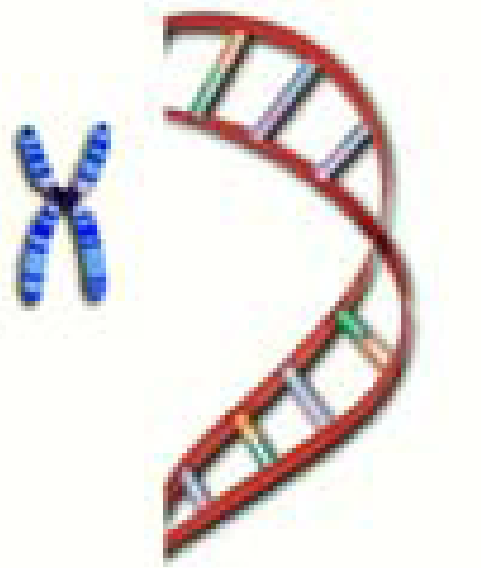
Genetics in dentistry 3

Odontogenesis

- Complex series of events including cell interactions, differentiation, elaboration of a unique extracellular matrix and mineralization are required to produce dentin
- Tooth formation is highly regulated at the molecular level
  - terminal differentiation of specific cell types
  - epithelial-mesenchymal interactions
  - secretion of specific extracellular matrices
  - controlled processing of those matrices
  - regulation of ion deposition
  - mineralization of the dental tissues

Tooth Formation depends on:

- Genetic Factors
  - Hundreds to several thousand genes likely involved (polygenic)
- Environmental Factors
  - Nutrition
  - Physical phenomenon
  - Infection



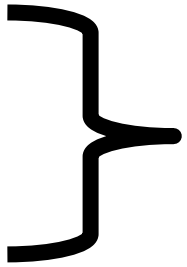
Stages Required for Tooth Formation

- Initiation
- Histodifferentiation
- Morphodifferentiation
- Apposition
  - – Secretory Phase
  - – Transition Phase
  - – Maturation Phase

- Stages are not discrete for any given tooth.
- Teeth develop over years beginning with the coronal portion of the crown.
- Can be mineralizing at the cusp tips while cervically cells are differentiating.

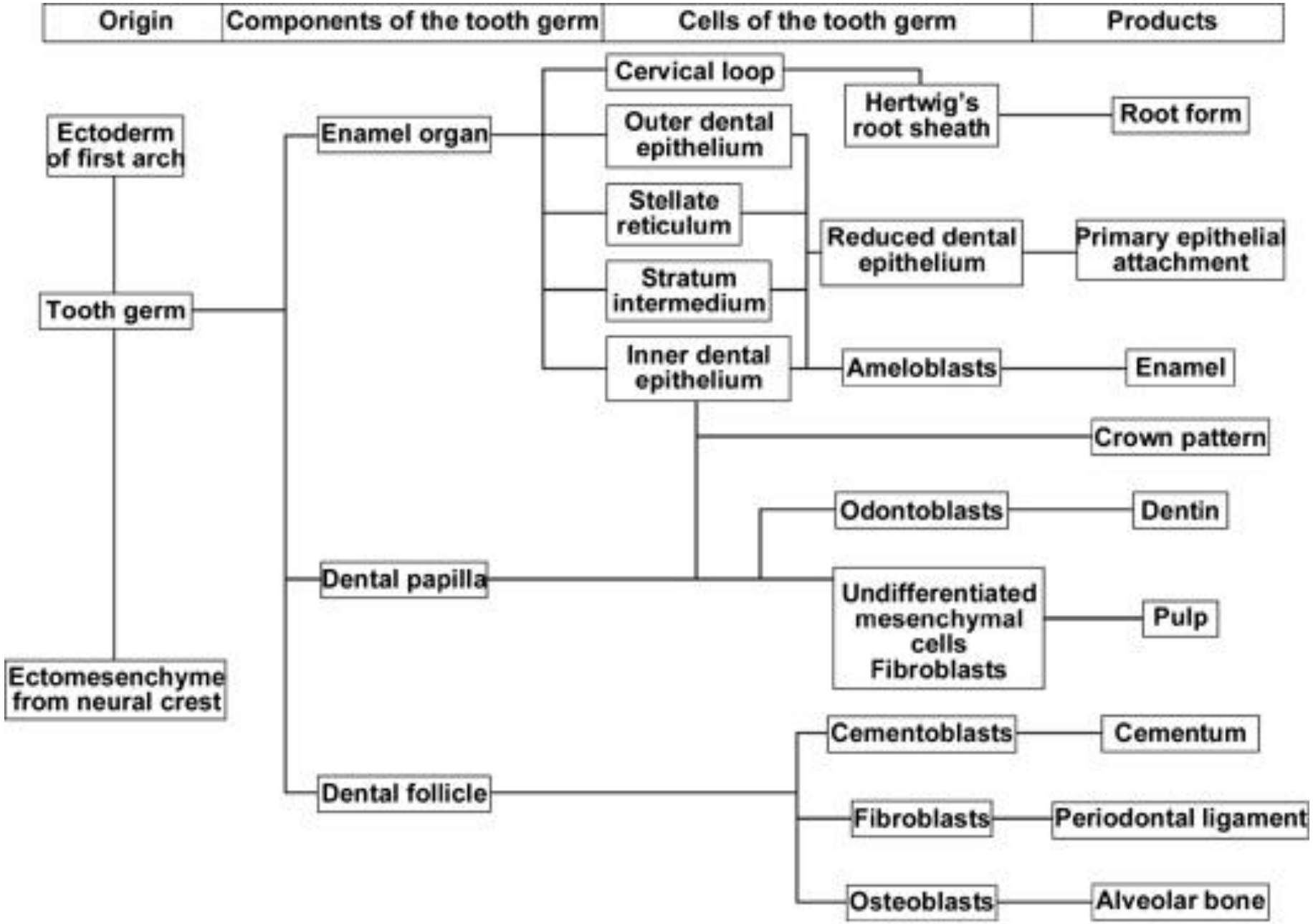
Odontogenesis

- Oral ectoderm
- Mesoderm
- Cells of neural crest



mesenchym
(ektomesenchyme
or mesoectoderm)

- Enamel evolve form ectoderm
- Other tissues from mesenchym



Tooth development

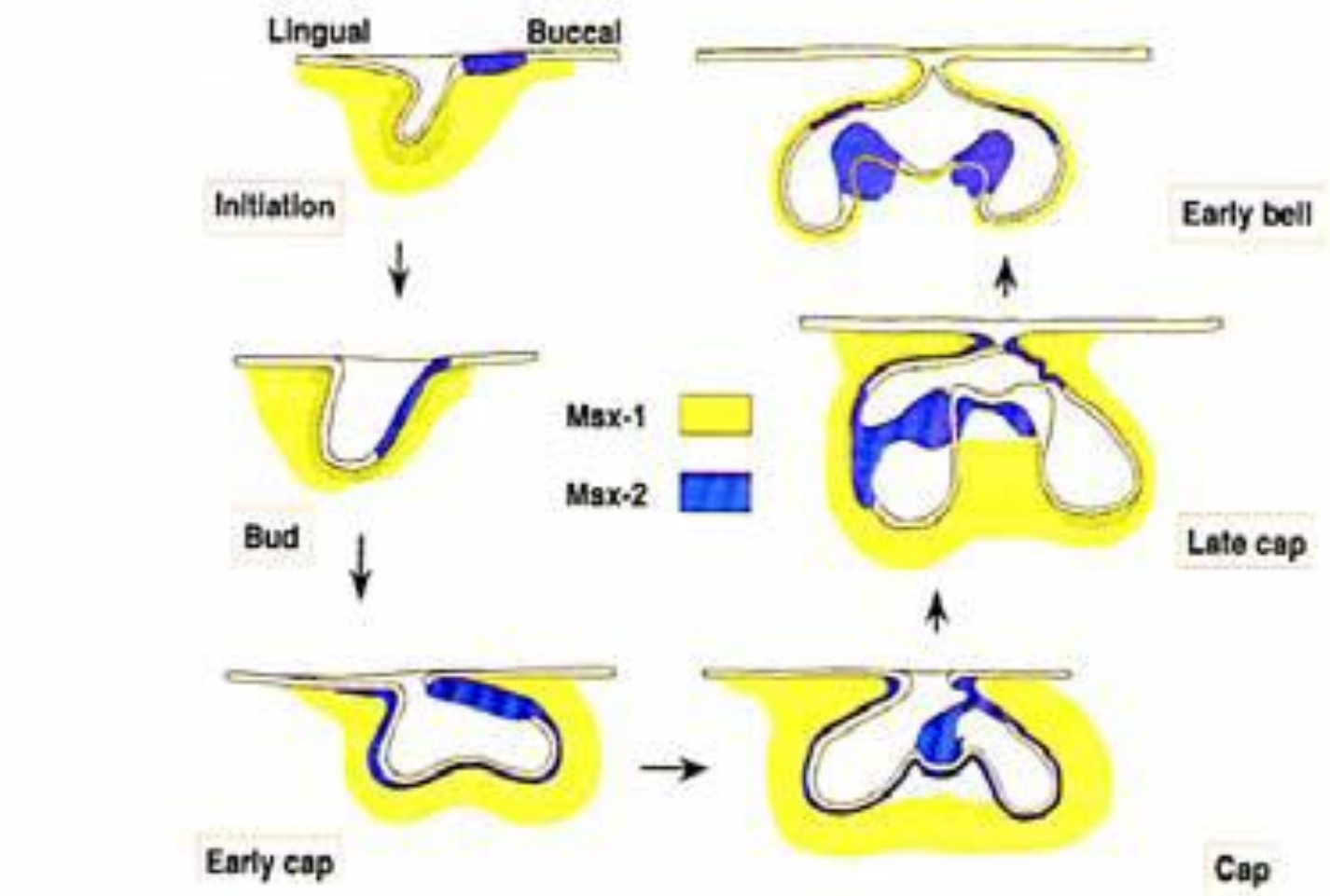


FIGURE 3 Diagrammatic summary of the expression of *Msx-1* and *Msx-2* in murine molar tooth development.

Molecular Determinants of Tooth Formation

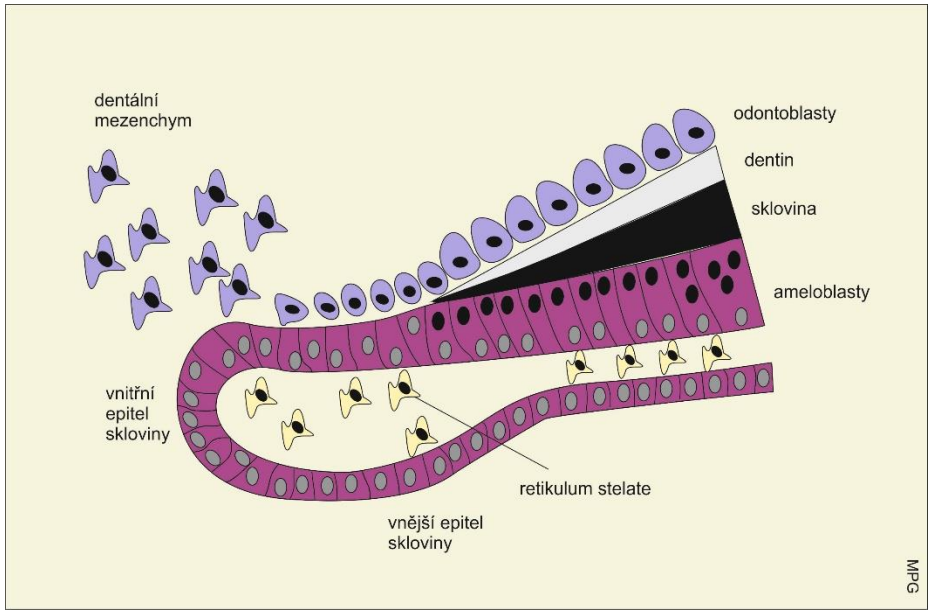
- Over 10,000 genes involved in making a tooth
  - Most genes involved in odontogenesis are expressed in non-dental tissues
  - Some genes are relatively specific for development of the dental tissues (e.g. amelogenin gene)

Types of dentin

- **Primary dentin**
  - rapidly produced dentin formed up to completion of root formation and beginning of tooth function
- **Secondary dentin**
  - normal physiological dentin production that proceeds slowly throughout the life of the tooth
- **Tertiary dentin**
  - dentin produced in response to external stimulus (e.g. caries, restoration etc.)
- **Normal Circumpulpal Dentin**

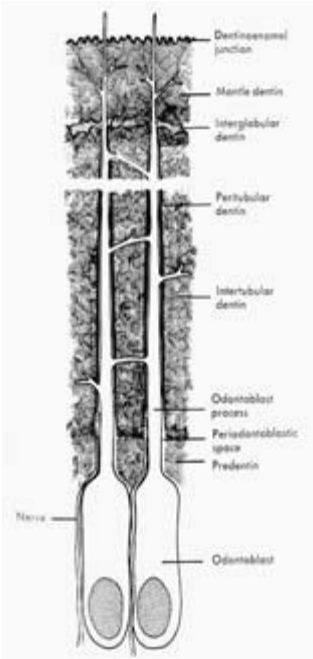
Dentinogenesis

- begins with differentiation of odontoblasts in teeth protuberances from peripheral parts of the dental papilla
- slightly precedes amelogenesis



Odontoblasts

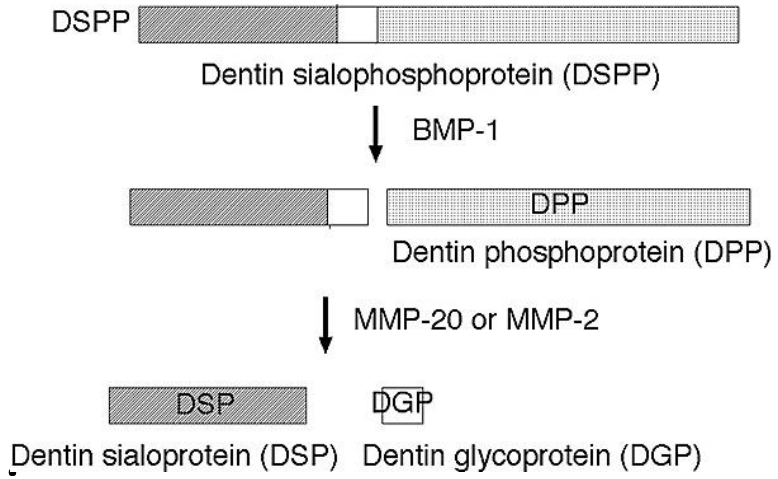
- Differentiated from mesenchymal cells condensing adjacent to the inner enamel epithelium
- Are tall columnar cells during active formation of primary dentin
- Height decreases and less organelles are present as cells become less active



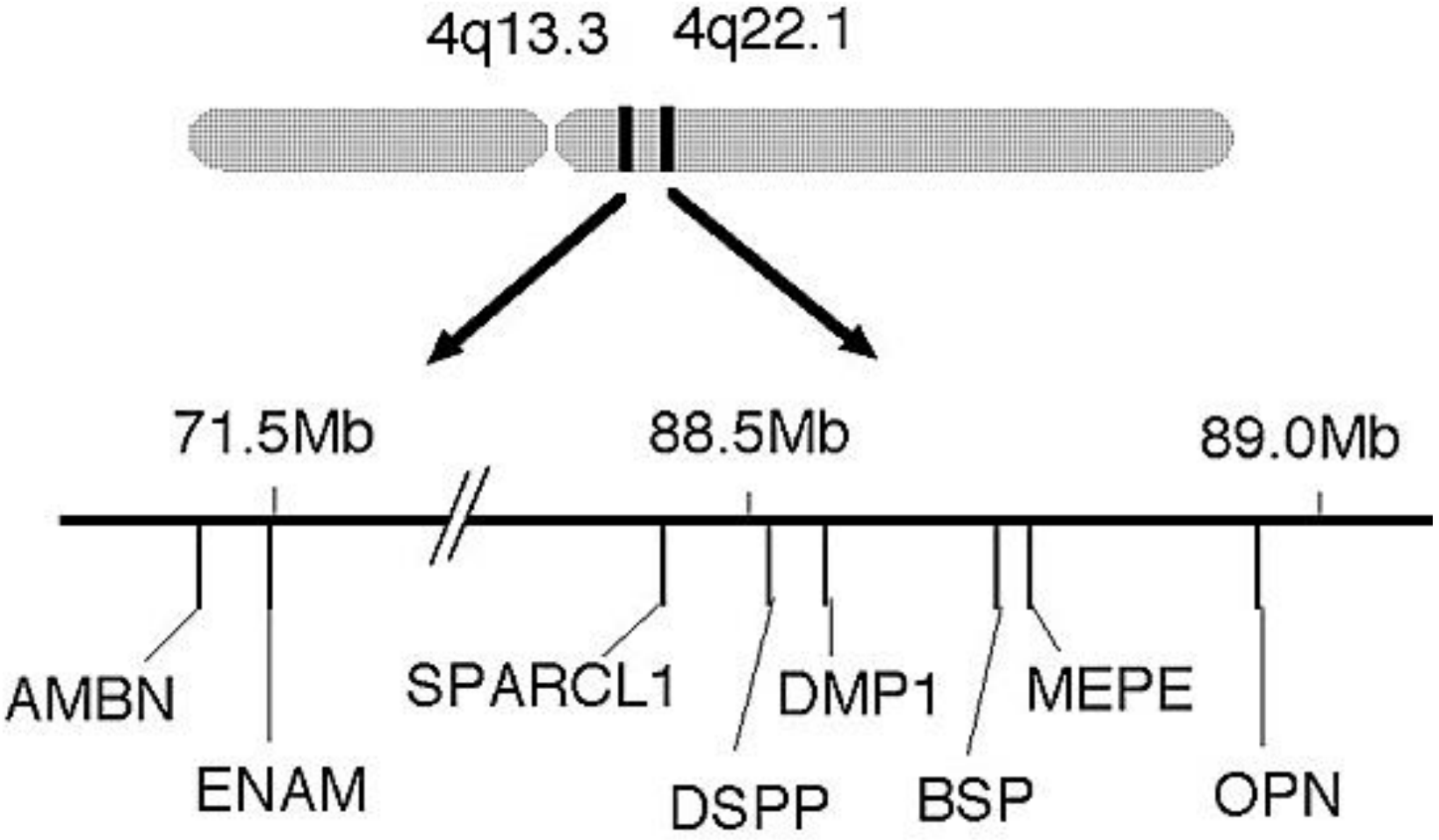
Dentin ECM components

- Type I collagen – most abundant
- Type III & type V – predentin, not normally in mature dentin
- Dentin sialophosphoprotein
  - Dentin phosphophoryn
  - Dentin sialoprotein

