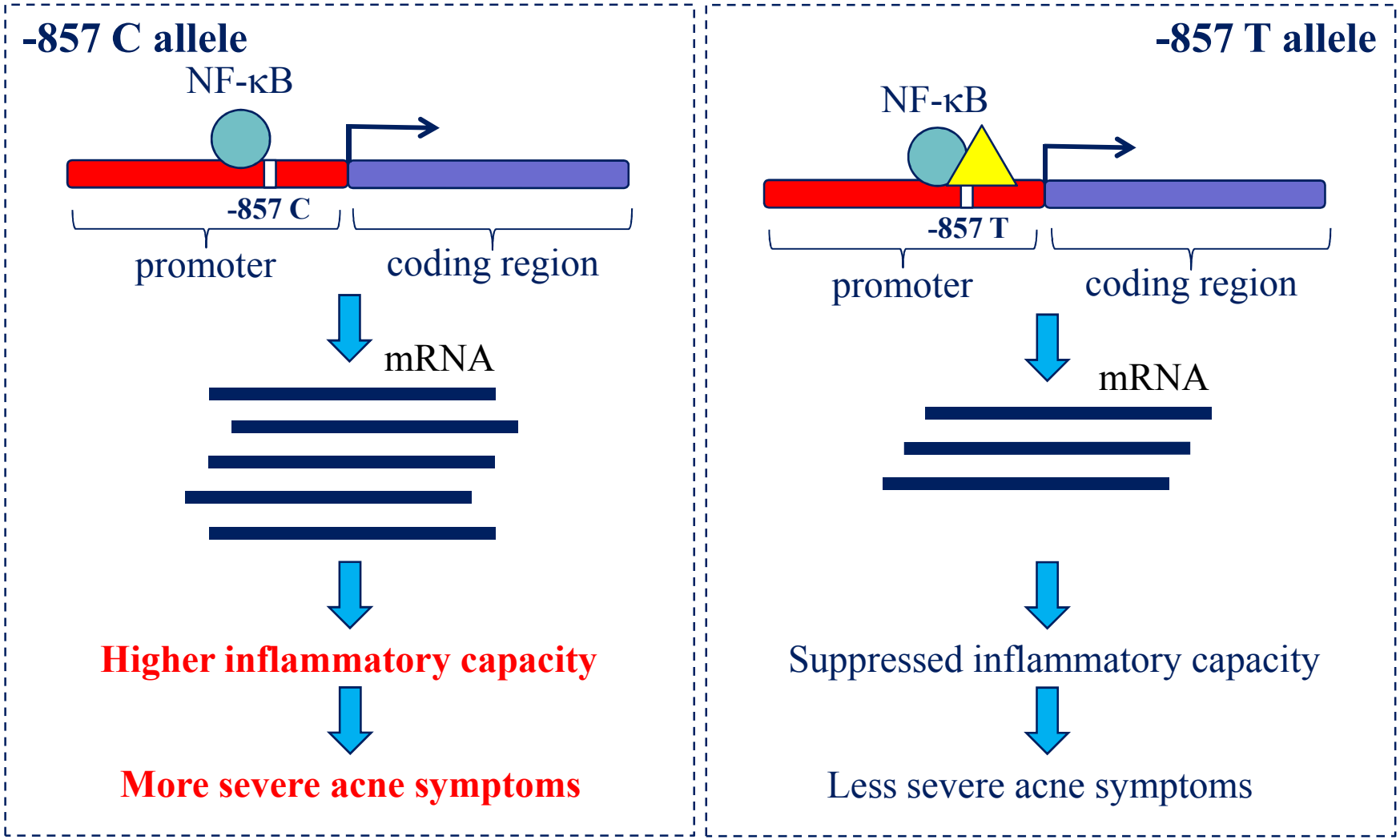


Transient transfection to an immortalized human keratinocyte cell line

Luciferase assay

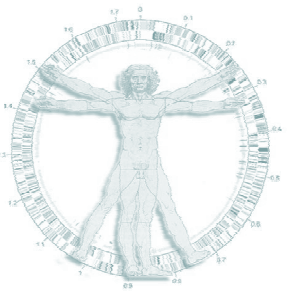


Proposed model of how the TNFA -857C>T SNP functions in keratinocytes



Conclusions I.

- Results of the **classical genetic studies** provided and still provides a lot of descriptive data on the development and timing of acne lesion formation, and on the prevalence of this common skin diseases in different populations.
- In the everyday clinical work they provide a lot of useful, epidemiologic information.



Conclusions II.

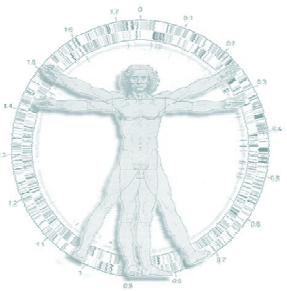
- Today, **molecular genetic studies** identify inherited disease causing and protective factors playing an important role in acne pathogenesis.
- These results provide a lot of important information about the exact molecular pathogenesis of this common multifactorial skin disease.
- Better understanding of the exact disease pathogenesis provide new possibilities to develop novell treatment modalities.

The more we know about a disease the better we can treat it!

Conclusions III.

- The available genetic evidence strongly suggests that acne is a genetically determined multifactorial disease and Westernized life style plays a pivotal role in the pathogenesis of the disease.

- Early education about the healthy life-style even before and during puberty is important to prevent the development of Westernized diseases.



Conclusions IV.

The introduced work also useful to train undergraduate and graduate students.

- How to come up with scientific questions they would like to study
- Design of the experimental plan (PCR, restriction digest, agarose gel electrophoresis, cloning, cell biology work, transient transfection, luciferase measurement)
- Execution of the various experiments
- Critical analysis of the gathered data
- Presentation of the data in conferences, meetings and in scientific papers.

Conclusions V.

PhD students:

Gábor Tax (3rd. place at the Hungarian National Student Conference)
Kornélia Kis
Krisztina Szegedi

Undergraduate students:

Bettina Tábori
Orsolya Megyesi

Foreign exchange students:

Csengelle Diószegi (Sweden)
Dragos Teodorescu-Brinzeu (Romania)
Giovanna Valenti (ERASMUS student, Italy)
Two other PhD student from 2012. (Italy)

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Thank you for your attention!

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