  Dentin Matrix Protein 1
- Proteoglycans – numerous species
- Gla proteins – e.g. osteocalcin



SIBLING-family genes



SPARCL1: SPARC-like protein 1, DSPP: dentin sialophosphoprotein, DMP1: dentin matrix protein 1, BSP; bone sialoprotein, MEPE: matrix extracellular phosphoglycoprotein, OPN: osteopontin. AMBN: ameloblastin, ENAM: enamelin

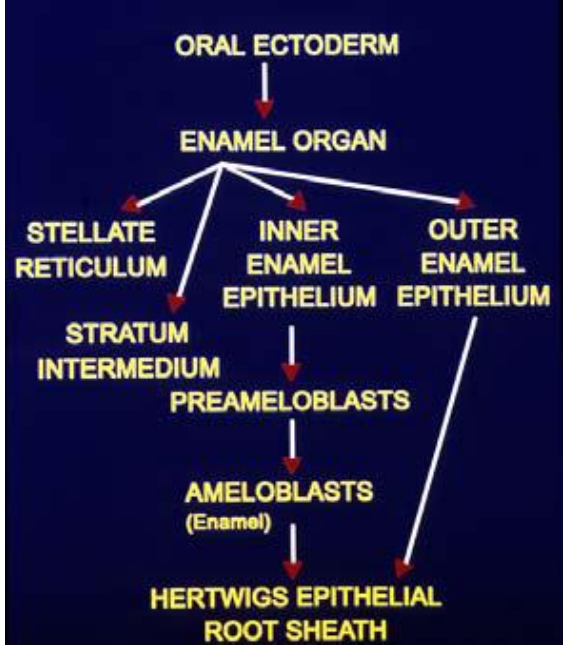
Predentin

- Unmineralized dentin matrix
- Always present in normal healthy teeth
- Usually 15 – 20 µm thick and is bounded by odontoblasts on pulp side and dentin on outside
- Predentin exists in a closed compartment with components being determined and regulated by the odontoblasts

Mechanisms of mineralization in Dentin

- Matrix Vesicles – initiates mineralization in mantle dentin
- Collagen/phosphoprotein complex – required to maintain and continue normal dentin mineralization

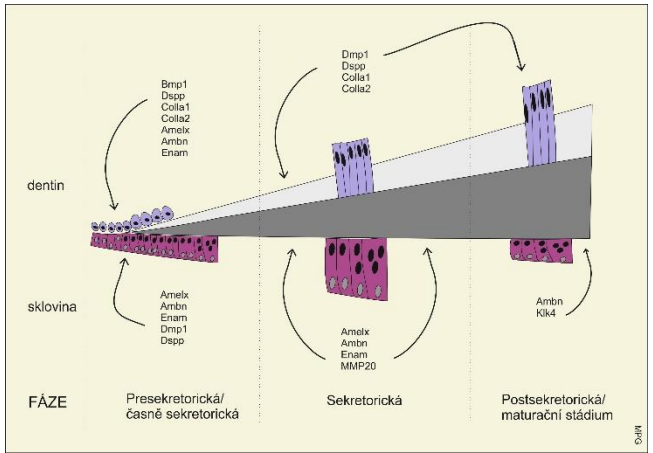
Ameloblasts

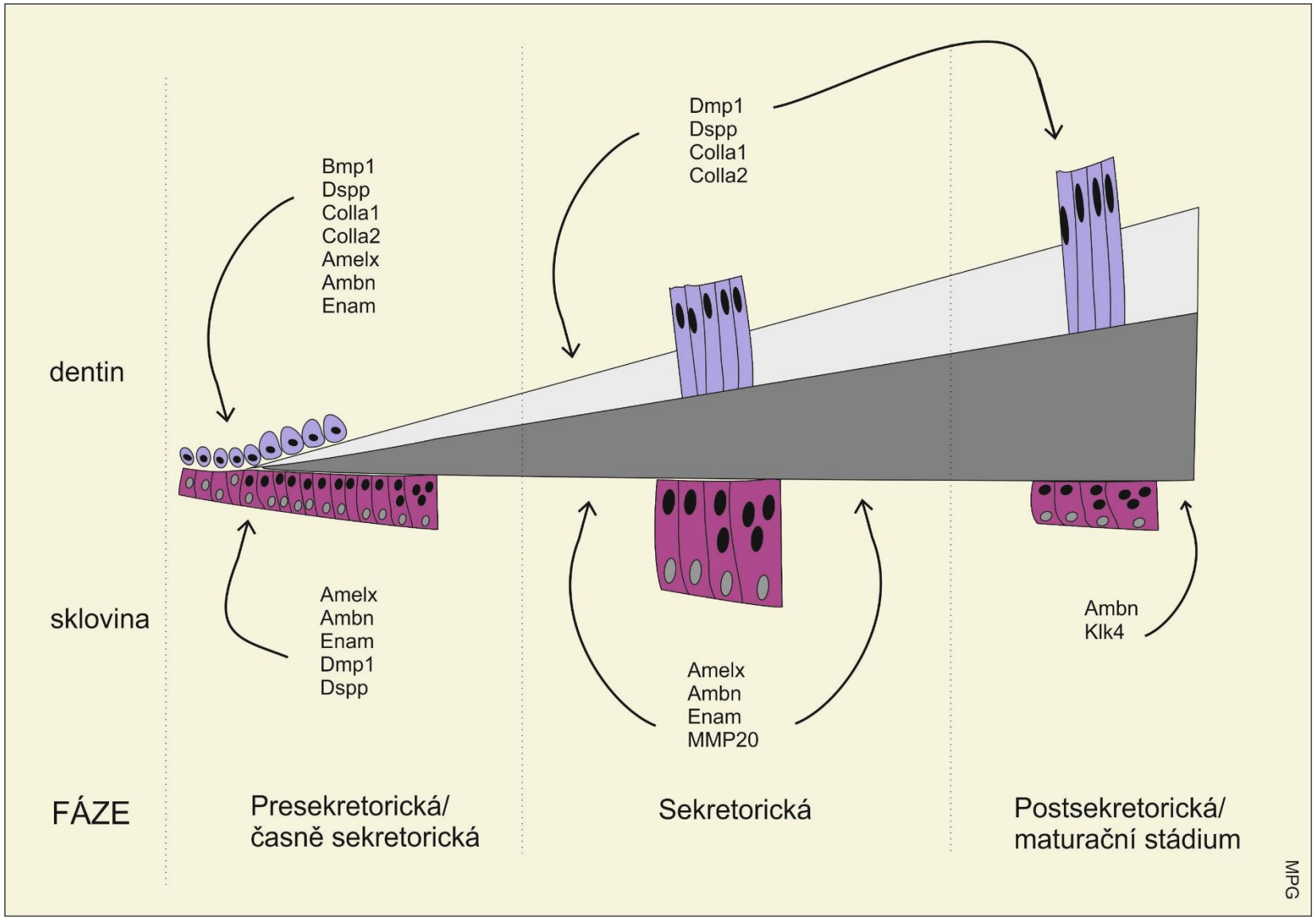


- migration to future pulp
- bud stage - odontoblasts and ameloblasts connected

- Secretion of proteins according to tightly regulated gene expression and programmed timing
- produce extracellular organic matrix of enamel - a skeleton, which itself regulates the initiation, growth and shape of inorganic hydroxyapatite crystals, which produce mature enamel

- thickness of the enamel depends on the length of secretory phase of ameloblasts



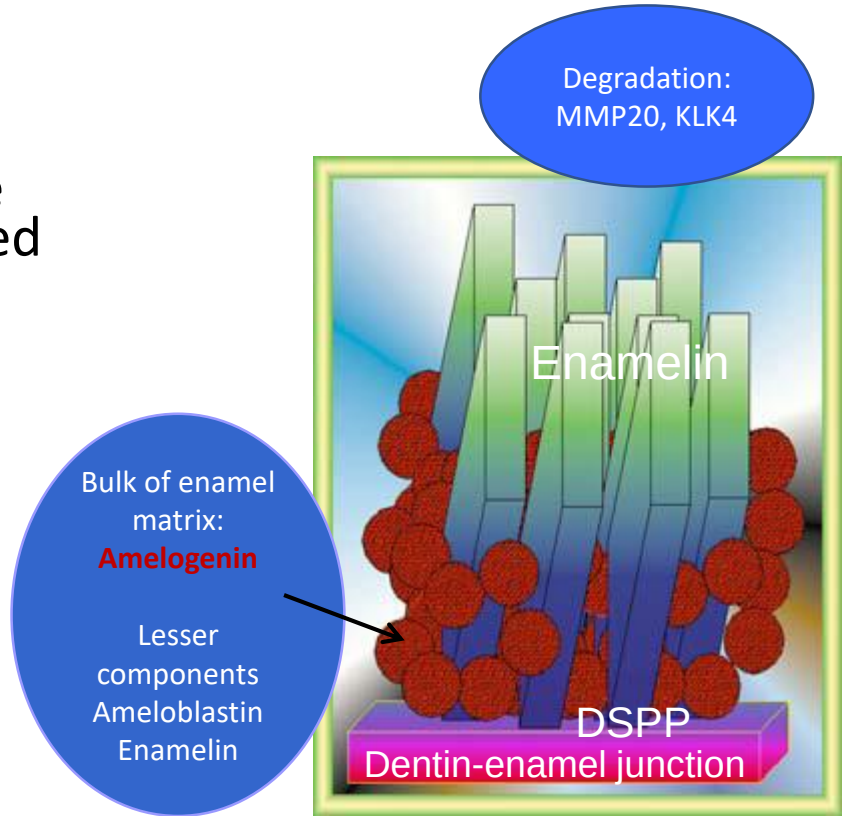

Bmp1
Dspp
Colla1
Colla2
Amelx
Ambn
Enam
Dmp1
Dspp
Colla1
Colla2
dentin
sklovina
Amelx
Ambn
Enam
Dmp1
Dspp
Amelx
Ambn
Enam
MMP20
Ambn
Klk4
FÁZE
Presekretorická/
časně sekretorická
Sekretorická
Postsekretorická/
maturační stádium
MPG

Environmental Influences of Amelogenesis

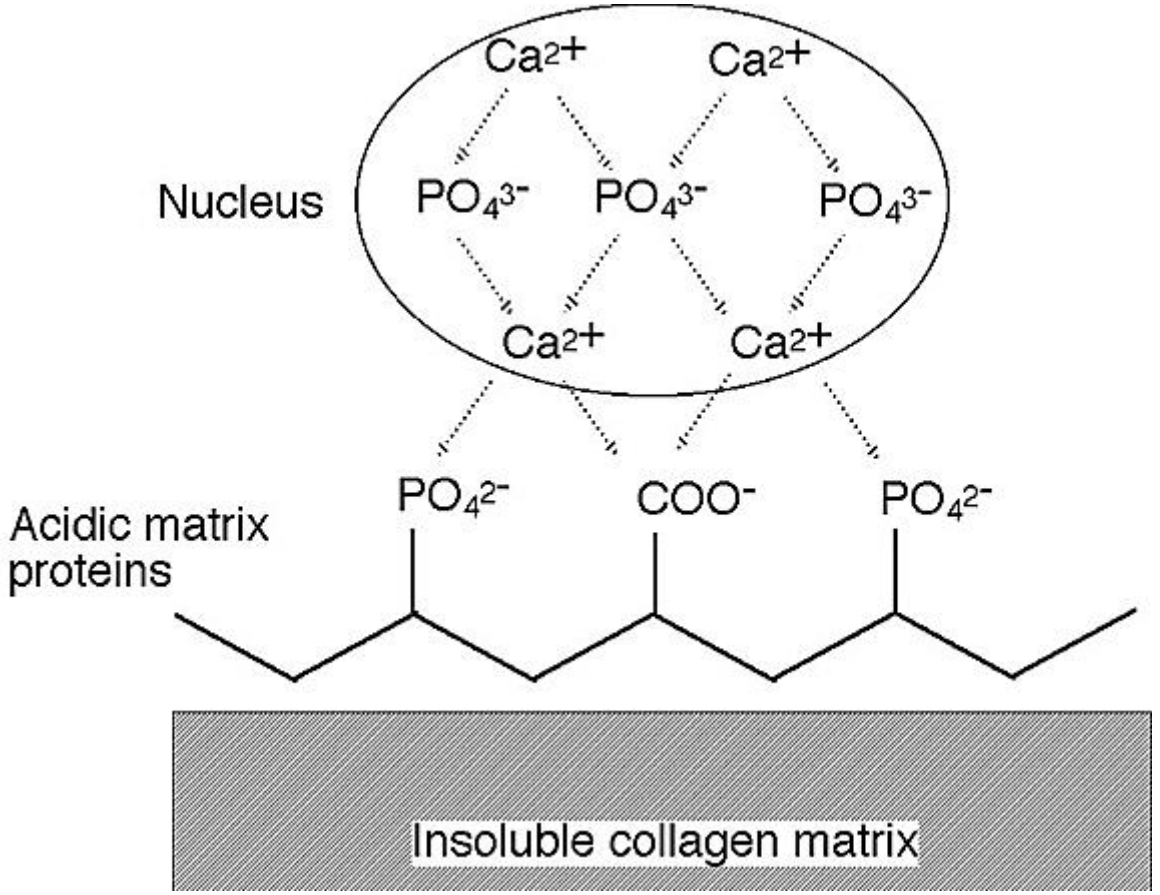
- Nutrition
  - Major and minor components
    - Calcium, phosphorus, protein, fluoride etc…
- Hypoxia
- Hyperthermia
- Infection
  - Congenital rubella, syphillus, CMV, etc…
- Physical Determinants
  - Space
  - Trauma
- Interactions

Enamel maturation

- secretory phase - enamel „maturation“
- culminate in deposition of ions on the sides of the enamel crystallites and replacement of extrusion matrix protein debris and fluid from the structure.
- almost all organic matrix proteins are cleaved and removed and are replaced by inorganic crystals.
- after secretory phase - no further formation of crystals or prolongation crystals already formed
- apoptosis of ameloblasts



Proteiny a ECM



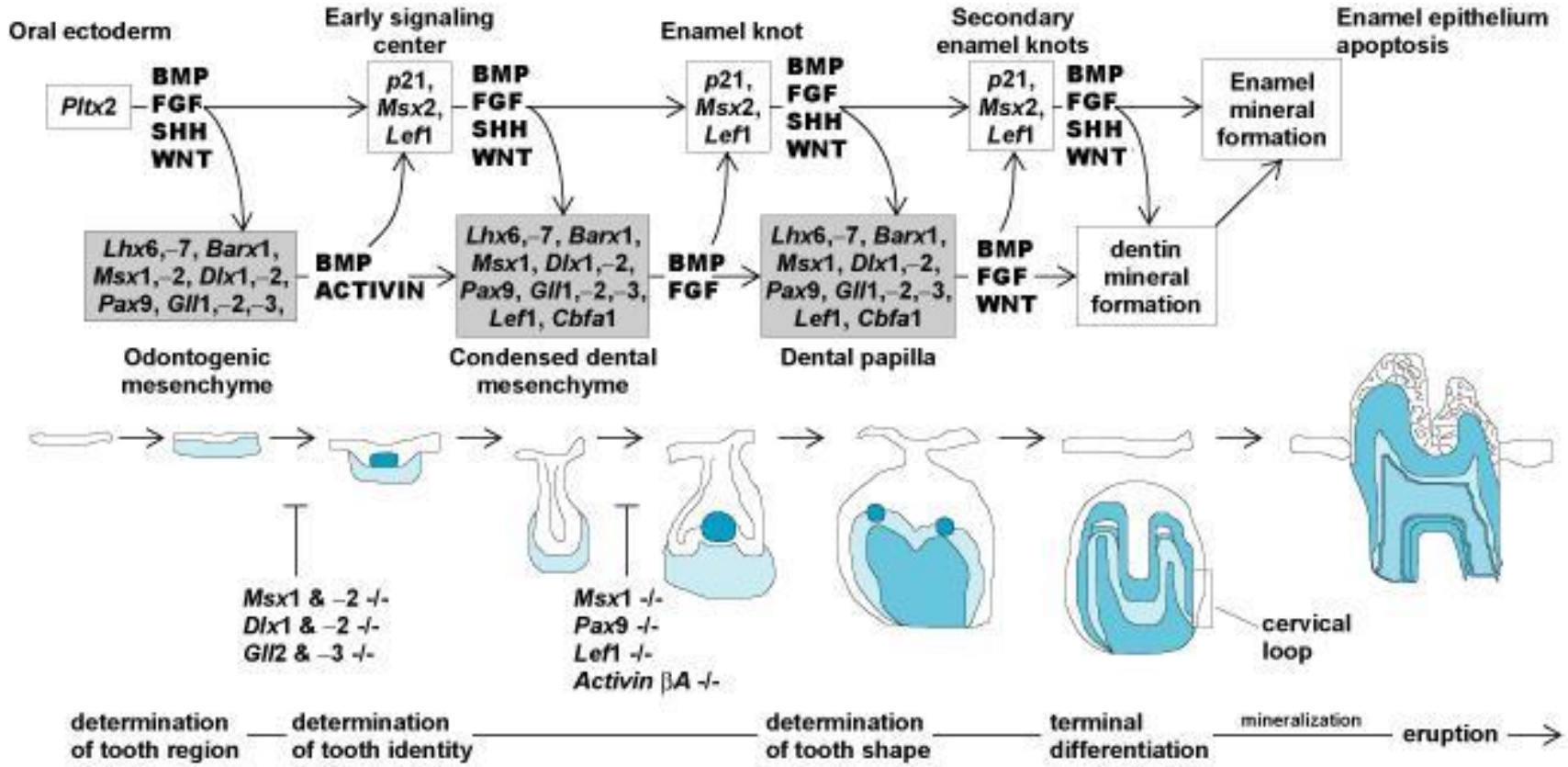
Nucleation of hydroxyapatite by acidic matrix proteins immobilized on insoluble collagen matrix. Some acidic matrix proteins, e.g. dentin phosphoprotein, have an affinity to collagen. The surface of the insoluble collagen matrix provides loci to reduce interfacial energy for nucleation. Calcium ions are bound to the acidic groups of the acidic proteins, and inorganic phosphates are attracted by the calcium ions. The ionic complex thus formed may constitute a crystal nucleus.

Genetics

Genes and pathways

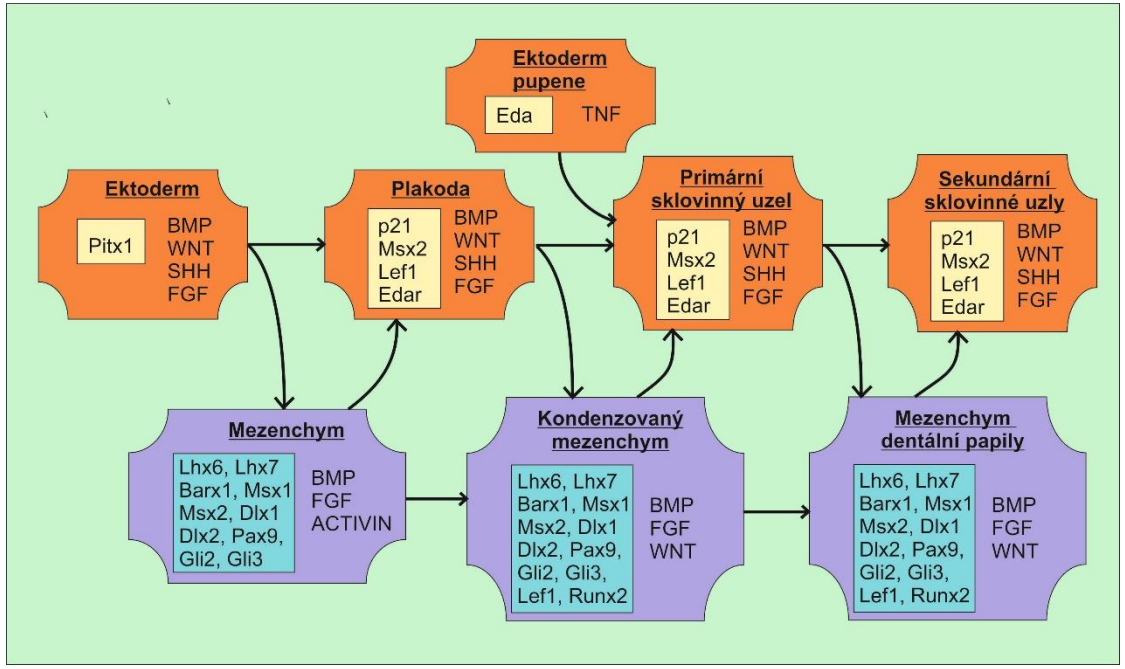
- more than 300 genes
- Signaling pathways:
  - *TGF beta* (transforming growth factor),
  - *FGF* (fibroblast growth factor),
  - *BMP* (bone morfogenetic protein),
  - *SHH* (Sonic hedgehog),
  - *Wnt*,
  - *TNF (tumor necrosis factor)*,
  - *EDA* (Ectodisplasin)

Genes and pathways



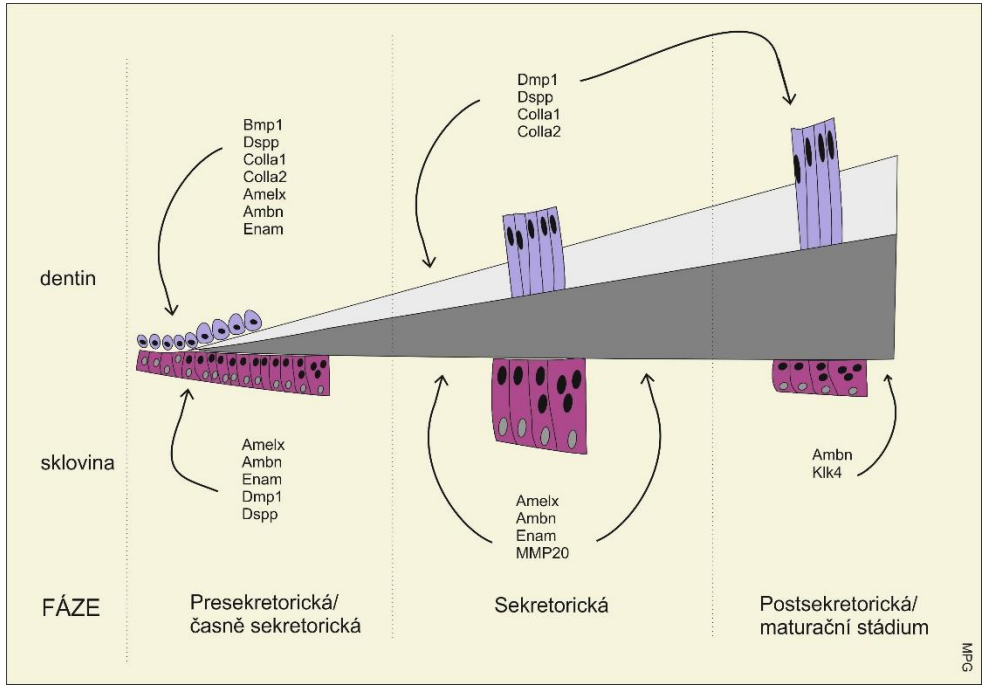
Gene regulation

- Modulators of signal pathways – e.g. Inhibitors of *BMP* and inhibitors of *FGF.*
- genes involved in the early stages of tooth development, have a different function in the later stages of development.
- multifunctionality allows induction or regulation by different transcription factors or regulators (*AMBN*, *BGLAP*, *IBSP* a *COLLA2*; *BGLAP*, *COLLA2;* complex Vitamin D3/-VDR-RXR: *CALB1*, *CST6*, *FOXO1*)



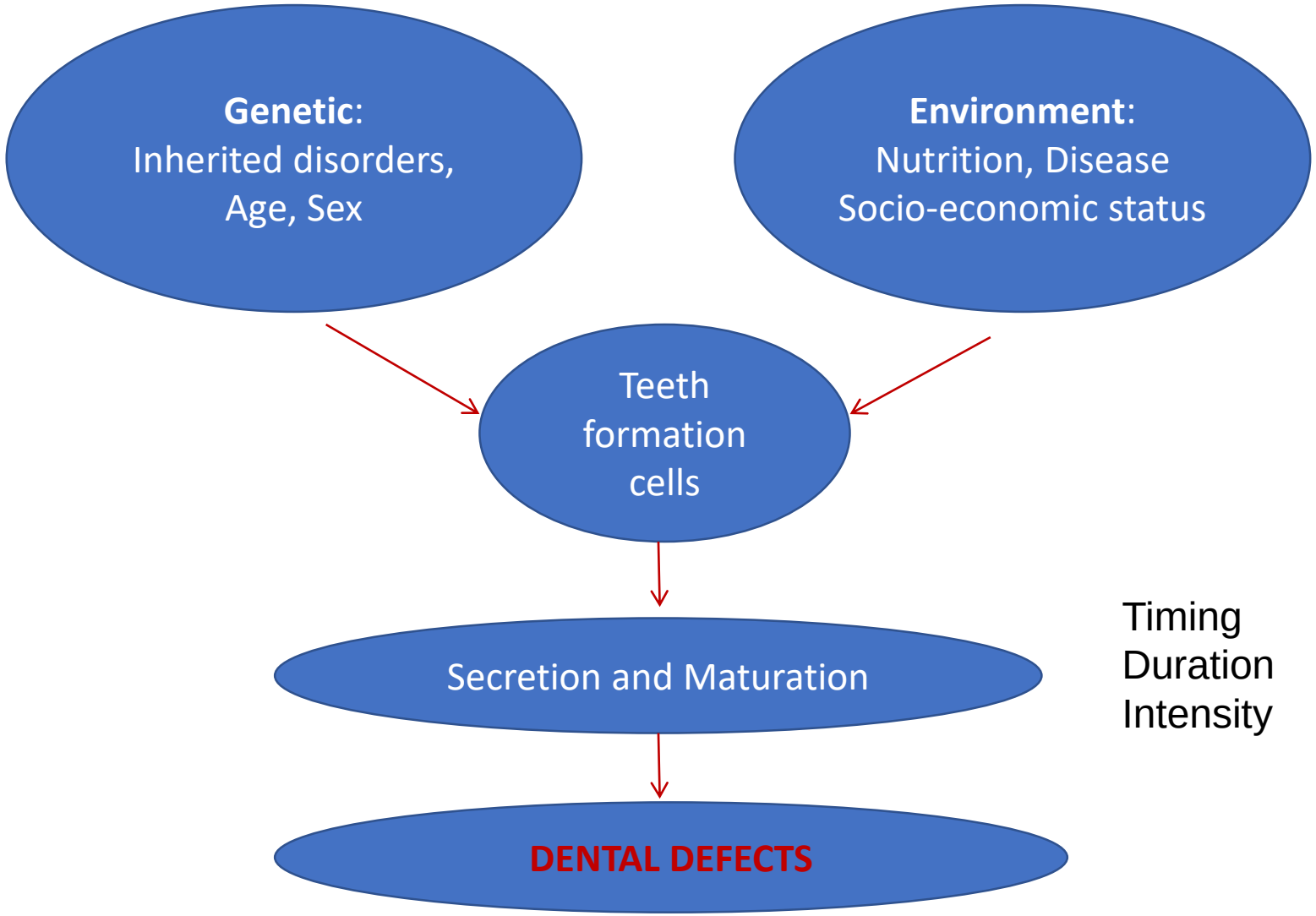
Genes and development of enamel and dentin

- amelogenin, enamelin, ameloblastin, a *MMP20 (matrix metaloproteinase 20)* – early secretory phase
- kalikrein 4 (KLK4), amelotin a odam (apin) – iniciation of maturation
- *KLK4* a *MMP20* – layers of enamel protein degradation
- *FAM83H* - reabsorption and secretion of enamel proteins
- homeobox genes *DLX* (Distal less);
  - *DLX1* and *DLX2* are specific for the development of molars



Abnormalities of Tooth

Teeth abnormalities



Diseases of hard dental tissues

• acquired	• congenital
• Abrasion • Erosion • Resorption • Discoloration of teeth	• genetic anomalies: - teeth - enamel - dentin

Congenital abnormalities

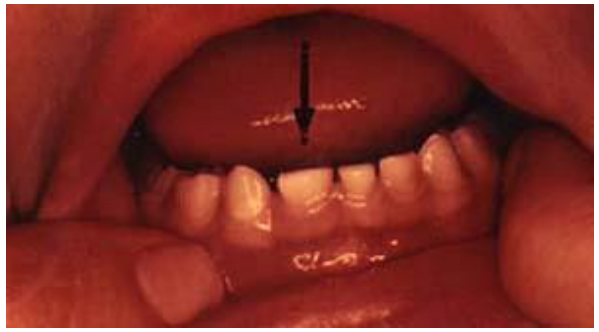
- scaling of teeth
- changes in the shape of the teeth
- changes in the number of teeth
- abnomal structure of teeth
- disorders in eruption

Dentes praelacteales

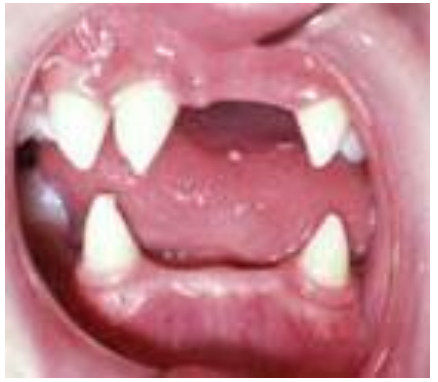
- Natal teeth (dentes natales) - erupted at birth
- Neonatal teeth (dentes neonatales) - erupted at 1. - 30. day after birth.
- From dentes praelacteales should be distinguished prematurely erupted deciduous teeth - dentitio praecox.

Changes in the number of teeth

- Anodoncia - total absence of teeth
- Hypodontia - reduced number of teeth, usually 3. molar
- Oligodencia - multiple teeth missing
- Hyperdoncia - excess teeth, erupted or retained, especially in the upper jaw
- Mesiodens - in the gap between the maxillary central incisors
  Gardner's syndrome – non-erupted excess teeth, colon polyposis, osteoma in the bones including the jaw, epidermoid cysts in the skin

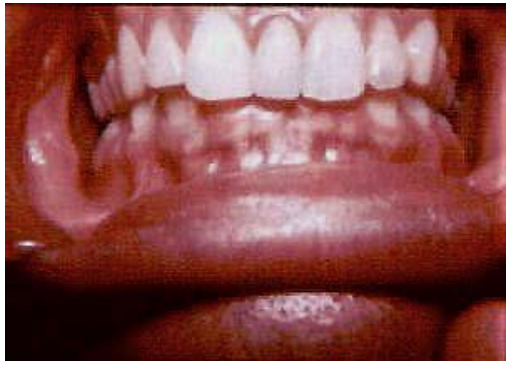


Fuze



Hypodoncie

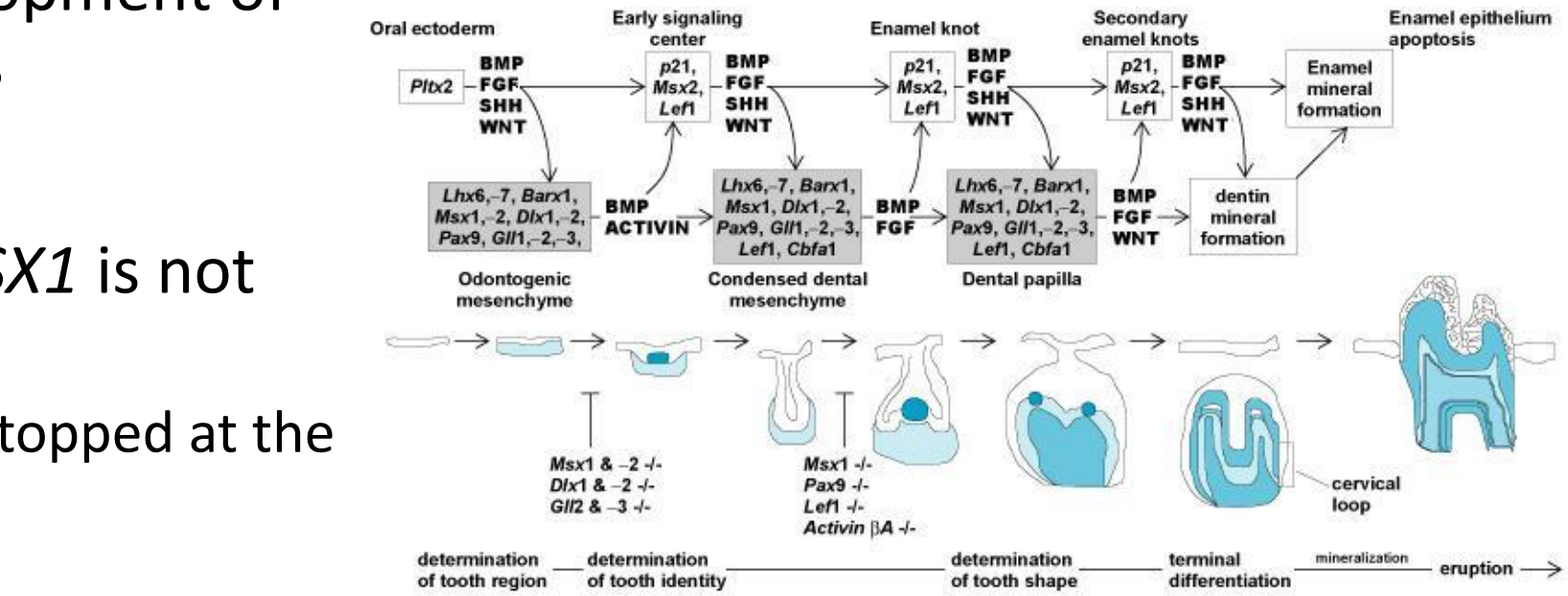
Mesiodens



Changes in the number of teeth

- autosomal dominant (AD) or autosomal recessive (AR)
- MSX-1 gene exons mutations – control of the development of all epidermal organs
- *PAX9* mutations -*MSX1* is not activated
  - tooth development stopped at the stage of bud

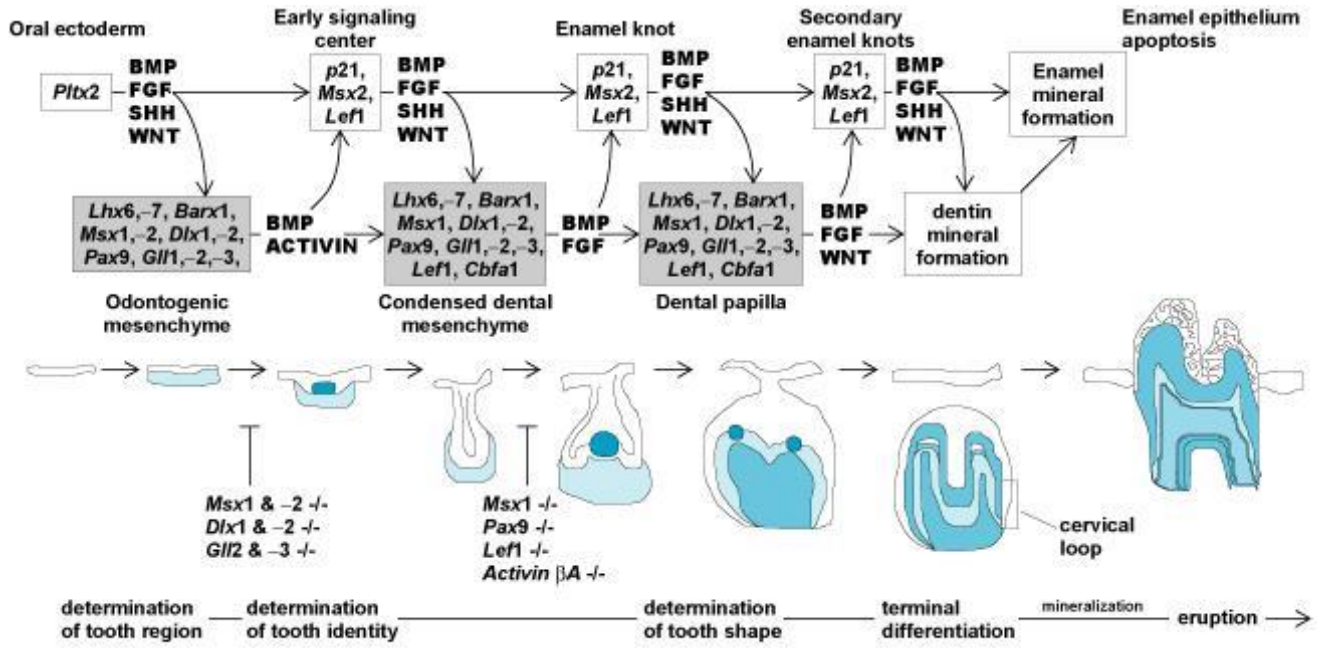
- MSX-1
- PAX-9



Changes in the number of teeth

- *RUNX2* – TF for osteoblast differentiation – connected with supernumerary teeth in the permanent dentition
- *AXIN2* - severe oligodontia. Leads to agenesis of most permanent molars, premolars, lower incisors and upper lateral incisors
- Dlx (distal-less homeobox) - failure of tooth development and loss of upper molars

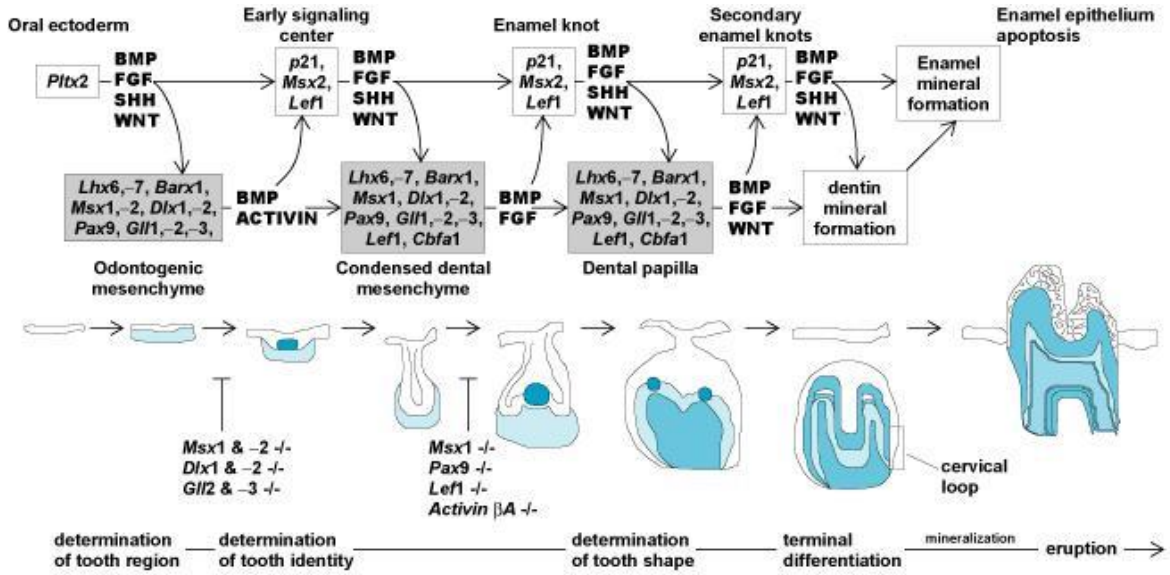
- RUNX-2
- AXIN-2
- DLX



Genes and abnormal position of teeth

- Abnormalities of tooth positions are often found together with tooth agenesis.
- mutations in genes associated with agenesis of teeth, especially MSX1 and Pax9 could also be the cause of an abnormal position of teeth.

- MSX-1
- PAX-9

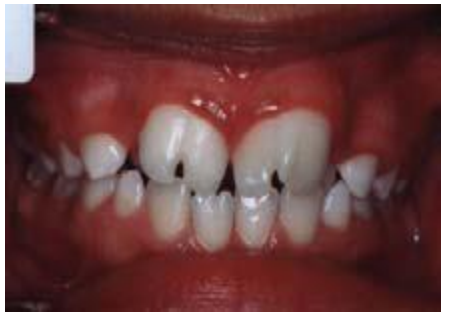


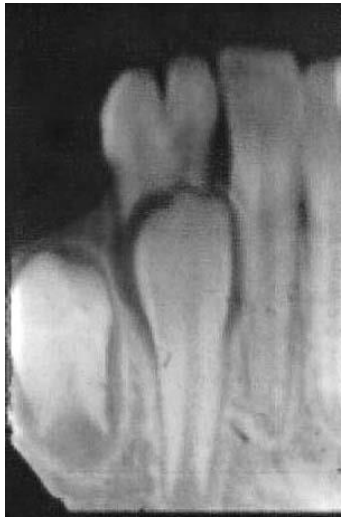
Changes in tooth size

• mikrodoncia	• makrodoncia
• generalized - all teeth are smaller (pituitary dwarfism, Down syndrome) • single tooth (mostly superfluous) • relative generalized (teeth normal or slightly smaller, larger jaw)	• generalized - all teeth larger (pituitary gigantism, acromegaly) • single tooth relative generalized (teeth normal or slightly larger, lower jaw)

Changes in tooth shape

- accessory cusps-molars, incisors
- accessory roots
- peg shaped teeth conical- shaped crown
- separate double- tooth crown and common root with the root canal (gemination, adhesions)
- taurodontia - abnormally large pulp cavity
- dens in dente - created by invagination of ectoderm into mesoderm of dental crown pulp before calcification of hard dental tissue - incorporated smaller tooth into the pulp of normal tooth





Eruption abnormalities

- premature or delayed eruption
- retention of teeth-especially 3rd molar
- teeth improperly developed or built atypically

Abnormalities of teeth structure

Amelogenesis Imperfecta

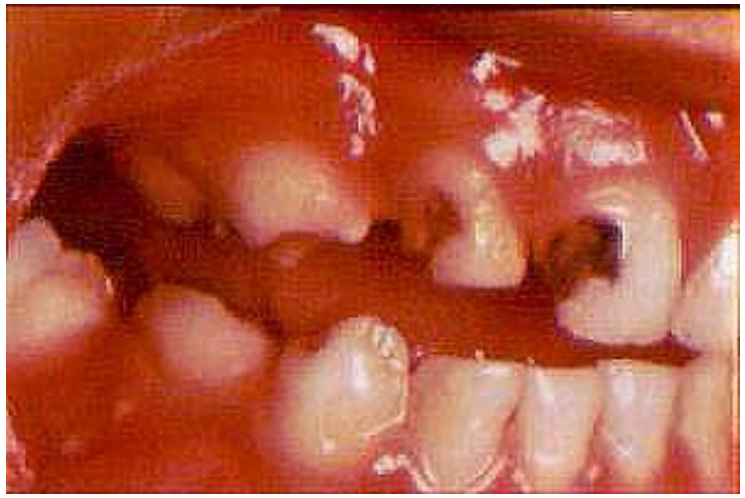
- Group of hereditary conditions caused by mutations in genes important in enamel formation.
- Phenotypes are highly variable depending on the mutation involved.
- Prevalence varies from 1:700 to 1:15,000 depending on population.

Abnormalities of teeth structure

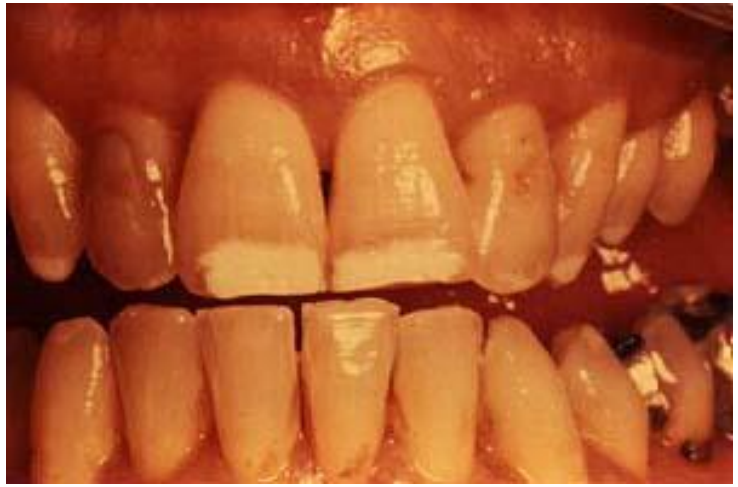
Amelogenesis imperfecta (AI)

- hereditary disturbance of enamel
- affected - temporary and permanent dentition

- **hypoplastic** - thin enamel of normal hardness
  - teeth not touching, the surface uneven, often pigmentation
- **hypomaturation** - soft enamel, normally strong, easily peeling off,
  brown-yellow-white speckled
- **hypocalciphication** - initially normal thickness, gradually decreasing, exposes the dentin
  soft, cheesy consistency



Hypoplazie skloviny



Hypomineralizace skloviny

AI and genes

- inheritance AI
  - X-linked,
  - autosomal recessive (AR) or autosomal dominant (AD).

Genes:

- autosomal dominant *AMLEX* (amelogenin),

  AMBN (ameloblastin enamel matrix protein),

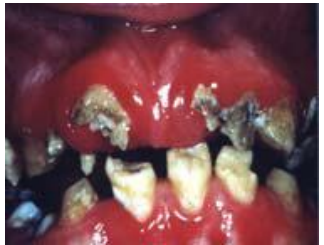
  ENAM (enamelin) a

  AMTN (amelotin),

  FAM83H,

  DLX3
- autosomal recessive
- MMP20

  KLK4



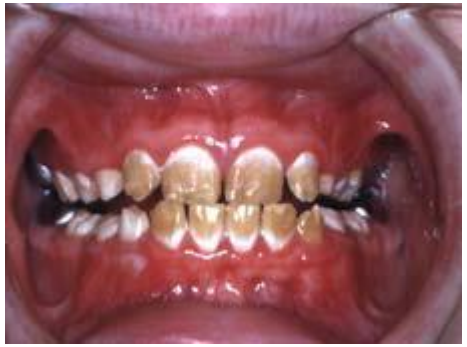
- AMELX make up 90% of the organic matter of enamel and is cleaved by the enzyme MMP20 and KLK4 degraded by the enzyme.
- ENAM makes up 5% of the organic matter of enamel;
  AMBN is essential for ameloblasts activity and
- AMTN is involved in the maturation of enamel.
- Mutations in individual genes leads to differences in phenotypic expression of the disease.

Amelogenin

- There are now 15 different mutations in the amelogenin gene (AMELX).
- The phenotypes vary from hypomaturation to hypoplastic defects depending on the type of mutation

Amelogenin

- **X – linked AI**



- Amelogenin gene mutation with C deletion at nucleotides g4114delC.
- Frameshift introduces premature stop codon (L167fsX173).
- Truncates amelogenin protein deleting 18 c terminal amino acids.

Non-Amelogenin Enamel Proteins

- The enamel extracellular matrix is a complex mix of multiple proteins, some of which are derived from amelobalsts (enamelin, ameloblastin) and others that are not (albumin).

- Enamelin
- Ameloblastin
- Amelotin
- Amelin
- Tuft protein
- Keratin
- Albumin

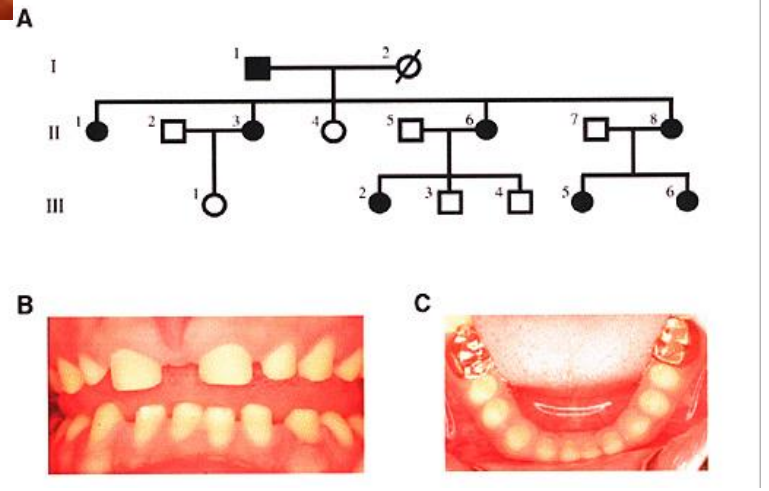
Enamelin

- Low abundance glycoprotein immunolocalized to the secretory face of the ameloblast Tome's process.
  - Parent protein is a 186 kD glycoprotein
  - Cleaved into multiple smaller polypeptides
  - May interact with amelogenin

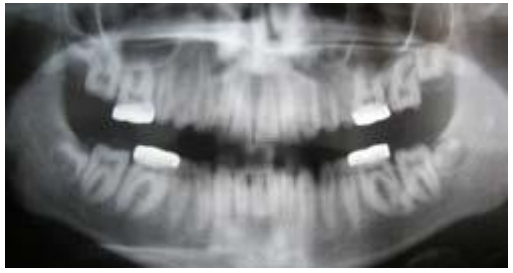
Enamelin

AD hypoplastic AI

- Exon 4
- introducing stop codon
- Predicted protein is 52 amino acids vs 1142 in wildtype

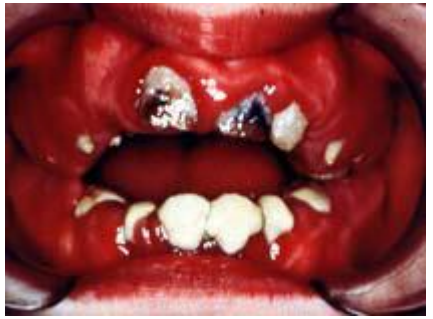


Single base deletion causes enamelin protein to be 270 AA vs wildtype 1142 AA in length.



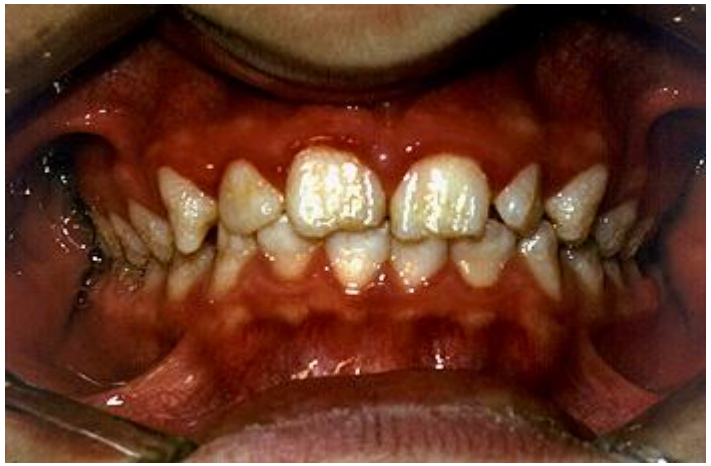
MMP20 - Enamelysin

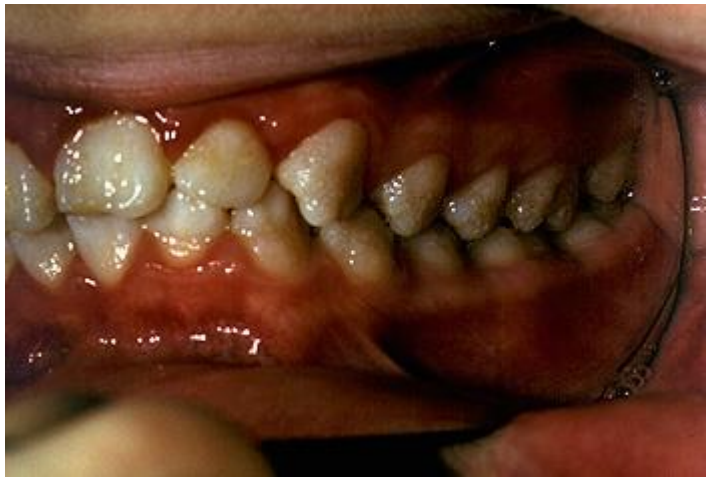
- Autosomal recesive AI
- matrix metalloproteinases – 483 amino acids (54kD).
- Expressed by ameloblasts and odontoblasts.
- Degrades amelogenin



Amelogenesis imperfecta

- Hypoplasia and hypomineralization
- Autosomal dominant hypoplastic hypomaturation AI with taurodontism

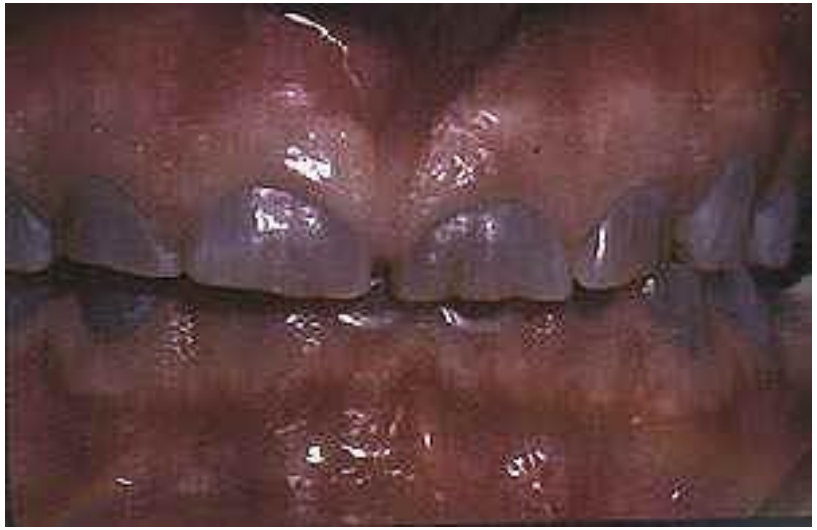




Abnormalities of teeth structure

⦿ Dentinogenesis imperfecta

- inadequate calcification of dentin, which almost fills the medullary cavity
- Teeth are gray, ocher-yellow, reduced mechanical resistance, abrasion occurs rapidly



Dentinogenesis imperfecta

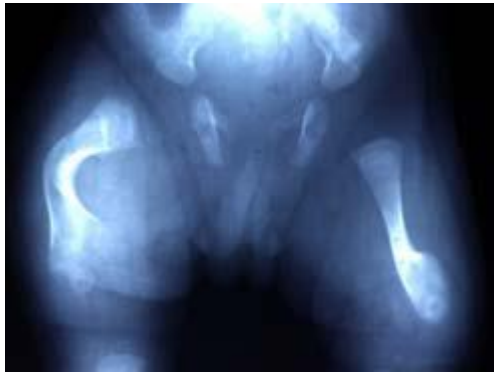
- Autosomal dominant clinically heterogenic disorders

Classification:

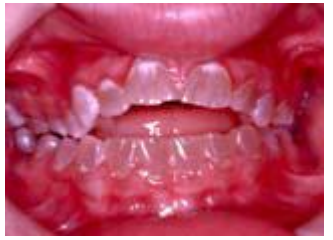
- Type I - Associated with osteogenesis imperfecta
  - COL1A1 and COL1A2 mutations
- Type II – Autosomal dominant condition
  - DSPP mutations
- (Type III – Autosomal dominant variant with large pulp chambers)
  - Allelic to type II (DSPP mutations)
- dentin dysplasia I (DD I),
- ⊙ dentin dysplasia II (DD II).

Osteogenesis Imperfecta

- Genetically and clinically heterogeneous group of hereditary disorders characterized by
  - Increased bone fragility
  - Blue sclera of eye
  - Hearing loss
  - Joint laxity
  - Dentinogenesis imperfecta (some families)



Dentinogenesis imperfecta



DGI I

- Molecular defects associated with OI include mutations in the alpha-chains of collagen type 1 and are phenotypically characterized by increased bone fragility. **Collagen type 1 is the product of genes COL1A1 and COL1A2.**
- less than 10% of cases the disease is caused by a recessive inheritance of the disorder in the genes for CRTAP, PPIB and LEPRE1
- ranging from the complete absence of marrow and roots developed normally up to the dentine

DGI II

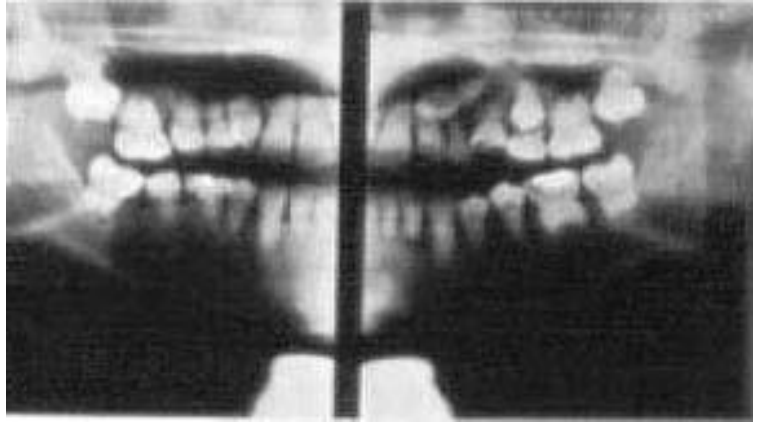
- Approximately 10% of the dentin organic material is formed by proteins other than collagen, especially proteins characteristic:
  dentin phosphoprotein (DPP), dentine sialoprotein (DSP) and dentine glycoprotein (DGP).

  Those are coded by chimeric protein DSPP (dentin sialofosfoprotein)

  In DGI II the temporary and permanent dentition is affected. Primary dentition impairment is far more serious than impairment of secondary dentition

- DSPP Mutation in Dentinogenesis ImperfectaShields Type II
  - Non-sense mutation (Bln45stop) in exon 3 of DSPP gene

Dentin dysplasia



DD-I

- Teeth are clinically of normal shape, form and consistency.
- X-rays - sharp conical roots with apical constriction.
- Pre-eruptive pulp obliteration, which leads to the remains of the pulp in a crescent shape parallel to the cement-enamel junction in the permanent dentition and to the total obliteration of the pulp in deciduous dentition

DD-II

- resemble symptoms in DGI-II,
- permanent dentition is either unaffected or shows mild radiographic deviations.
- X-ray - no temporary tooth pulp,

  in permanent teeth - funneled-shaped marrow space with growths emerging as pathologically calcified pieces of marrow.
- Dental roots in both dentitions are normal.

Ectodermal Dysplasia

- Clinically and genetically diverse group of conditions affecting development of tissues derived from ectoderm incl. teeth.
- Two affected tissues (e.g. hair, fingernails, teeth, skin).

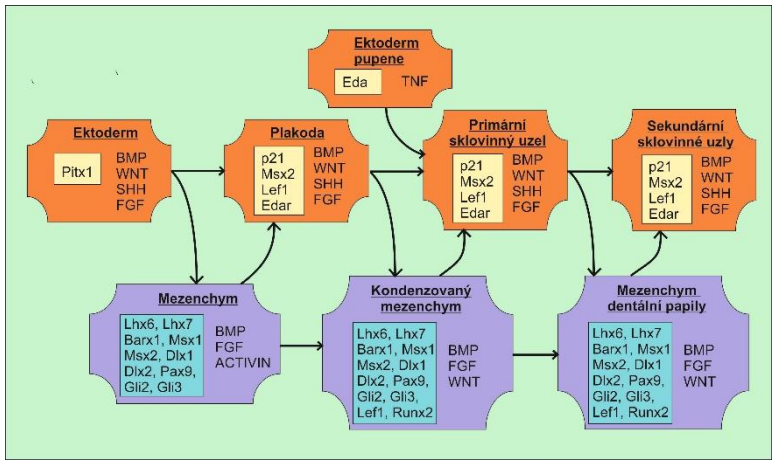
- Two main types:
  - Hypodidrotic (lack of sweat gland, hair, hypodontia)
  - Hidrotic – normal perspiration levels (variable hair, teeth, nail abnormalities)

Ectodermal Dysplasia Molecular Defects

- First ED gene defect was reported in 1996
- • Molecular defects have now been identified
- in 10 to 20 ectodermal dsyplasias
  - Hypohidrotic X linked ED
  - Reiger Syndrome
  - Tricho Dento Osseous Syndrome
  - Autosomal Dominant/Recessive ED
  - Clouston ED
  - Incontinentia Pigmenti

Ectodermal Dysplasia Molecular Defects

- X-linked hypohidrotic ED
  - transmembrane protein (ectodysplasin-A)
  - Gene defect - can not run normally signals required for interaction ectoderm-mesoderm
- Autosomal dominant and recessive hypohidrotic ED
  - tumor necrosis factor receptor (Downless DL)
  - mutation in gene for *GJB6*
- Rieger Syndrome
  - homeobox gene (RIEG, PITX2)
- Tricho-dento-osseous syndrome
  - homeobox gene (DLX3)



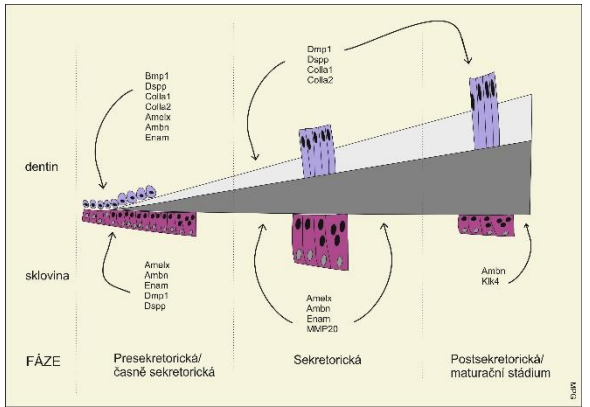
Other genes and dysplasia

Witkop syndrome (Tooth-and-nail syndrome; TNS)

- rare autosomal dominant ectodermal dysplasia
- manifests as defects hypodontia and nail beds with normal sweat glands and normal patterns of hair.
- type of non sense mutation in the gene for MSX1.

Mutations in the **p63 gene**

- are involved in the pathogenesis of several syndromes that include ectrodactylia, syndactyly, ectodermal dysplasia and cleft.
- Expression of the p63 gene is important for the development of ectodermal organs including teeth



Syndromes asociated with orophacial defects

- **Van der Woude syndrome** is an autosomal dominant disorder that is characterized by a cleft lip or palate and the obvious defects of the lower lip - gene IRF6 (interferon regulatory factor).
- **Crouzon syndrome** is an autosomal dominant disorder that is characterized by the premature closure of cranial sutures, maxillary hypoplasia and maxillary pseudo-cleft - with mutations in the FGFR2 and FGFR3
- **Apert syndrome** is an autosomal dominant genetic disorder that causes abnormal development of the skull - with mutations in the FGFR2 gene
- **Treacher Collins syndrome** is a disorder characterized by structures derived from the first and second branchiálního arc: hypoplastic zygoma and mandible, micrognathia, Coloboma, ear defects, the Lateran facial cleft, cleft palate. - Mutations in the gene for TCOF1
- **Down's Syndrome** - an underdeveloped facial bones, characterized by delayed development and eruption of teeth (eruptions tarda), missing teeth, incorrect position - chromosomal aberrations
- **Pierre-Robin syndrome** is an autosomal recessive disorder with heterogeneous etiology. It is already known X-linked form of disorder. It is a syndrome characterized by a cleft palate, hypoplastic mandible and tongue hypertrophy
- **Marfan syndrome** is a rare autosomal dominant genetic disorder of connective tissue. Characteristic maxillarní / mandibular retrognacie, micrognathia, narrow arched palate with stěsnanými teeth, and symptoms similar to symptoms in dentinogenesis imperfecta - mutations in the gene for fibrillin-1 (FBN1). FBN1

Caries

- Multifactorial disease with endogenous and exogenous factors

- predisposing factors:

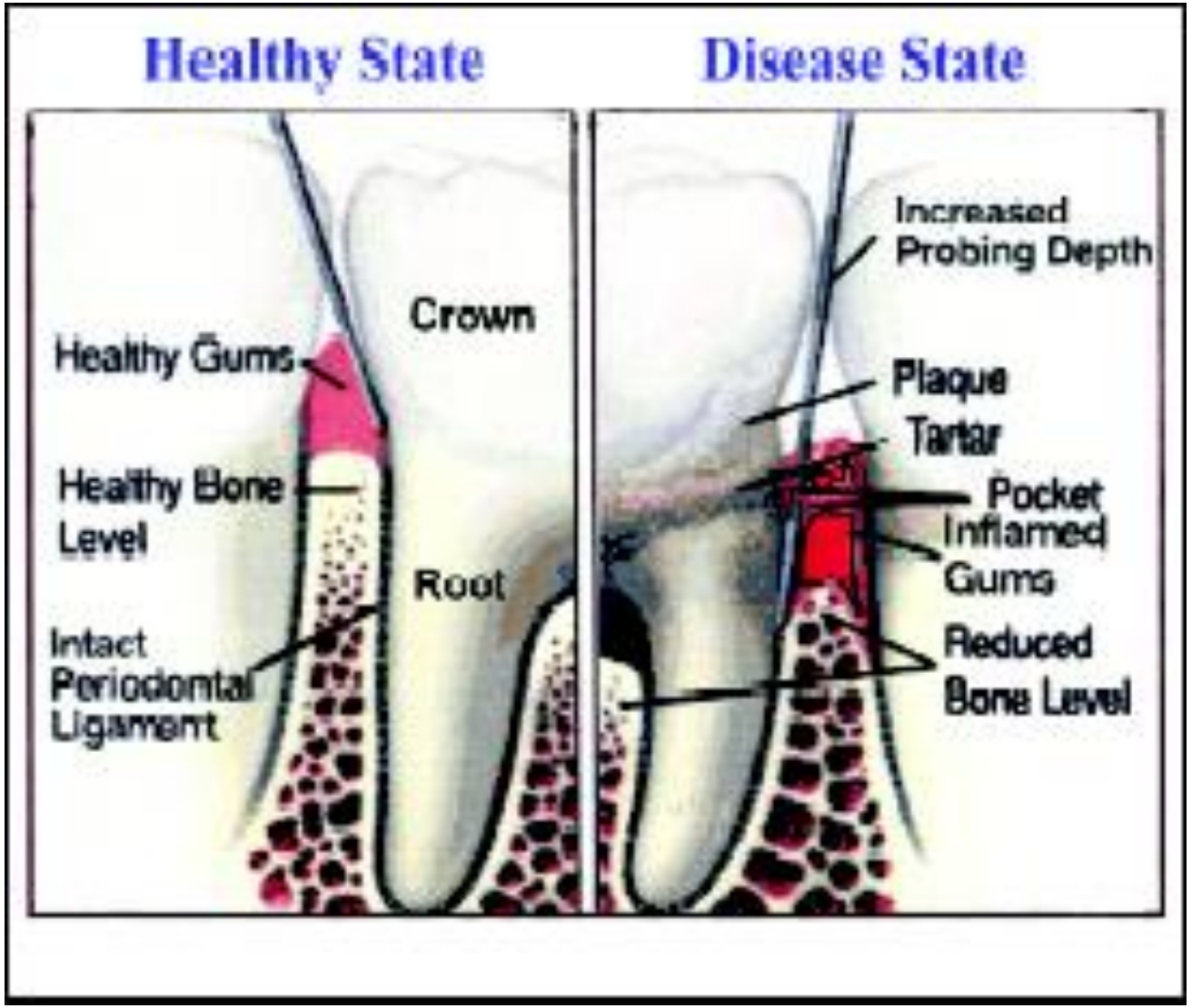
  - inheritance- relation to caries or caries resistance
  - diet rich in carbohydrates
  - fluorine content in drinking water-resistant dental caries have higher fluorine content in hard tissues
  - personal hygiene-enhanced plaque formation by a lack of hygiene

  - tooth- shaped teeth with fissures – more caries

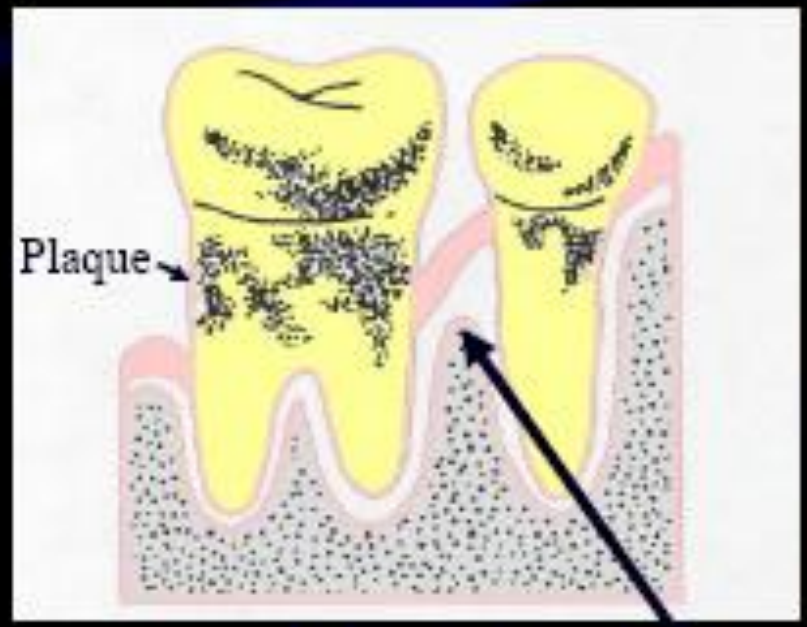
Caries (caries dentium)

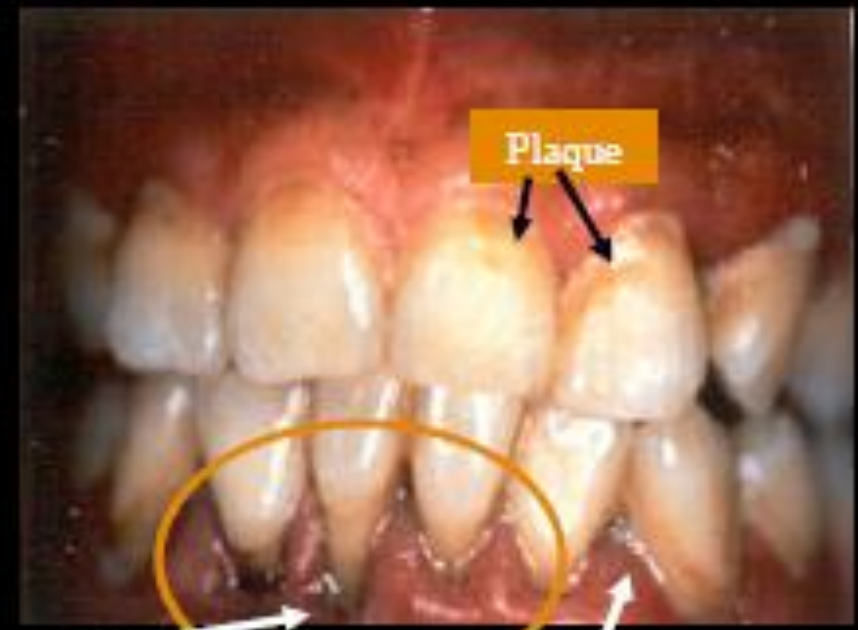
- dekalcification of hard dental tissues
- bacteria-Lactobacillus acidophilus and Streptococcus that break down sugars, food debris, forming organic acids causing decalcification-proteolytic enzymes break down organic ingredients
- plaque-thin coating of bacteria, mucus and desquamated epithelial cells
- other effects-type food composition of saliva, pH
- Decalcification of enamel-whitish chalk stain, softening, a cavity filled with food residue and bacteria-spreading further decay process affects dentin, spreads thrue the dentin tubules until marrow - occurs pulpitis
- **Mutations in the genes that cause structural failure of enamel formation (AMLEX, Enam, KAL4 and MMP20) lead to the formation of the enamel more susceptible to tooth decay.**

Periodontal disease



Periodontal Disease





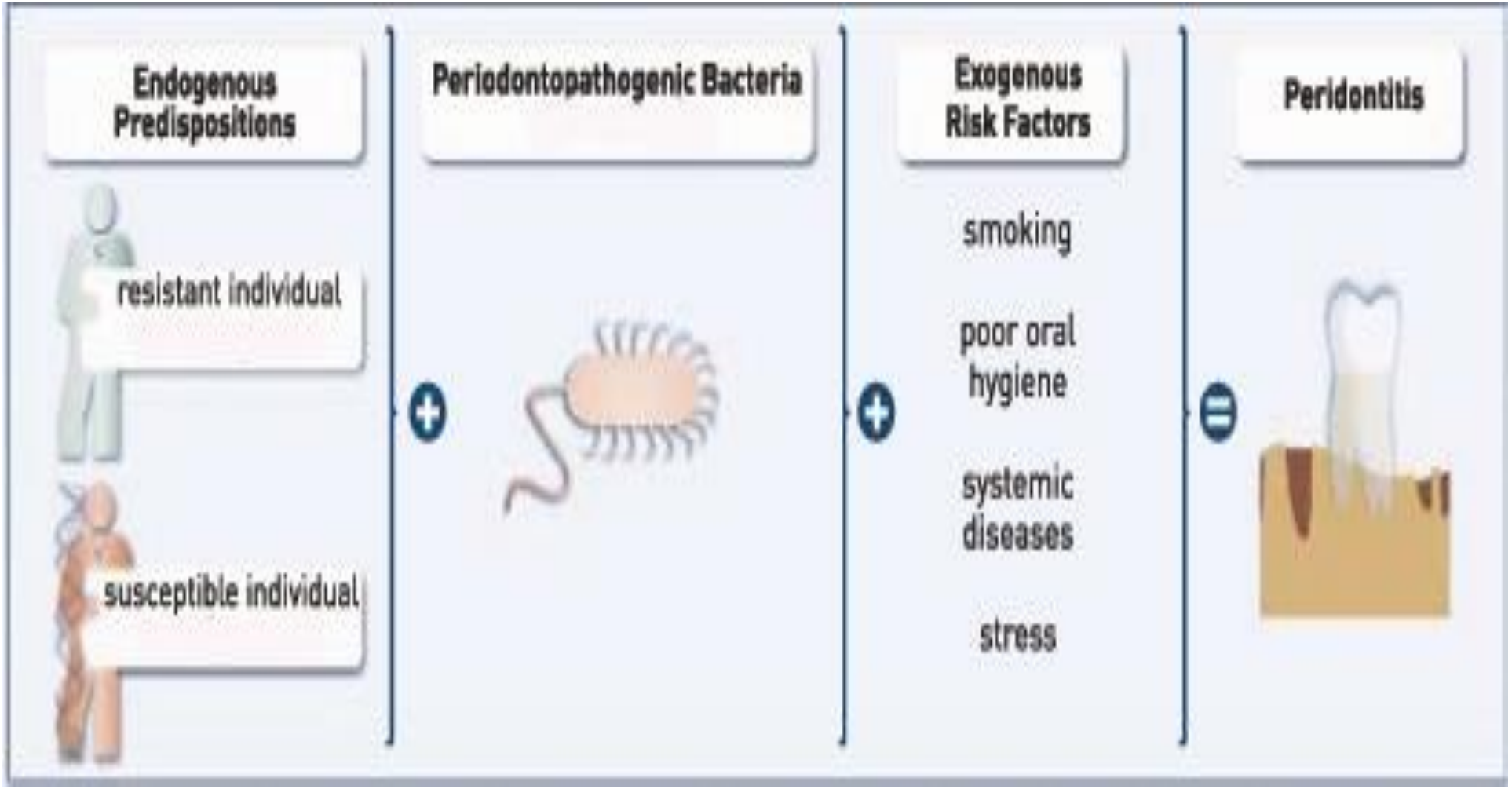
Periodontitis

- Advanced gum inflammation
- Bone loss
- Destruction of ligaments

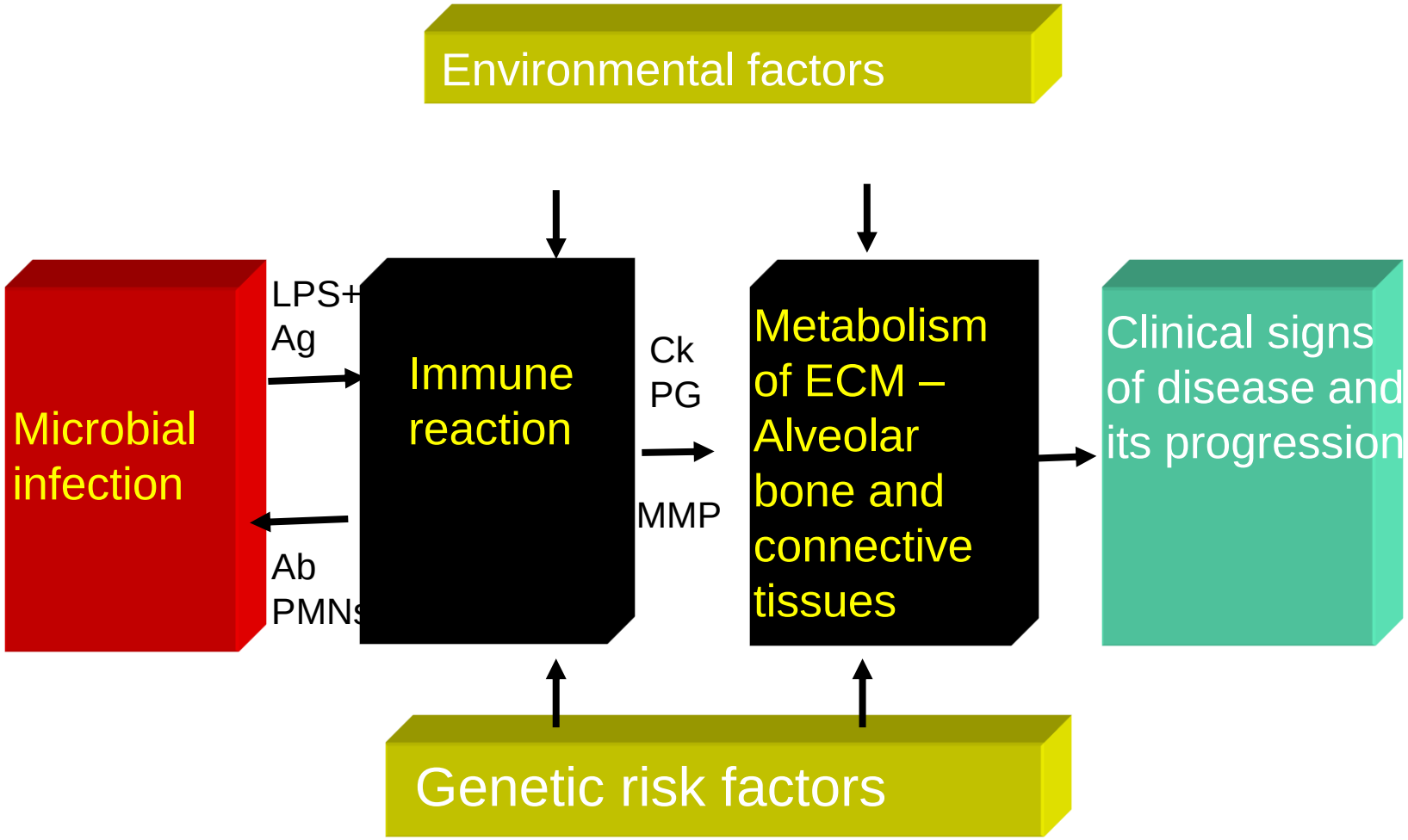
Gingivitis

- Inflamed Gums

Multifactorial disorder



Conception of periodontal disease



Page & Kornman

Syndromic Forms of Periodontitis

- Severe periodontitis presents as part of the clinical manisfestations of several monogenetic syndromes.

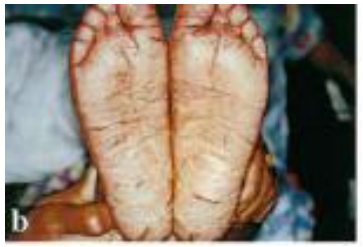
- Significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis.

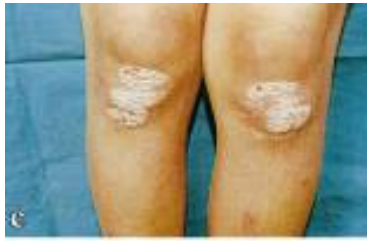
TABLE 2

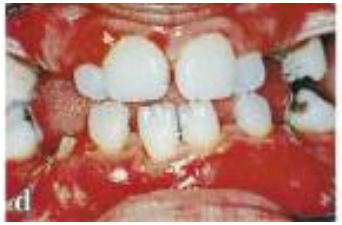
Examples of Syndromic Forms of Periodontitis in Which Inheritance is Mendelian and Due to a Genetic Alteration at a Single Gene Locus

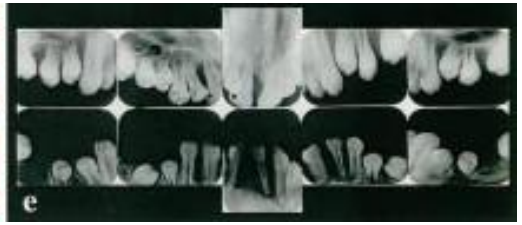
Condition	Biochemical/Tissue Defect	Inheritance	OMIM
Papillon-Lefèvre syndrome	Cathepsin C	AR	245000
Haim-Munk syndrome	Cathepsin C	AR	245100
Ehlers-Danlos syndrome type 4	Collagen	AD	130050
Ehlers-Danlos syndrome 8	Collagen	AD	130080
Cyclic neutropenia	neutrophil elastase	AD	162800
Chronic familial neutropenia	Defect unknown	AD	162700
Chediak-Higashi syndrome	lysosomal trafficking regulator gene	AR	214500
Congenital disorder of glycosylation type IIc	GDP-fucose transporter-1	AR	266265
Leukocyte adhesion deficiency type 1	Leukocyte chain adhesion molecule CD18	AR	116920

Papillon LeFevre Syndrome









- Clinically characterized by:
  - Palmoplantar hyperkeratosis
  - **Severe early onset periodontitis** that results in premature loss of the primary and secondary dentition (distinguishes PLS from other plamoplantar keratoderma)
- Prevalence 1/ 4million
- No gender or racial predilection

CTSC gene encodes for Cathepsin C protease

- **CTSC gene lies on chromosone 11q14-q21; seven exons encoding for lysosomal protease cathepsin C.**

- **It is expressed at high levels in a variety of immune cells including polymorphonuclear leucocytes, macrophages, and in epithelial regions commonly affected by PLS, including the palms, soles, knees, and oral keratinized gingiva (RT-PCR) (Hart et al., 1999).**

- **Cathepsin C is a protease enzyme that processes and activates a number of granule serine proteases critical to immune and inflammatory responses of myeloid and lymphoid white blood cells**

Mutations in CTSC gene

- **Mutations in Cathepsin C (CTSC) gene are implicated for PLS**

- **For example:**
  - **One exon 1 nonsense mutation (856C→T): introduces a premature stop codon at amino acid 286.**
  - **Three exon 2 mutations:**
    - **single nucleotide deletion (2692delA) of codon 349: introduces a frameshift and premature termination codon,**
    - **2 bp deletion (2673-2674delCT): introduces a stop codon at amino acid 343, and**
    - **G→A substitution in codon 429 (2931G→A): introduces a premature termination codon.**

- **Truncated or altered conformation of the protein may not be transported to the organelle and may not be able to activate protein kinases**

- **In other words, Cathepsin C activity in these patients is nearly absent**

Polymorphism Studies on Periodontitis

- Host response is predominantly influenced by genetic make-up.
- Several features of host's innate immune response may contribute to susceptibility to AgP and include epithelial, connective tissue, fibroblast, and PMN defects.
- Aspects of the host inflammatory response namely cytokines are crucial variants influencing host respone in periodontitis.

Immunological Polymorphisms

- MHC or HLA genes determine our response to particular antigens.
- Japanese study of AgP pts found a significant association for pts with atypical BamH1 restriction site in the HLA.DQB gene (Takashiba et al. 1994).
- Hodge & Kinane (1999) found no assoc. in caucasian AgP pts and this restriction site.

IL-1 Gene Polymorphisms

- In 1997 Kornman et al found an association between polymorphisms in genes enconding for IL-1a(-889) and IL-1B(+3953) and an increased severity of periodontitis.
- The specific genotype of the polymorphic IL-1 cluster (called PST-periodontitis susceptibility trait) was associated with severity of PD in only non-smokers, and distinguished individuals with severe periodontitis from those with mild disease.

Genetic control of IL-1: Genes and Locus of SNPs associated with controlling IL-1 biological activity

Genes	Polymorphism Locus	Current Locus assessed with test	Controlled product
IL-1A	Allele 2 -889	Allele 2 IL-1A +4845	IL-1 alpha
IL-1B	Allele 2 +3953	Allele 2 IL-1B +3954	IL-1 beta
IL-1RN			Protein receptor antagonist (impedes IL-1 alpha and beta)

Genetic Susceptibility Test for periodontitis: tests for the presence of at least one copy of allele 2 at the IL-1A +4845 loci and at least one copy of allele 2 at the IL-1B +3954 locus.

**IL-1A +4845 is being used because it is easier to identify than IL-1A -889 and it is essentially concordant with it.*

*** IL-1B +3953 has been now renumbered as IL-1B +3954 because the current convention indicates that the numbering of the transcription should begin at +1 instead of zero.*

Interleukin 1

- A proinflammatory multifunctional cytokine.
- Enables ingress of inflammatory cells into sites of infection
- Promotes bone resoroption
- Stimulates eicosanoid (PGE2) release by monocytes and fibroblasts
- Stimulates release of MMP’s that degrades proteins of the ECM.
- Forms IL-1α and IL-1B

IL-1 as modulator for Periodontitis

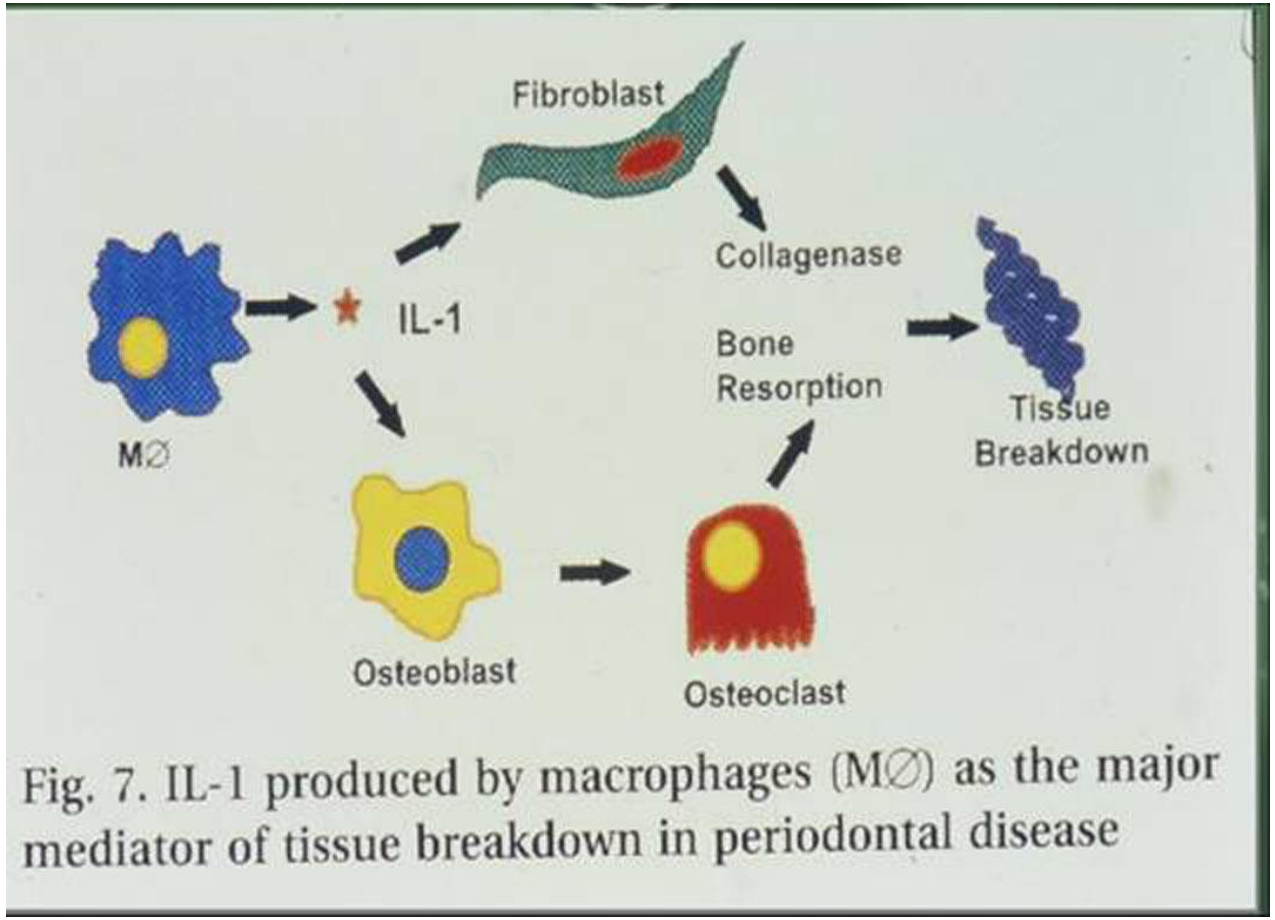


Fig. 7. IL-1 produced by macrophages (MØ) as the major mediator of tissue breakdown in periodontal disease

- **Kornman et al. (1997)**
  - Genotype-positive non-smokers → 18.9 times more likely to have severe periodontitis (when compared with genotype-negative non smokers)
  - No significant association between periodontal status and genotype detected when smokers were included in the statistical analysis.
  - 86% of the severe periodontitis patients were accounted for by either smoking or IL-1 genotype
  - Presence of allele 2 at IL-1A -889 or IL-1B +3953 did not significantly increase the risk of periodontitis among smokers and non-smokers.

+IL-1 genotype and IL-1 protein

- The specific periodontitis-associated IL-1 genotype consists of a variant in the IL-B gene that is associated with high levels of IL-1 production.(Poiciot et al 1992)

- Patients positive for composite IL-1A (+4845) and IL-1B (+3954) periodontitis-associated genotype has higher level of IL-1B in GCF, but not in gingival tissue before and after treatment (Kornmann et al 1999)

- Carriage of allele 2 in the (-889) locus resulted in an almost four fold increase of IL-1 protein levels in chronic periodontitis patients (Shirodaria et al., 2000)

- **Attachment loss**

  Kornman et al. (1997)

  - Reported 18.9 times greater risk (OR=18.9) of finding severe periodonitis among non-smokers genotype-positive.

  McDevitt et al. (2000)

  - Among non-smokers or former smokers, genotype positive individuals had 3.75 greater odds of having moderate to advanced periodontitis than genotype-negative.

- **Genotype and tooth loss**

  McGuire and Nunn (1999)

  - Genotype-positive individuals had a 2.7 greater chance than genotype-negative patients of losing a tooth.

  - Combined effect of being genotype-positive and heavy smoker increased the odds of tooth loss to 7.7 compared with genotype-negative non-smokers.

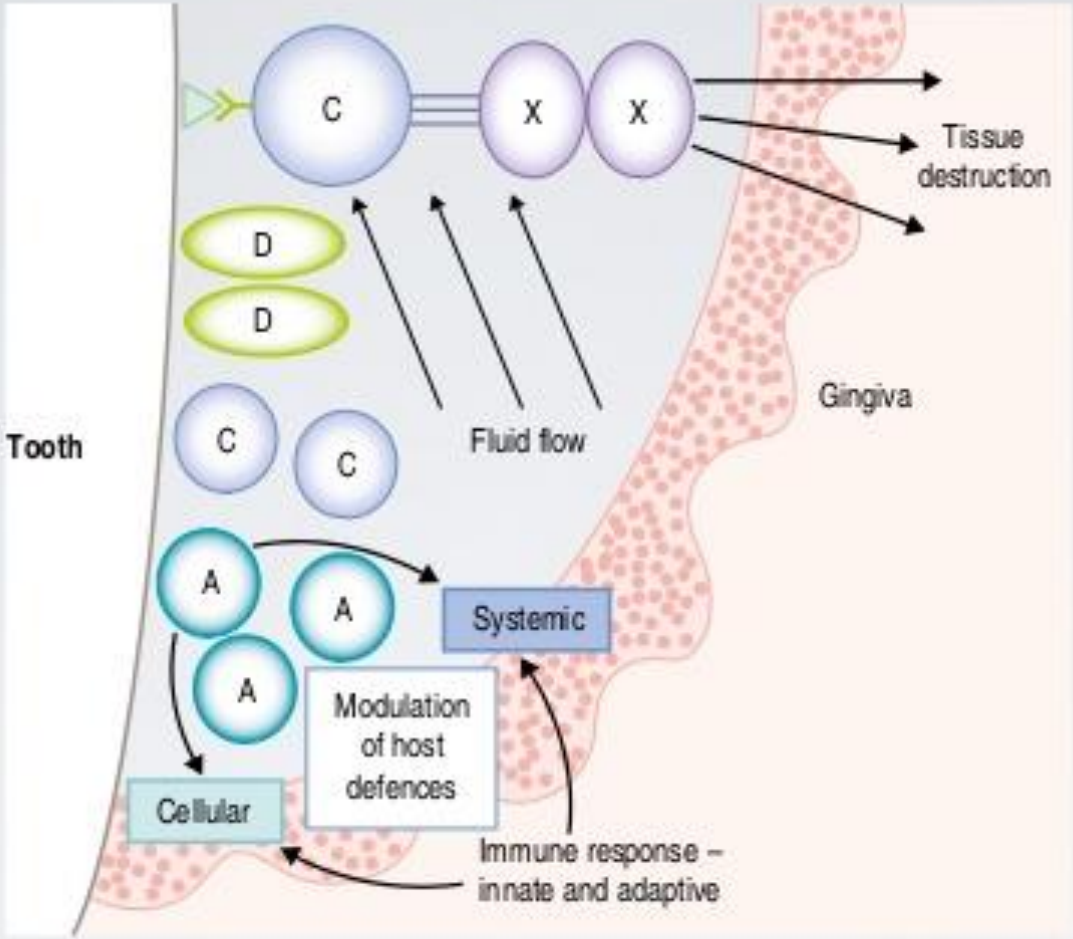
  - PST can be helpful in treatment planning.

Prevalence of genotype positive individuals in different ethnic groups

- Frequency of many genetic alleles varies between ethnic groups, therefore, it is necessary to establish allele frequencies in populations before genetic test can be evaluated and used.

  - **Caucasions:**
    - 29% of northern european caucasions were genotype positive (Kornman et al., 1997)
  - **African Americans:**
    - 14.5% of non-diseased individuals and 8% of patients with localized form of aggressive periodontitis were genotype-positive. (Walker et al., 2000)
  - **Chinese:**
    - 2.3% of sample of 132 mod-severe periodontitis cases were genotype-positive (Armitage et al., 2000)
  - **Hispanics:**
    - 26% of hispanic individuals with peridontitis were genotype-positive (Lopez et al., 2005)

Take home message: Dissimilarity in the prevalence of genotypes in different ethnic groups precludes extrapolating data from one group to another.



Pathogenic synergy in the aetiology of periodontal diseases. Bacteria capable of causing tissue damage directly (e.g. species X) may be dependent on the presence of other cells (e.g. organisms C and D) for essential nutrients or attachment sites so that they can grow and resist the removal forces provided by the increased flow of GCF. Similarly, both of these groups of bacteria may be reliant for their survival on other organisms (e.g. A and C) to modulate the host defences. Individual bacteria may have more than one role (e.g. organism C) in the aetiology of disease.

Microbial factors

- *Porphyromonas gingivalis*
- *Bacteroides forsythus*, newly *Tanarella forsythensis*

- *Actinobacillus Actinomycemcomitans*
- …….

BUT

- The bacteria themselves are not able to cause disease:
  - A wide range of host susceptibility
  - Differences in prevalence and extent between the teeth

Dental plaque biofilm infection

- Ecological point of view
  - Ecological community evolved for survival as a whole
  - Complex community of more than 400 bacterial species
- Dynamic equilibrium between bacteria and a host defense
  - Adopted survival strategies favoring growth in plaque
  - “Selection” of “pathogenic” bacteria among microbial community
  - Selection pressure coupled to environmental changes
  - Disturbed equilibrium leading to pathology
  - Opportunistic infectio

Reaction of the organism to bacterial infection

- Gate input - usually mucosal surfaces (violation of integrity)
- The fate of the host depends on:
  - Immunity (largely genetically determined)
  - Pathogenicity of bacteria (invasive ability, production of toxins, the ability to resist the defense mechanisms of the host)

non-invasive - multiply the point of entry into the body
threatening in case of toxin production
(defense - only neutralizing Pt)

Invasive - penetrate into the organism (x extracellular intracellular)
(defense are Pt, complement, phagocytosis vs. macrophages)

  - The size of the infectious dose

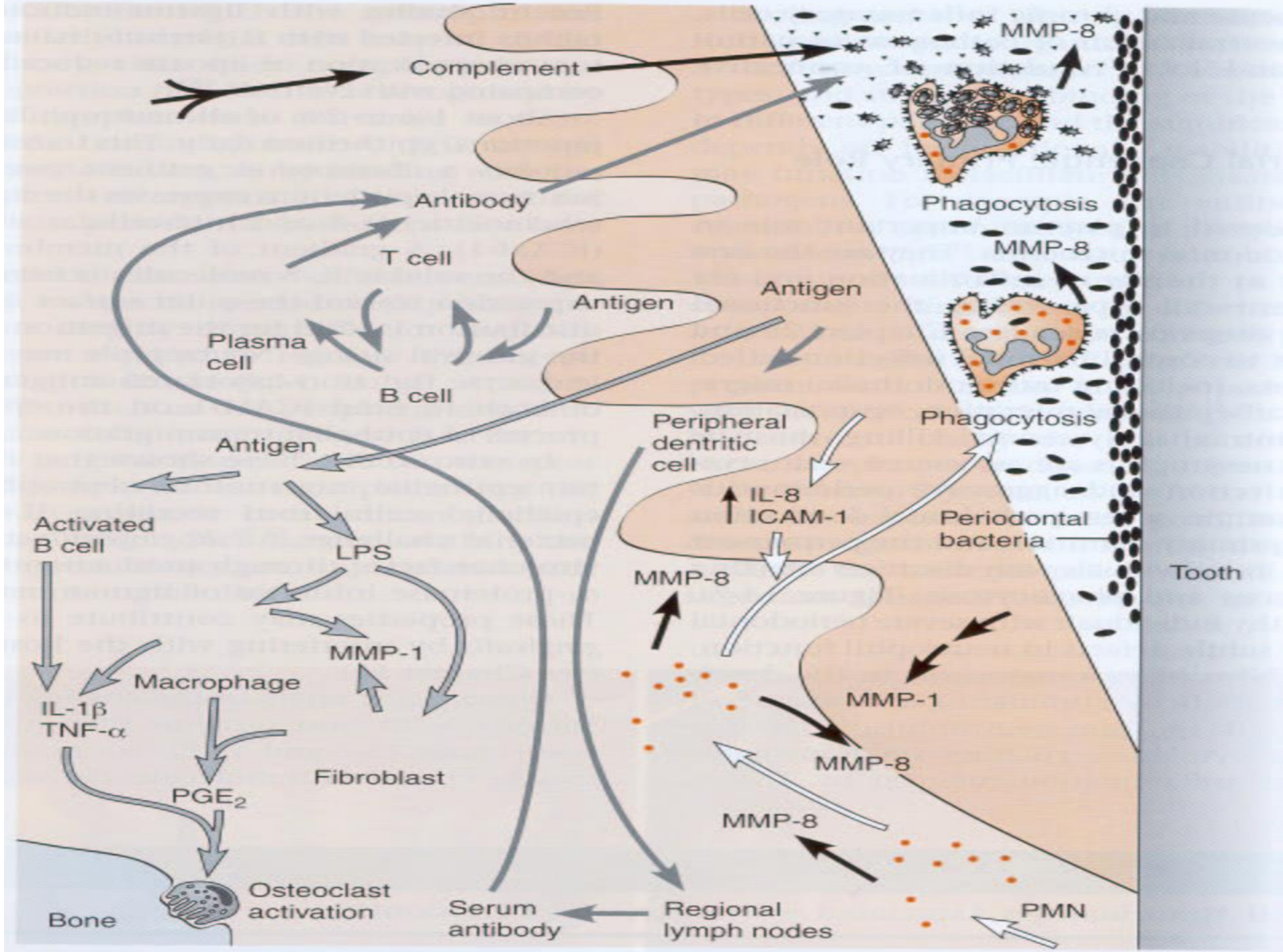
Identifying virulence factors

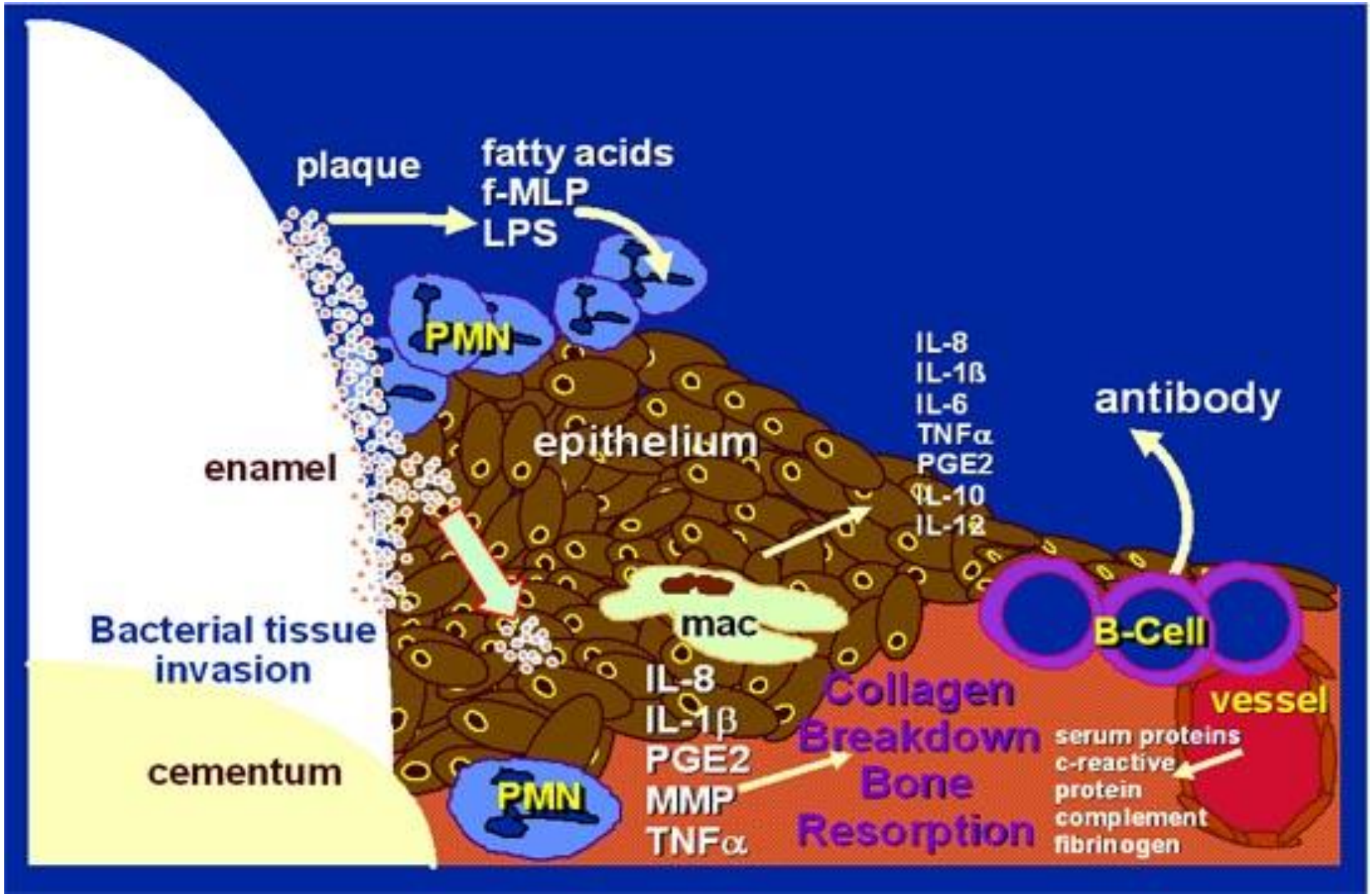
- Microbiological and biochemical studies
    - *In vitro isolation and characterization*
    - *In vivo systems*
- Genetic studies
    - Study of genes involved in virulence
    - Genetic transmission system
    - Recombinant DNA technology
        - Isogenic mutants
        - Molecular form of Koch’s postulates (Falkow)

Koch’s postulates

- A Molecular form of Koch’s postulates
  - The phenotype should be associated with pathogenic species (strains)
  - Specific inactivation of genes associated with virulence should lead to a decrease in virulence
  - Complementing inactivated genes with the wildtype genes should restore full virulence

Falkow, 1988


Complement
MMP-8
Antibody
Phagocytosis
T cell
MMP-8
Antigen
Antigen
Plasma cell
B cell
Phagocytosis
Peripheral dentritic cell
Antigen
IL-8
ICAM-1
Periodontal bacteria
Activated B cell
LPS
Tooth
MMP-8
MMP-1
Macrophage
MMP-1
IL-1β
TNF-α
MMP-8
Fibroblast
PGE2
MMP-8
Osteoclast activation
Bone
Serum antibody
Regional lymph nodes
PMN


plaque
fatty acids
f-MLP
LPS
PMN
epithelium
enamel
IL-8
IL-1ß
IL-6
TNFα
PGE2
IL-10
IL-12
antibody
Bacterial tissue
invasion
mac
B-Cell
IL-8
IL-1β
PGE2
MMP
TNFα
Collagen
Breakdown
Bone
Resorption
vessel
serum proteins
c-reactive
protein
complement
fibrinogen
cementum
PMN

TLRs

- First described as a gene for type I transmembrane receptor

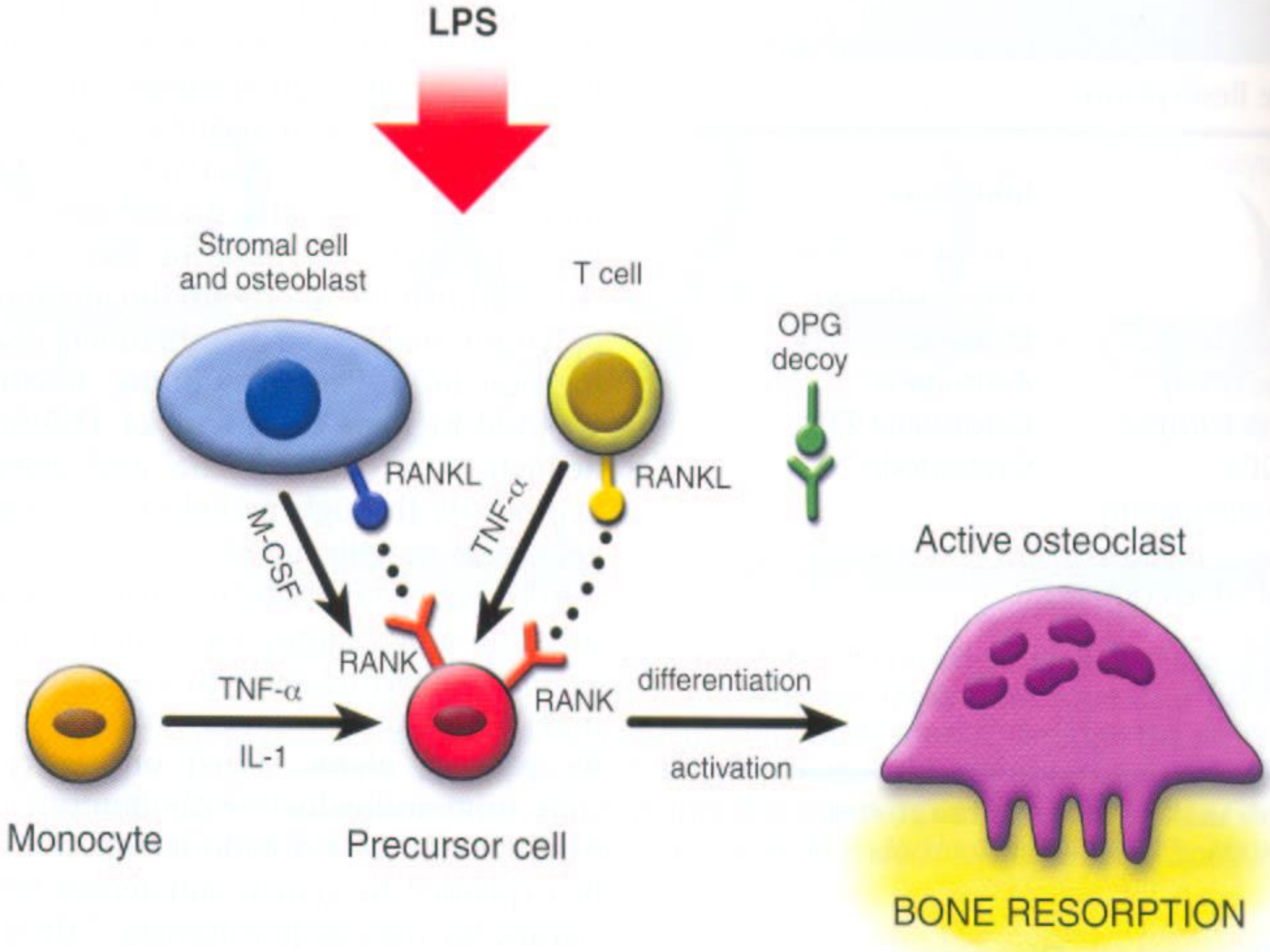
$\rightarrow$ important role in dorzoventral embryonic development of Drosophila

$\rightarrow$ absence of tolls has led to a serious brake-down of defense against fungi and bacteria G+

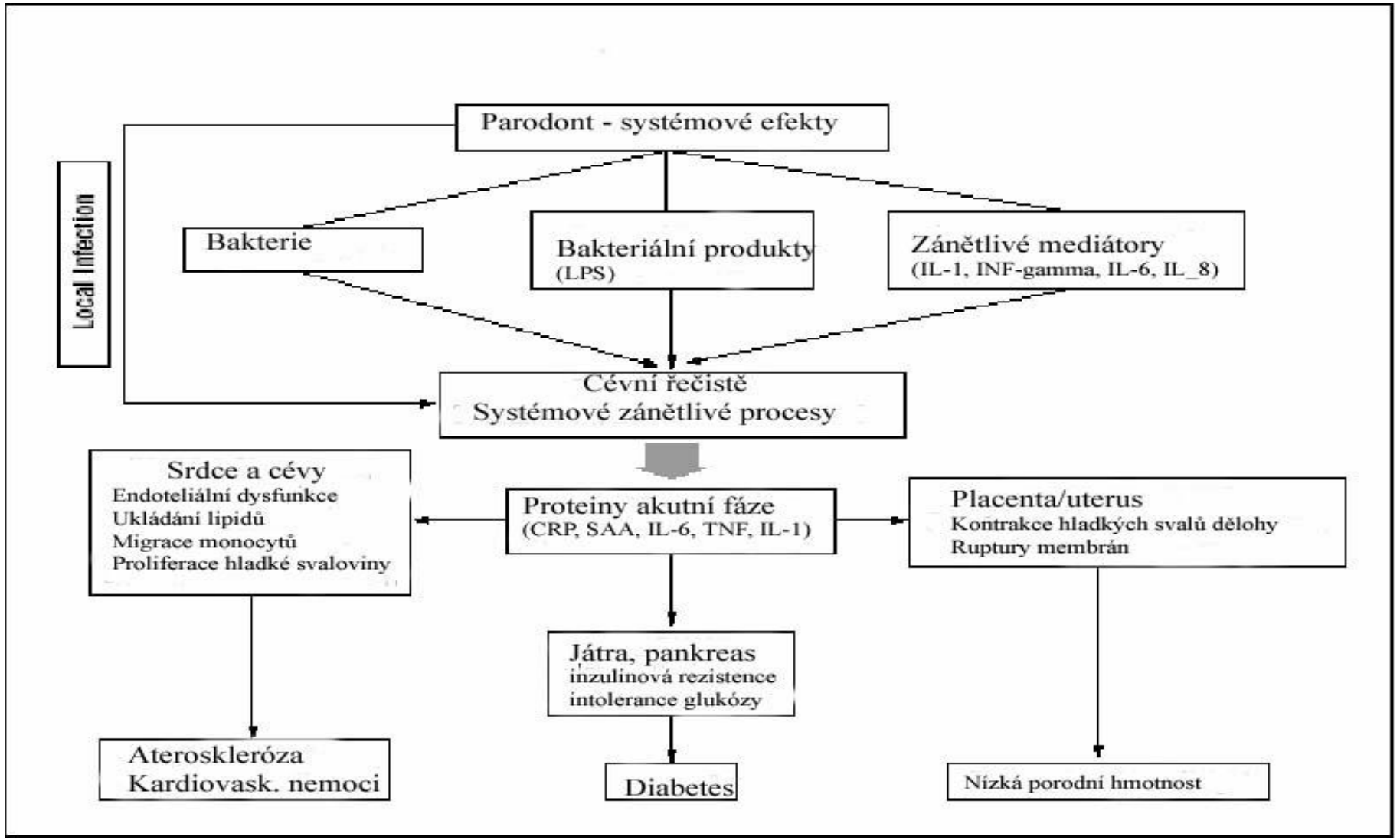
$\Rightarrow$Mammalian homologues - similar role??

TLRs receptors and ligands in periodontal disease

PRR	PAMP	Periodontal Pathogen
TLR-2	Lipoproteins	*Bacteroides forsythus**
	Atypical LPS	*P. gingivalis, C. ochracea*
	Outer membrane proteins	Oral treponemes
	Fimbriae	*P. gingivalis*
	Nonendotoxic glycoprotein	*P. intermedia*
TLR-4	HSP-60 (GroEL)	*P. gingivalis*
	LPS	*A. actinomycetemcomitans, F. nucleatum*
TLR-9	CpG-containing DNA	*A. actinomycetemcomitans, P. gingivalis, P. micros*


LPS
Stromal cell
and osteoblast
T cell
OPG
decoy
RANKL
RANKL
M-CSF
TNF-α
RANK
RANK
TNF-α
IL-1
differentiation
activation
Active osteoclast
Monocyte
Precursor cell
BONE RESORPTION

Local problem?



Periodontitis

- follows the loss of pulp vitality - the dead tooth

- Infection is usually odontogenic
- Trauma - one-time or repetitive microtrauma
- Acute periodontitis (apical)
- Hyperemia and serous exudation
- Suppuration, osteoclastic bone remodeling

- Strong pain at all stages, swelling in the later stages

- **Relationship of polymorphisms in genes IL1B, IL1RN, FcγRIIIb, VDR and TLR4 with an aggressive type of periodontitis.**

Periodontitis

Chronic periodontitis (periapical)

- Secondarily from acute periodontitis
  Primarily chronic (more frequent)
- Forms:
  Granulomatous
  Granulomatous progressive - fistula (mucosal and cutaneous)
  Diffusion - dismantled and alveolar bone

- granulation tissue
  macrophages
  Possibility of creating radicular cysts

- The type of chronic periodontitis is associated with polymorphisms in genes for IL1B, IL1RN, IL6, IL10, VDR, CD14, TLR4 and MMP1.

- Meta-analysis of published data have associated variants ofpolymorphisms IL1A-889, IL1B 3954, IL1B-511, TNFA-308 and IL6-174 to aggressive and chronic periodontitis.